Advances and Challenges in Plant Breeding, Biotechnology and Conservation is compilation of papers presented in National Seminar on Patents, Plant Breeding, Biotechnology and Conservation, MBGIPS, Kozhikode, 3-5 December, 2015

Malabar Botanical Garden and Institute for Plant Sciences
Kozhikode, Kerala, India
An institution of Kerala state council for Science, Technology and Enironment

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Editors

R. B. Smitha
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PINARAYI VIJAYAN
CHIEF MINISTER

GOVERNMENT OF KERALA

Secretariat
Thiruvananthapuram-695 001

5th July, 2016

Message

It gives me immense pleasure to note that the Malabar Botanical Garden and Institute for Plant Sciences (MBGIPS), Kozhikode is publishing a book titled "Advances and Challenges in Plant Breeding, Biotechnology and Conservation" which contains the proceedings of the three day National Seminar held at the institution during 3rd–5th December, 2015 in association with the Gregor Mendel Foundation. I understand that the MBGIPS is one of the fast upcoming prestigious botanical research institutions of our country with excellent conservatories for aquatic/wetland plants, Lower plant groups, medicinal plants etc, as a sincere attempt to conserve our rich plant diversity.

I am happy to note that the present book is on the most relevant topics of the day, ie., Plant Breeding, Biotechnology and Conservation with 78 research papers on topics relevant to agriculture and its conservation efforts.

I appreciate the scientists of MBGIPS, Kozhikode and Gregor Mendel Foundation, Calicut University for bringing out this volume, which will be released on the 21st July, during the birthday celebrations of Fr Gregor Johan Mendel.

I wish you all success.

Pinarayi Vijayan

Shri. R. Prakashkumar
Director,
Malabar Botanical Garden & Institute for Plant Sciences,
Kozhikode

M S Swaminathan

Preface

Gregor Mendel Foundation (GMF) was established in 1991 at University of Calicut by Prof K Pavithran, then Professor of Genetics, to commemorate the memory of the pioneering geneticist Gregor Johan Mendel, "the Father of Genetics". The GMF organizes Mendel's birthday lecture on every 21st July and a National level Seminar on Plant Breeding and Genetics related topics alternatively. A National Seminar with the topic "Patents, Plant Breeding, Biotechnology and Conservation" was organized at the Malabar Botanical Garden and Institute for Plant Sciences (MBGIPS) with an overwhelming participation and active, meaningful deliberations. There were invited lectures by senior scientists across the Country prior to each session followed by paper presentation by participating scientists. The papers are scrutinized, peer-reviewed and edited in the form of a book and is presented here as *Proceeding of the GMF* seminar.

Genetics is the branch of biology which has revolutionized the scientific world leading to the production of high yielding crops through plant breeding leading to the Green Revolution which has virtually saved the world from the feared famine and poverty and the consequent unrest and violence which could have been erupted out of hunger and poverty.

The name, Gregor Mendel should always be thankfully remembered for the great services he has made to mankind by evolving the basic principles of genetics i.e., dominance, segregation, and independent assortment which later has grown into the noble science of Genetics, Plant breeding and Crop improvement. In the present book there are 78 articles/papers which include five invited papers, 17 papers on Plant Breeding, 31 papers on Biotechnology and 25 on Conservation.

I congratulate MBGIPS and the GMF on this appropriate tribute to Mendel.

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Contents

Western Ghats – Biodiversity Conservation

N. Preetha, K.P. Laladhas and Oommen V. Oommen*

The biodiversity that exists today is the result of over 3.5 billion years of evolution, involving speciation, migration, extinction and human interactions. Just 17 of the world's 190 countries contain 70 percent of its biodiversity, making them "megadiverse" countries. India is a megadiverse country, accounting for 7-8% of the species of the world, including about 91,000 species of animals and 45,500 species of plants, that have been documented in its ten bio-geographic regions. It is home to 8.58% of mammalian, 13.66% of avian, 7.91 % of reptilian, 4.6% of amphibian, 11.72 % of fish, documented so far. India is recognized as one of the nine Vavilovian centres of origin of crop plants. Western Ghats are part of the Western Ghats- Sri Lankan global hotspot stretching nearly 1600 km in length from Tapti river in north to Kanyakumari in the south. Western Ghats traverse through Gujarat, Goa, Maharashtra, Karnataka, Kerala and Tamil Nadu and run parallel to the west coast at a distance of 40 km, on an average, from the shore line. The Western Ghats form the major watershed in Peninsular India and as many as Fifty Eight major Peninsular Indian rivers originate from it.

Biodiversity of Western Ghats

The Western Ghats are second to the Eastern Himalaya as a treasure trove of biological diversity in India and is considered one of the eight, hottest hot spots` of biodiversity. The high western slopes of the Ghats harbor evergreen forest, and the vegetation changes to moist and dry deciduous forest types from western to eastern slopes. A diversity of vegetation types, including scrub forest in the low lying rain shadow areas and plains, deciduous and tropical rain forests upto about 1,500 mts. and rolling grasslands above 1,500 mts. are encountered here. The southern parts of the WG are diverse and have high endemicity compared with the central and Northern parts. Western Ghats harbor more than 7388 species of flowering plants, of which 5584 species are indigenous. Out of this 2242 species are Indian endemic and 1261 are Western Ghat endemics.

There are four major forest types in the Western Ghats: evergreen, semi-evergreen, moist deciduous and dry deciduous and shola-grassland complexes in the higher elevations (1,900-2,200 meters). The highest levels of endemism are found in the evergreen forests occuring within a 200-1,500 meter elevational range and 2,500- to 5,000 millimeter rainfall range. The Wayanad evergreen forests of Kerala represent

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a transition zone to the northern drier dipterocarp forests. The moist deciduous forest type occupies the largest area within the Western Ghats at an elevational range of 500-900 meters in areas with mean annual rainfall of 2,500-3,500 millimeters.

Among the invertebrate group about 350 ant species (20% endemic) 330 butterfly species (11% endemic), 174 odonate species (40% endemic) 269 mollusc species (76% endemic), 288 species of fishes (41% endemic) and amphibians 220 species (78% endemic) 225 species of reptiles have been reported, The western ghats are unique for caecilian diversity harboring 16 of the country's know 20 species all of which are endemic. Among reptiles, Cochin forest cane turtle and Travancore tortoise are endemic to evergreen forests of southern Western Ghats. The primitively burrowing snakes of the family Uropeltidae are mostly restricted to the southern hills of the Western Ghats. The monotypic agamid genus *Dravidogecko* is reported from Anamalais only. The amphibians reported from WG include the recently discovered *Philautus ponmudi, Philautus anili, Philautus tuberohumerus, Philautus neurostegona, Philautus ochlandrae, Philautus akroparallagi and Philautus chromasynhysi*. The type locality for *P. ochlandrae* is the reed brakes of Kakkayam dam site, the only known site for this species. The recent discovery of a new genus of frog, *Nasikabactrachus sahyadrensis*, with Indo-Madagscan affinity, in the southern Western Ghats affirms the importance of the region in harbouring these ancient Gondwanan lineages. The Anamudi peak area is the habitat of a *Raorchestes resplendens*, a frog species only found here. *Tomopterna parambikulamana*, an endemic frog of Parambikulam and *Garra surendranathanii*, an endemic sucker fish contributes to biodiversity of the area.

Butterflies of Kerala include, the Crimson Rose, Malabar Banded Swallowtail and the Danaid Eggfly which are placed under Schedule -I of the Wildlife conservation Act. Thirteen endemic butterflies which are dominant in the higher elevations are found in the shola. Many bird species are endemic to Western Ghats such as Blue winged parakeet, Malabar grey hornbill, Grey headed blue bill, White bellied Treepie, rare species like Three toed forest kingfisher, Ceylon Frogmouth, Crimson-Throated Barbet, Bee-Eater, Sunbird, Shrike, Fairy Blue Bird, Grey Headed Fishing Eagle, Black Winged Kite, Night Heron, Grey Heron, Malabar Trogon and Malabar Grey Hornbill.

Protected Areas of Western Ghats

To conserve the rich biodiversity of Western Ghats protected areas have been established. The Eravikulam-Rajamala Wildlife Sanctuary, Kerala is habitat of Neelakurinji (*Strobilanthes kunthianam*), which blooms once in 12 years. The park represents the largest and least disturbed stretch of unique Montane Shola-Grassland vegetation in the Western Ghats. Forests of Aralam and adjacent areas

represent the only compact protected patch of vegetation belonging to the unique vegetation sub-type viz; the *Dipterocarpus-Mesua-Palaquium* sub-type in Kerala. Chinnar Wildlife Sanctuary is the only protected area in Kerala State located in the rain shadow region of Western Ghats and represents a large number of plants and animals unique to the thorny vegetation. The Mannavan Shola is the largest shola forest patch in South India. The giant fern, *Cyathia crinita* reaching up to 6m height is a unique character of Mannavan Shola. A very important rare and endemic medicinal plant named as Arogyapacha (*Trichopus zeylanicus*) is very widely distributed in Shendurney Wildlife Sanctuary. This plant is known to have anti-fatigue properties and is widely used by tribals and locals. Myristica swamps are unique ecosystems rich in biodiversity. Eighty species of butterflies belonging to 5 families were recorded from these swamp forests with a land area of less than 1.5 km^2 (149.75 ha).

Mendelian Inheritance and Agrodiversity

Farmers started selective breeding of crops for desirable characteristics much before the basis of genetics was discovered. Gregor Mendel's discoveries explaining how traits pass from parents to offspring shed new light on the matter. Mendel's pioneering work showed that genes separate during the formation of gametes, and unite randomly during fertilization; he also showed that genes are transmitted independently of one another to offspring. This understanding of the way that plants and animals acquire traits form parents created the potential for people to selectively breed crops and livestock. Gregor Mendel's discovery revolutionized agriculture by launching the development of selective cross breeding with a comprehensive understanding of the underlying mechanisms of inheritance.

Agricultural Diversity of Western Ghats

India is one of the eight vavilovian centres of origin of crop diversity and also a major centre of crop plant domestication with 25 crop species domesticated here. In India eighteen agro-biodiversity hotspots has been identified, including Travancore/Malabar area of Kerala. The wild relatives of cultivated plants constitute a rich reservoir of genetic variation and this diversity is of immense value to breeders.

The ecosystem of Western Ghats is extremely complex and people have been existing in harmony with nature in the Western Ghats. The Western Ghats harbour a number of wild relatives of cultivated plants, including pepper, cardamom, mango, jackfruit and plantain. The commercially important species as teak and plantations of coffee, cardamom, tea, *Acacia* and *Eucalyptus,* cashew, rubber, bananas, arecanut, coconut, etc. occupy large area and also make the forest

landscape highly mosaic. These wild relatives of crop plants play a vital role as genetic sources in plant breeding programmes. The traditional land use in WG was paddy cultivation and evolution and natural selection over centuries have resulted in a large number of traditional varieties possessing special traits like resistance to biotic and abiotic stresses, ability to adapt to drought or floods, quality attributes like medicinal value, aroma and resistance to diseases. Wild relatives of cultivated rice play a very important role in rice breeding practically and theoretically. The traditional rice varieties were found to have several characters as disease resistance, flood and drought tolerance. The traditional varieties of rice from Wayanad includes drought-tolerant and short-duration varieties like *Kalladiyaran, Thonooranthondi* and *Urunikaima*; scented varieties like Jeerakasala, Gandhakasala, *Kayama* and *Mullankayama* medium to long-duration varieties such as *Gandhakasala* and *Jeerakasala*; flood-tolerant, long-duration varieties like *Chettuveliyan*, medicinal varieties like Njavara.

The two hot spots of global biodiversity viz. North Eastern Himalayas and Western Ghats are particularly rich in wild relatives of tropical root and tuber crops. Most of the species of Yam, *Dioscorea alata* L. are ethnically important as a delicacy for tribal people in the interior areas in the Western Ghats. Edible roots, tubers and rhizomes of 24 wild plant species/varieties are eaten by the tribal communities in Wayanad. The tribals collect *Dioscorea* from almost all landscapes, but most frequently from the forests. *D. oppositifolia*, *D. pubera*, *D. bulbifera*, *D. hamiltonii*, D. *entaphylla, D. tomentosa, D. kalkapreshadii, D. wightii, D. wallichi, D. hispida* and *D. spicata* are some of the important species (Edison and Sheela 2009).

Piper nigrum is believed to have originated in India in the submontainous tracts of the Western Ghats. Seventeen Piper species are reported from Western Ghats, of which 12 are endemic. Considerable variation exists among the landraces with respect to an array of plant morphological characters giving them the status of distinct plant types, each with its own characteristic features. Much variability has been noticed among the cultivars for important quality characters also. The cultivar diversity is enormous with more than 100 cultivars available. Wild Pepper species are present in certain localized regions of Western Ghats. The Piper species are found growing extensively in the moist evergreen and the semi-evergreen forests of Western Ghats.

Western Ghats are considered as secondary center of origin of *Cinnamomum verum*. (Edison and Sheela 2009). The diversity of *Cinnamomum* included besides *C. verum, C. cassia, C. camphora* (an economically important tree yielding camphor oil), a lemon grass oil smelling *Cinnamomum* spp. collected from Munnar (Idukki Dt.) *C. malabatrum, C. perrottetti, C. wightii, C. macrocarpum, C. sulphuratum, C. riparium* and *C. tamala.*

Cardamom (*Elettaria cardamomum* Maton) is indigenous to evergreen forests of Western Ghats in South India. Eight species has been described from the Western Ghat and adjacent areas. Kerala and the Western Ghats form the secondary centre of mango origin in the world. *E. superbum*, *Kalluvazha* a rare medicinal wild banana found in Western Ghats region is used by traditional healers to treat diabetes, leucorrhoea and kidney stone.

Agrodiversity provides plant breeders with the basic genepool to be incorporated for crop improvement. Any crop improvement programme depends to a great extent upon the effective use of both wild and improved agricultural biological diversity, which constitutes an indispensable input to modern plant-breeding science. The Western Ghats harbour a number of wild relatives of cultivated plants, including pepper, cardamom, mango, jackfruit and plantain which can provide plant breeders with the necessary building blocks for breeding programme**s.**

Conclusions

Economic growth can be inclusive only if the natural resources are sustainably managed. An integrated natural resource management policy has to be adopted whereby the interlinkage between water, soil and forest has to be adopted. Proper management of water and maintaining a balance between supply and demand is the most pressing natural resource challenge of this century.

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Edison S, Sheela MN (2009) Diversity of Tuber crops and wild relatives, in Agrobiodiversity Hotspots Access and Benefit Sharing Eds S Kannaiyan, Narosa Publishing House, Kolkata

Science of Plant Breeding-Milestones

K. V. Peter* and T. Pradeep Kumar[1]

The art and science of plant breeding are partitioned into four periods- knowledge of plant breeding before 1900 (Pre-Mendalian), application of genetic principles (Mendalian) 1900-1940, plant breeding research after 1940 (post Mendalian) and the recent developments including molecular breeding, transgenics, designed crops, biofortification and bioremediation. The major milestones in genetics and breeding of crop plants are listed below:

AD 1 - 1900

1665- Hooke - Described the cell.

1675- Malpighi - Conducted anatomical investigations in plants; repeated the observations of Hooke.

1676 -Millington - Anthers function as male organs.

1694- Camerarius - First to demonstrate sex in plants. Suggested crossing as a method to get new types. Worked with mulberry and maize. Systematic plant breeding was established at this point. Slow progress because workers did not recognize difference between genetic and environmental variation.

1716- Mather - Observed natural crossing in maize. Noted xenia, where ears of yellow maize planted next to red and blue maize had red and blue kernels in them the first year.

1719- Fairchild - Created first artificial hybrid of Carnation x Sweet William, commonly known as Fairchild's mule.

1753- Linnaeus - Published "Species Plantarum". Binomial nomenclature of plant taxonomy officially begins with his general list of plant species.

1761-66- Koelreuter - Demonstrated that hybrid offspring received traits from both parents (pollen and ovule transmit genetic information), and were intermediate in most traits. First scientific hybrid produced (tobacco). Demonstrated the identity of

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reciprocal crosses. Noted hybrid vigor, segregation of offspring (parental and non-parental types) from a hybrid.

1778 -Herbert - Suggested winter hardiness was inherited after observing the research of Koelreuter.

1779 - Knight - Emphasized the practical aspects of hybrids. He worked on crop improvement of garden pea. Noted the advantage of outcrossing to produce new forms.

1785- Van Mons - Started systematic selection of horticultural plants. Seed catalogue in 1825, listed 1050 cultivars.

1793- Sprengel - Classic publication on the role of insects in pollination of angiosperms. Studied flower structure and color in relation to pollination and insect habits.

1801- Lamarck - Theory of evolution through inheritance of acquired characters.

1812- Gauss - Suggested the theory of least squares. Had begun the idea of "probable error" by 1820.

1819- Shirreff - Utilized pure line selection (progeny test) to develop a new oat cultivar (released in 1824), and a new wheat cultivar (released in 1832). Had first nursery plots, with 70 families by 1857. Suggested crossing parents with desirable characteristics to obtain progenies of value.

1820- Goss - Reported dominance and recessiveness in peas.

1820 - Knight - Noted that male and female parents contribute equally to the F_1, with segregation following in the F_2. Made first wheat variety cross in Europe.

1827 - 49- Von Gartner - Made the most extensive crossing experiments to date, with 10,000 crosses in 700 species from 80 genera.

1831- Brown - Discovered the eukaryotic cell nucleus.

1837-38- Schleiden and Schwann - Developed the cell theory of higher plant organization.

1844-63- Godron - Used sterility of interspecific hybrids as evidence that the parents were distinct species.

1844- Unger - Showed that cells come from the division of preexisting cells.

1846- Quetelet - Described biological traits in quantitative terms.

1847- Sutton - Developed cultivars of beets, peas and potatoes for production in Great Britain.

1848- Hofmeister - Observed dark staining bodies (chromosomes) in the cell nucleus.

1855- Virchow - Established that the egg of one generation comes from the egg of the previous generation; the "continuity of heredity".

1858- Hofmeister - Discovered the female gametophyte.

1859-89-Darwin- Published "Origin of Species"; noted inbreeding, sterility, and differences in reciprocal crosses.

1860- Louis Vilmorin - Stressed the value of self-pollination in breeding stable cultivars of wheat and sugar beets.

1860-Naudin - Did research much like Mendel, describing the separation of essences in various proportions in germ cells and recombination in the offspring of a cross. Used *Datura* for his rather complex experiments. Thought the whole plant was a mosaic of the two parents.

1861- Pasteur - used heat to kill bacteria.

1861- Schultze - Found that protoplasm was an essential element in the cells of all organisms.

1866 -Mendel - Published "Experiments in plant hybridization"; discovered unit factors (genes), segregation of F2, recombination of two or more genes, and dominance of one allele over another; formulated the laws of inheritance.

1875-77- Strasburger - First adequate description and drawing of chromosomes; nuclei arise only from nuclei; suggested the terms "gamete" and “chromosome".

1878- Henry Vilmorin - Did the first extensive work with interspecific crosses: *Triticum vulgare* x *T. polonicum* and *T.durum.*

1885-87- Weismann - Wrote 12 essays on heredity and evolution, important in directing the trend of biology and thought; summarized current knowledge and pointed out the broad implications: continuity of germplasm, equal inheritance from parents, and non-heritability of acquired characters.

1884- Strasburger - Demonstrated fertilization and showed the fusion of the two' nuclei to form the zygote.

1888- Strasburger - Demonstrated reduction division in plants.

1889- Galton - Wrote book on natural inheritance.

1894- Pearson - Made contribution to mathematical aspects of testing; introduced the term "standard deviation".

1900- Correns (Germany) and DeVries (Holland) and von Tschermak (Austria) - Independently rediscovered Mendel's laws of heredity.

1900-Bateson - Introduced the terms "allelomorph", "homozygote", "heterozygote", "F_1" and "F_2".

AD 1901 - 1940

1901-02-Bateson and Punnett - Called public attention to the importance of Mendel's work; first report of linkage (using garden pea).

1902- DeVries - Proposed the mutation theory of evolution from evidence in *Oenothera.*

1902 - Biffen - First inheritance studies on disease resistance; found that stripe rust resistance was due to a single gene.

1903- Johannsen - Developed the pure line theory of selection.

1904- Hannig - Contributed to the idea of embryo culture.

1904-05- East and Shull - Began inbreeding experiments with maize.

1905- Shamel - Reported yields of maize inbred lines for 3 generations and their hybrids; first report of hybrid made by crossing inbred lines.

1905-07- Williams - Developed the remnant seed breeding plan for maize.

1906- Bateson - Introduced the term "Genetics".

1908-09- Hardy and Weinberg - Developed law of equilibrium for populations.

1908-10- East - Published his work on inbreeding and proposed the idea of multiple alleles at a locus.

1910- Morgan - discovered genes on chromosomes.

1910- Bruce, Keeble and Pellew - Proposed theory for hybrid vigor due to favorable dominant genes.

1910- Wood and Stratton - Suggested check plots to correct for soil variability in yield trials.

1911- Collins and Kempton - Demonstrated linkage in maize.

1911- Mercer and Hall - Introduced questions about number, plot shape and size, and replications in field trials.

1912- Jennings - Selfing reduces heterozygosity by one-half in each generation.

1912-Harris - Suggested the use of Chi-square for testing the goodness of fit for segregation ratios.

1913- Sturtevant - Provided the experimental basis for the concept of linkage vs. map distance.

1916- Jones and Gilman - Released yellow resistant cabbage.

1916- Winkler - Introduced the term "genome".

1916- Shull - Introduced the term "heterosis" to replace "heterozygosis".

1917- McFadden - Described wheat-rye hybrids (triticale).

1917- Jones - Proposed that hybrid vigor was due to linkage of favorable dominant alleles; developed the first commercial hybrid of maize, ëBurr-Leamingí (4-way hybrid).

1917- Winge - Proposed the theory of polyploid origin of plant species.

1918- Sakamura - Classified chromosome numbers in wheat species.

1918- Fisher - Introduced the ideas of quantitative inheritance and correlation.

1920- East and Jones - Produced the first tobacco cultivar by hybridization.

1920- Jones - Proposed the 4-way hybrid for commercial cultivars.

1920- Blakeslee - Discovered trisomics in *Datura* and coined the term "monosomics" for diploid missing one chromosome.

1920- Wright - Published "Biometrical relations between parent and offspring".

1920- Harland and Pope - Described the backcross breeding technique for small grains.

1920- Bridges - Discovered duplications, deficiencies and translocations in chromosomes.

1925- Sturtevant - Found first chromosome inversion.

1925- Fisher - Introduced analysis of variance and other statistical methods.

1926- Sumner - Isolated the first enzyme in crystalline form (protein).

1926- Muller - Reported artificial mutations in animals by X-rays.

1928- Griffin - discovered genetic transformations in bacteria.

1928- Stadler - Described the mutagenic effects of X-rays in barley.

1929- McClintock - First to report and number ten chromosomes in maize.

1931- Stern, Creighton and McClintock - Provided the cytological proof of crossing-over.

1932- Davis - Suggested the inbred-variety cross method for testing maize inbreds.

1932- Jenkins and Brunson - Used the topcross method to give comparative tests for combining ability.

1933- Rhoades - Discovered cytoplasmic sterility in maize.

1933- Morgan - Awarded Nobel Prize for theory of the gene.

1934- Dustin - Discovered Colchicine.

1935- Vavilov - Contributed much to knowledge of species origin in "The scientific bases of plant breeding".

1936- Fisher - Published book "Statistical methods".

1937- Nebel and Ruttle - Used Colchicine to double chromosome number.

1937- Dobzhansky - Published book "Genetics and the origin of species".

1939- Harvey - Showed that different genotypes of maize and tomato have different nutrient requirements.

Genetics and plant breeding research after 1940

1941- Beadle and Tatum - Published their classic study on the biochemical genetics of *Neurospora*.

1942- Atkins and Mangelsdorf - Suggested the use of isogenic lines for comparative studies of plant characters.

1943- Jones and Clarke - Described the inheritance of male sterility in onion; first use of male sterility in commercial hybrid production.

1944- Avery, MacLeod and McCarty - Described the transforming principle and suggested that DNA, not protein, is the hereditary material.

1945- Hull - Proposed recurrent selection.

1946- Muller - Received Nobel Prize for his contribution to radiation genetics.

1946- Delbruck and Bailey - Demonstrated genetic recombination in bacteriophage.

1946- Lederberg and Tatum - Demonstrated genetic recombination in bacteria.

1946- Auerbach and Robson - Showed that chemicals could induce mutations.

1948- Borvin, Vendrely and Vendrely - Showed that the quantity of DNA and chromosome number were related.

1948- Frandsen and Frandsen - Described the polycross method in detail (the method had been used before).

1948- Comstock - Suggested reciprocal (half-sib) recurrent selection.

1948- Green and Green - Described crossing over between alleles at the *lz* locus of *Drosophila*.

1951- Kihara - Reported production of seedless watermelon through triploidy.

1952- Zinder and Lederberg - Described transduction in *Salmonella*.

1953- Watson, Crick and Wilkins - Proposed a model for the structure of DNA.

1955- Benzer - Demonstrated fine structure of the genetic material of phage T4 of *E. coli*; coined the terms "cistron", "recon" and "muton".

1955- Mather- Proposed disruptive selection.

1956- Demerec and Hartman - Showed that gene order and biochemical sequence were related in certain instances.

1956- Ochoa, Kornberg - Synthesized RNA and DNA polymers.

1957- Taylor, Woods and Hughes - Made the first successful attempt to determine the mechanism of chromosomal duplication using tritiated thymidine and autoradiography.

1957- York- Exploited self incompatibility for commercial production of F_1 hybrid seeds in cabbage.

1958- Beadle, Tatum and Lederberg - Received the Nobel Prize for their work in biochemical genetics.

1958- Peterson and Weigle - Advocated use of gynomonoecious lines for the production of hybrid seeds in cucumber.

1958- Sanger - Received the Nobel Prize for being the first to determine the sequence of amino acids in a protein molecule (insulin).

1959- Nieuwhof- Proposed family selection method for cabbage.

1959- Ochoa and Kornberg - Received the Nobel Prize for their research on the *in vitro* synthesis of nucleic acids.

1960- Peterson and Anhder - Suggested use of gynoecious lines for the production of hybrid seeds in cucumber.

1966- Davis- suggested utilization of marker genes for hybrid seed production in onion.

1968- Shimotsuma - developed reciprocal translocation stocks in water melon for production of seedless fruits.

1968- Ogura- reported male sterility in radish.

1970- Berg, Cohen and Boyer - Introduced recombinant DNA techniques.

1970 - Cross- Reported male sterility in carrot.

1970- Pochard- In bell pepper *ms-509* male sterile line was developed.

1973- Stanley N. Cohen and Herbert Boyer-created first Genetically Modified Organism (GMO).

1975 - Casali and Tigchelaar – Proposed single seed descent method (SSD).

1976- Rick- Suggested single cell culture and cell hybridization for tomato improvement.

1981- Tanskley- Demonstrated usefulness of isozyme markers in vegetable breeding.

1983- Karry Mullis-Invented polymerase chain reaction (PCR).

1991- Georgiev – Successfully exploited *ms-10* gene for hybrid seed production in tomato.

1994- FlavrSavr tomato introduced.

1995- *Bt* corn introduced.

1996- Monsanto-commercially launched glyphosphate resistant transgenic soyabean.

2002- More - Developed tropical gynoecious parthenocarpic lines in cucumber.

Development of transgenic technology

Transgenic technology provides the means to make "crosses" which were unthinkable earlier. Organisms which have until now been completely outside the realm of possibility as gene donors can be used to donate desirable traits to crop plants. These organisms do not provide their complete set of genes, but rather donate only one or a few genes to the recipient plant. A single insect-resistance gene from the bacterium *Bacillus thuringiensis* can be transferred to a tomato or cabbage plant to make *Bt* tomato or *Bt* cabbage. Transgenic plants were first created in the early 1980s by four groups working independently at Washington University in St. Louis, Missouri, the Rijksuniversiteit in Ghent, Belgium, Monsanto Company in St. Louis, Missouri, and the University of Wisconsin. On the same day in January 1983, the first three groups announced at a conference in Miami, Florida, that they had inserted bacterial genes into plants. The fourth group announced at a conference in Los Angeles, California, in April 1983 that they inserted a plant gene from one species into another species.

The Washington University group, headed by Mary-Dell Chilton, produced cells of *Nicotiana plumbaginifolia*, a close relative of ordinary tobacco, which were resistant to the antibiotic kanamycin. Schell and Marc Van Montagu, working in Belgium, produced tobacco plants resistant to kanamycin and to methotrexate, a drug used to treat cancer and rheumatoid arthritis. Robert Fraley, Stephen Rogers, and Robert Horsch at Monsanto produced petunia plants that were resistant to kanamycin. The Wisconsin group, headed by John Kemp and Timothy Hall, inserted a bean gene into a sunflower plant. These early transgenic plants were

laboratory specimens, but subsequent research has developed transgenic plants with commercially useful traits such as resistance to herbicides, insects and viruses.

Science based plant breeding got momentum with increase in productivity and resistance to biotic and abiotic stresses. Advances in biotechnology, biochemistry, bioinformatics and instrumentation boosted the once art of plant breeding into the present designer crops to meet the nutritive, energy and aesthetic requirements with free flow of genes across organisms evolutionary different. Biosafety of GMO's and transgenic are ensured through regulation in many developed states.

Reference

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Genetics after Mendel-the Genome and Genomic revolution

G. M. Nair *

Genetics and Genomics, the two sides of the same coin, are the cardinal realms controlling existence and management of biological organisms. The in depth study and analysis of the two complementary disciplines have created unprecedented opportunities in understanding and manipulating organisms relevant to human welfare. Though the complex functioning of the cells and the organisms are deciphered to a great extent, there are several missing links yet to be understood about "life". The genomic revolution, as we know it today, is stronghold in solving many such mysteries. Comparative genomics, which compares whole genome sequences from a range of organisms, will advance our understanding of the natural world and the role genes play in complex human diseases. There is no doubt that the genomic technologies will change our lives for the better.

When Austrian monk Gregor Mendel's mid-19th century experiments led to the discovery of the basic mechanisms of heredity, the science of genetics was born and humanity took its first small steps towards deciphering the genetic code. Mendel helped set in motion a golden age as scientists around the world grappled with the biological underpinnings of heredity. The focus of scientific inquiry has since moved from Mendel to molecules and from genetics—the study of individual genes and the way traits pass between generations—to genomics, the study of an organism's entire complement of DNA. Today the landscape is dominated by the Human Genome Project, an international research consortium that completed the first draft of the human genetic code in June 2000. The end product—the complete sequence of all 3.1 billion base pairs of DNA contained in almost every human cell—is an encrypted blueprint for human life. No one could have predicted that only a century after Mendel, scientists would begin to master the DNA molecule itself. Technologies that enabled scientists to see and manipulate specific DNA sequences also evolved. A crucial breakthrough was the invention of polymerase chain reaction (PCR) by Kary Mullis in 1983, a process that generates trillions of copies of a specified segment of DNA in a matter of hours. PCR transformed molecular biology by making genetic material in quantities large enough to allow experimentation.

All these discoveries set the stage for the first sequencing of an entire genome, that of a tiny virus called PhiX0174, in 1977. The sequence itself unveiled many

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unknowns about genes and gene structure, a theme that played out over and over as more genomes were sequenced: a bacterium in 1995; the first higher organism, the roundworm *C. elegans*, in 1998; the fruit fly in March 2000; and three months later, the human being. Now that the human genome has been sequenced, the emphasis is shifting to proteomics: the study of all the proteins for which genes code. The approximately 25,000 - 30,000 genes defined by the Human Genome Project translate into 300,000 to 1 million proteins. While a genome is relatively fixed, the proteins in any particular cell change dramatically as genes are turned on and off in response to their environment, directing an astonishing range of biological functions with exquisite precision.

Although attempting to address all that have happened after the Human Genome sequencing will be cumbersome and exhaustive, this will focus on the methods relevant to genome sequencing and some of the application prospects in terms of species identification through DNA barcoding, fingerprinting for solving disputes, metagenomics for gene mining as well as control of gene expressions through gene silencing practiced in the Genomics Centre at the University of Kerala

Plant Breeding

Hari Har Ram *

J. M. Poehlman defines plant breeding as "the art and science of improving the heredity of plants for the benefit of mankind". N. I. Vavilov defines plant breeding as 'evolution directed by the will of the man'. Bernardo (2002), however, offers the most versatile definition as 'plant breeding is the science, art and business of improving plants for human benefit'. Plant breeding historically started with domestication of crop plants and has now become more complex and sophisticated. Domestication is a selection process carried out by man to adapt plants and animals to their own needs, whether as farmers or consumers. Successive selection of desirable plants changed the genetic composition of crop plants. This happened about 11000 years ago in so called Fertile Crescent, a hilly region in south-western Asia (Xu 2010). Primitive farmers had no formal knowledge of genetics and/or plant breeding but could get huge accomplishments in a short time. The early improvements were achieved by interfering with the natural process of evolution. In other words, domestication was nothing more than directed evolution and in view of this, plant breeding can also be defined as directed evolution. The key to domestication is the selective advantage of rare mutant alleles, which are desirable for successful cultivation but not necessarily for survival in the wild.

Breeding Procedures for Self-pollinated Crops

This is a procedure where seeds of improved lines/superior germplasm collected from one ecological area are transported and evaluated into another ecological area. These introductions are either utilized directly as cultivars or are introduced into the crosses to be used a donors for certain desirable traits available in the introductions. While introducing germplasm for direct or indirect use, due consideration should be given to the characters needed in the cultivars for a particular location as per need of the consumers and adaptation to that particular location. As far as possible, attempts should be made to introduce lines from geographical locations having almost similar latitudes where they are to be tested and used. This minimizes the problem of too early or too late maturity for the new germplasm lines in the new area. This could be illustrated with example in soybean. Soybean is short day plant *i.e.* is photoperiod sensitive and has a narrow range of latitudes in which it is adapted. Based on this simple premise, one can conclude that soybean cultivars introduced into India from China, Japan, Taiwan and USA should not run into flowering problems as these geographical regions fall on almost similar

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latitude belts. One should keep a thumb rule in mind that as we move away from equator, the maturity duration of same soybean cultivar will keep on increasing.

In India, large scale soybean germplasm introductions were made in early seventies in which about 3500 lines were obtained from USDA and these were evaluated at G. B. Pant University of Agriculture and Technology, Pant Nagar. From these early introductions, several promising cultivars, namely, Bragg, Clark 63, Hardee, Lee and Monetta were released for commercial cultivation in India. This worked as a trigger and now soybean breeding and production in India are expanded considerably and India plants more than 10 million ha of soybean. The starting acreage was hardly 50,000 ha. Introductions should be used as a potential breeding option in early stage of any breeding programme.

Introduction has been very effective in case of major vegetable crops in the early stages of vegetable breeding in India and a few exceptionally successful and promising cultivars came through the route of introduction where Plant Introduction Division and Division of Vegetable Science at Indian Agricultural Research Institute made early headways. A few of these include vegetable pea variety Arkel, watermelon variety Sugar Baby, cauliflower Pusa Snow Ball 16, etc.

Pure-line Selection

Pure-line selection is utilized in these lines where superior looking individual plants are selected at maturity and harvested separately. Next year, plant-to-progenies are planted and single plant selection may be again repeated in the superior progeny rows if they still show variation, otherwise, superior progeny rows, looking uniform are selected and they produce from a single progeny row is bulk harvested. There could be several progeny row-bulks for further evaluation followed by selection of a few superior types or only one progeny row bulk. In principle, this is a seed purification exercise.

Pedigree Method of Breeding

This method of breeding is the most common method being used in large number of self-pollinated crops, like wheat, rice, and soybean, various types of legumes, tomato, eggplant, okra, hot-pepper, sweet pepper and cucurbits which though are cross-pollinated but behave like sef-pollinated crops genetically. This method includes selection of superior individual plant progenies between rows and then selection of superior individual plants within selected progeny rows during inbreeding following crosses among selected parents. In nut-shell, this is selection between and within rows in segregating generations. Selection of superior parental cultivars is the most important step of this procedure. F2 is the first segregating

generation where individual plants are selected based on desirable characters like days to maturity, plant height, plant type, freedom from lodging and shattering, and resistance to diseases. Knowledge of qualitative genetics of the target characters plays a critical role in handling the segregating generation under this method. For example, resistance to bacterial pustules in soybean has been reported to be governed by a single recessive gene and therefore if the susceptible plants are discarded in the F2 itself, the future generations are bound to be free from this disease. A flow chart of this procedure is given in Table 1 (Ram 2011).

Table 1: Pedigree method of breeding in a typical self pollinated crop

Year	Generation	Activity
1	Parents	Crossing of two selected parental lines
2	F1	Growing of F1 in bulk, in 1-2 rows, 75 x 20 cm/appropriate distance apart, elimination of possible self based on marker traits, if applicable
3	F2	Growing of F2 plants, quite apart than the normal commercial planting, selection for plant type, height, maturity, resistance to diseases and shattering and fruit shape, size, colour and quality as applicable
4	F3	Planting of individual plant progeny-rows (about 100), 5 m long, appropriately spaced, selection for above traits
5	F4	Planting of families of selected plants in plant to progeny row fashion, selection for above traits
6	F5	Planting of families of selected plants in plant to progeny row fashion, selection for above traits with emphasis on yield components
7	F6	Planting of families of selected plants in plant to progeny row fashion, selection for above traits and bulking of selected progeny rows, each bulk to be labeled as a new selection/breeding line
8	F7	Yield evaluation of new selections/breeding lines along with standard checks in station initial varietal trial, RBD x 2 replications, 5 m long 5 row/plot
9	F8	Yield evaluation in station advance trial as above but with 4 replications
10	F9	Coordinated multi-location initial trial
11	F10	Coordinated multi-location advance trial-I of the breeding lines selected from above initial trial
12	F11	Coordinated multi-location advance trial-II of the breeding lines selected from above advance trial-I and parallel evaluation by agronomist and other stake holders to identify the best combination of a superior cultivar and the production technology
13	F12	Submission of release proposal with all the relevant data as per prescribed pro-forma to the state/central variety release committee for release and notification of the new cultivar

Bulk Method of Breeding

Plants from a segregating generation are harvested in bulk and a sample of the seed is used to plant the next generation. In F5, individual plants are selected and harvested separately to grow plant to progeny rows in F6 from where further procedure is similar as given in case of pedigree method of breeding. Natural selection can cause changes in the characteristics of a bulk population during inbreeding. Plants with tall height, late maturity, lodging susceptibility and with branching tend to increase during inbreeding. Procedures for minimizing undesirable shifts towards late maturity have been investigated. Empig and Fehr (1971) evaluated a procedure referred to as maturity group bulk that consisted of sub-dividing populations into early, mid-season and late maturity sub-groups in soybean. Plants of each maturity group were handled separately. This process helped increasing the frequency of early maturing lines. For successful practical implementation of bulk breeding, it is essential that the population be cultivated on as large scale as possible and be grown under conditions appropriate for the variety to be bred. This is the only way to realize all gene combinations with an assured probability and only with this method will natural selection also proceed in the direction intended by the breeder.

The amount of time needed for bulk breeding can be decreased by application of the so called single- seed descent method where only one seed is chosen at random from each plant for further use. Since the number of plants to be cultivated is considerably reduced by such a procedure, the individual generations can be grown in greenhouses, where two-three generations per year can be achieved (Kuckuck et al. 1991).

In each bulk breeding, it is absolutely necessary that the breeder pay utmost attention to the natural selection processes within the bulks, in order to prevent natural selection from working against the desired goal. For example, in cereals, plants producing many small grains will prevail much quicker than those producing only few, but larger grains. Thus, nature breeds varieties with low 1000-kernal weight, whereas the breeder often desires those with a higher one. By simply passing the seed through a sieve after each harvest, however, it is possible to exclude the small grains from further propagation and by such a selection, to balance the unwanted consequences of natural breeding selection. Also, the breeder's observation that different genotypes all develop equally well cultivated in monoculture, but that in mixed cultivation one genotype is completely suppressed by another due to competition, is of importance for the right direction of natural selection in bulks.

Some breeders prefer to space-plant the hybrid populations and select and bulk only the superior plants in each generation. This procedure has sometimes been called as 'modified mass method' although typical modified mass selection in cross-pollinated crops is applicable in different context. J. B. Harrington in 1937 proposed a tem mass-pedigree method in which the hybrid populations are grown in bulk until circumstances are favourable for expression of important characters when single plant selections could be made and carried forward by the pedigree method.

Mass Selection

Desirable segregates can be increased in frequency for some characters like seed size if mass selection is applied in association with the bulk method. Direct selection for seed size in soybean has been found to be effective in changing the frequency of segregates with large or small seeds. Seeds harvested in bulk can be passed over sieves to obtain the desired seed size. Indirect selection for specific gravity has been used to change the frequency of segregates for protein and oil composition of soybean seed (Smith and Weber 1968).

Single Seed Descent (SSD) Method of Breeding (Brim 1966)

There are three procedures under this as given below:

(i) Single Seed Procedure

(ii) Multiple Seed Procedure

(iii) Single Hill Procedure

Composite Crosses

Composite crosses are bulk progenies composed of many different F1 progenies and these have been developed to increase the chance of formation of new combinations. If, in addition, male-sterile genotypes are used, the number of new combinations within a bulk can increase. Application of gametocides and incorporation of foreign genes (an RNase coding gene coupled to a pollen-specific promoter which causes such a high level of RNase in the pollen grain that it deteriorates) has been used with varying degrees of success.

Backcross Breeding

This procedure is usually used to transfer a few desirable characters from a non-recurrent donor parent to an adapted cultivar which lacks in those desirable traits which are present in the non-recurrent parent. As the name implies, this method makes use of a series of backcrosses to the variety to be improved during which the

character (or characters) in which the improvement is sought is maintained by selection. At the end of backcrossing the gene (or genes) being transferred unlike all other genes will be heterozygous. Selfing after the last backcross produces homozygosity for this gene pair and coupled with selection will result in a variety with exactly the adaptation, yielding ability, and quality characteristics of the recurrent parent, but superior to that parent in the particular characteristics for which the improvement programme was undertaken. It is apparent that this method, in contrast to pedigree and bulk population methods of breeding, provides the plant breeder with a high degree of genetic control of his populations (Allard 1960).

General plan of a backcross breeding programme can be outlined as follows (Allard 1960).

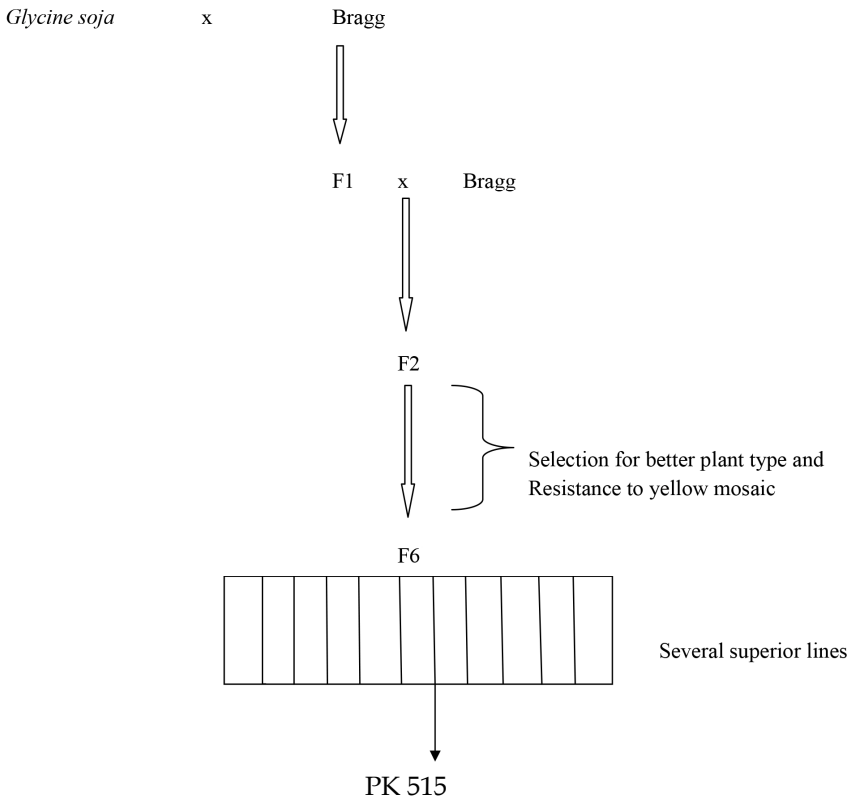
1. The selection of parents. The recurrent parent (*A*) is generally a dominant variety in the area. The donor parent (*B*) is selected because it possesses in high degree some character in which *A* is deficient.
2. The F1 of *A* x *B* is backcrossed to *A*. Selection is practiced for desirable character of parent *B*. Selfed seeds from the selected plants are used to produce a large F2 in which intensive selection is practiced for the desirable character of *B* and for general features of parent *A*. The selected plants are used to produce F3 lines, among and within which further selection is practiced for the type of recurrent parent.
3. The selected plants are backcrossed to parent *A* to produce second backcross seeds (*A*3 x *B*). The second backcross plants are again backcrossed to parent *A* to produce third backcross seeds (*A*4 x *B*). Procedure of (2) is repeated.
4. The fourth, fifth and sixth backcrosses are made in succession, with an F2 and F3 being grown after the sixth backcross and intensive selection being practiced for the character being transferred and for the plant type of the recurrent parent.
5. A number of lines homozygous for the character from parent *B* and as similar as possible to the recurrent parent, are bulked, increased and released for commercial production.

If minimum number of plants are grown at each step in the foregoing programme, the transfer of a single dominant gene can theoretically be accomplished (through six backcrosses) with 53 plants from backcrossed seeds, 96 F2 plants, and 68 F3 rows of 24 plants each. These numbers are based on a probability of 0.99 of having at least one *Aa* plant after each backcross and at least one homozygous *AA* progeny in the F3. In species where artificial hybridization is difficult, the number of hybrid seeds necessary can be greatly reduced by growing F2 and F3 populations after each backcross and crossing on homozygotes. Somewhat smaller total populations are required to transfer incompletely dominant or recessive genes because

homozygotes can be recognized in the F2 generations, making F3 progenies unnecessary.

Use of limited backcross is quite effective in soybean for transferring yellow mosaic virus resistance from *Glycine soja*. Here F1 is backcrossed once with the adapted cultivar and the resultant BC1 is routed through pedigree method of breeding. For example, at G. B. Pant University of Agriculture and Technology, Pant Nagar PK-515, a line resistant to yellow mosaic and moderately resistant to Bihar hairy caterpillar has been developed by this method as given in Fig. 1 (Ram et al. 1984; Ram 2011).

Figure 1: Limited backcrossing to transfer YMV resistance from a wild soybean



Limited backcrossing is useful and effective for yield improvement towards large seeded cultivars. A cross between a large and small seeded parent does not provide an adequate frequency of large seeded progeny. Under this situation, a backcross with the large seeded parent results in the necessary large seeded segregates (Weber 1966). When separate recurrent parents are used, this procedure is referred to as 'modified -backcross'.

Mutation Breeding

The process of hereditary change is termed mutation and the individuals affected by the change are called mutants. Besides recombination, mutations are causes of genetic variation from which every breeding activity proceeds. By means of a mutation, the multiplicity of types of a population is not only increased by one genotype, but also by a large number of further new genotypes due to possibility of subsequent recombination of the mutant with the existing genotypes.

To use mutation in breeding successfully, the breeders must understand the different forms of mutations, their development, manifestation, and genetic behavior and above all, the necessary knowledge to increase the frequency of their spontaneous occurrence by experimental intervention. Broadly mutations are of three different types. These are genome mutations, plastidome mutations and Plasmon mutations. Genome mutations comprise the mutations within the nucleus and are subdivided as point mutations, chromosome mutations (changes in chromosome structure) and ploidy mutations (changes in chromosome number). Plastidome mutations are due to changes in the plastids. Plasmon mutations are as a consequence of hereditary changes of the cytoplasm.

Normally point mutations are used in breeding programmes. In classical genetics, genes have been defined as unit of function (cistron) for formation of specific characters, as units of recombination (recon) and as units of mutation (muton). Therefore, a true gene mutation must follow Mendelian inheritance after a cross between the mutant and the original type. Mono-hybrid segregation takes place in the F2. In general, mutation only takes place in one allele and usually from dominant to recessive one, for example, an AA genotype is changed to Aa and Aa into aa genotype. If mutation takes placed in the generative tissue, 'a' gametes are formed in the original AA genotype resulting in generation of some Aa progenies. If A is completely dominant over a, the process of mutation after selfing will only be observed in the following generation, in which 25 % aa genotypes will be observed as segregants. If a mutation occurs within the somatic tissue, it can only be transferred to the progeny by sexual multiplication, if it has developed into L2 layer from which generative tissue is formed. Somatic mutations in the L1 can manifest themselves, for example, in the flower in the form of colour altered sectors. These mutants (originating in L1) can only be multiplied by vegetative propagation. If several characters have clearly changed at the same time, the mutation is called pleiotropic.

X-rays, gama-rays, neutrons, ethyl methane sulfonate (EMS), ethylamine (EI) and nitroethylurethane (NEU) are the common mutation inducing agents used in

mutation breeding. First one should know the radiation dosage or concentration of the chemical compound as well as the duration of the treatment that promises the greatest yield in the usable mutations. The mutation rate increases with increasing dose, however, simultaneously non-genetic primary damage increases as well resulting in a decreasing ability to germinate, increasing inhibition of growth and other morphological and physiological disorders during plant growth. For this reason, usually weaker dosages are recommended so that not more than 20-25% of the plants die off. With increasing dosage, the proportion of chromosomal mutations with conspicuous morphological and physiological changes usually increases. However, these mutants are generally of no breeding significance due to their simultaneously diminished vitality.

Analogous to the symbols used in Mendelian genetics, the first generation developing after a mutagenic treatment is called M1 and the subsequent generation with segregating recessive mutant is called as M2. If in the M2, individual ears derived from the progeny of an M1 plants are grown separately, segregation of a recessive mutation can be observed in few progenies. Since the majority of point mutations is recessive, the search for deviating phenotypes does not begin until the M2 generation. The determination as to whether a detected variant is a mutant or a non-heritable modification can only take place in the M3 after testing their progeny. In very rare cases only, it will be possible to prove the presence of a point mutation in the M2 by identifiable Mendelian segregation. A large number of phenotypically normal plants must be selected from the M2 and the individual plants must be cultivated as M3 lines. The comparison of M3 lines with the original type can establish the presence of a true point mutation. The significance of differences in quantitative features can be ascertained by applying biometrical methods. Further cross of mutant with the original one gives a good source of variability for further selection of desirable types. Also the cross of two mutants can occasionally break the undesirable pleotropic effects and valuable transgression in other features can be obtained.

Double Haploid (DH) Breeding

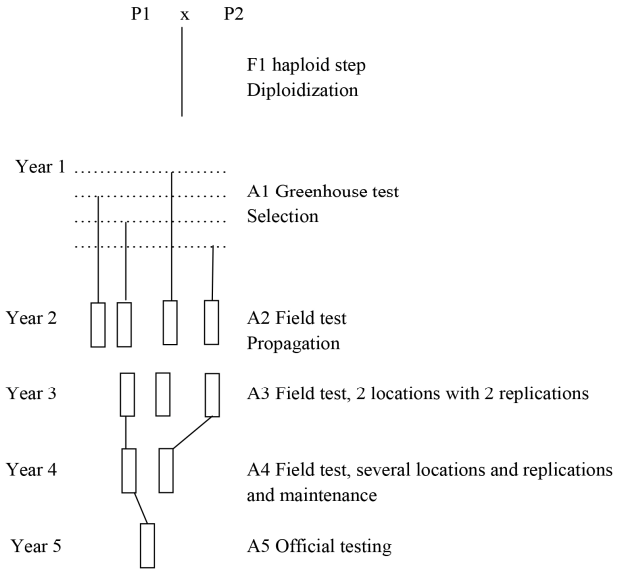
In self-fertilizing crops, the commercial product must have to be uniform and completely homozygous. Breeding for homozygosity is particularly time consuming for traits with a dominant inheritance, as an AA genotype cannot be phenotypically distinguished from the Aa type. Techniques were very much needed to speed up this process. Such suggestions were made by Blakeslee and Belling (1924), soon after Bergner discovered the first haploid higher plant in 1920. Melchers and Labib continued this discussion and added practical breeding aspects after new methods were developed in the 1970s with which sufficient numbers of haploids could be produced (Kuckuck et al. 1991). In the meantime the technique

has been improved to such an extent that in crops like potato, rapeseed, rice, barley, wheat and maize haploids have become an integral part of commercial breeding programmes.

Haploids arise from the unfertilized egg cell (a special case of parthenogenesis) or from young pollen grains (microspore androgenesis). For breeding purposes, androgenesis is more in use. In most crops, functional plants can be produced by culturing microspores. They are normally cultured within the anther, the technique is often called as anther culture. Haploids are completely sterile, smaller than diploids, and slow growing. During regeneration of plats from structures of haploid origin, a high percentage spontaneously becomes diploid or their genome may be doubled by colchicines (0.5%) treatment. Completely homozygous plants are the result of such doubling procedures. In colchicines treatment, the most commonly used procedure is treatment with an aqueous solution of 0.5 % colchicines, supplied via the root system to the entire plant or with cotton wool plugs that are placed after axillary buds are excised.

In barley the first variety, "Mingo" of haploid descent (by the *Hordeum bulbossum* procedure) was licensed in Canada in 1980, in wheat the first variety "Florin" based on use of androgenic haploids was licensed in France in 1985. Breeding scheme for the introduction of mono-and polygenically inherited traits from related varieties into inbreeders using a haploid step is illustrated in Fig. 2 (Kuckuck et al. 1991).

Figure 2: Breeding scheme for recombination breeding using double haploids



Breeding Methods in Cross Pollinated Crops

Maize is highly cross-pollinated crop and breeding methods applicable in this crop are typically applicable to all the cross pollinated crops. Its population comprises of freely interbreeding individuals and is heterogeneous. As a consequence, the plants in a maize population are heterozygous at most of the loci. From breeding viewpoint, maize is thus highly adapted to heterozygosity, which is an essential feature of maize cultivars. Therefore, heterozygosity has to be maintained or restored in the end commercial product in the cross-pollinated crops. Inbreeding leads to inbreeding depression resulting in reduction in vigour, size and yield and appearance of lethals and sub-lethals. Therefore, inbred lines cannot be adopted as commercial products. The heterozygosity has to be brought back through breeding processes in the commercial products. Ultimately the commercial cultivar may be open-pollinated population of hybrid. The choice of cultivar depends upon resources, stage of breeding programme, infrastructure and manpower for seed production and socio-economic factors. Hybrid cultivars have the advantage of higher yield potential and uniformity. They are preferred over open-pollinated populations subject to higher level of heterosis. However, seed production of hybrids is costlier and a bit tedious and complicated (Dhillona and Prasanna 2001) and in those situations population breeding methods are sometimes resorted to. Maize is taken as a typical example to illustrate breeding methods in cross-pollinated crops. Moreover, historically, most of the breeding methods applicable to cross-pollinated crops basically originated in maize at least on theoretical basis.

Basically, maize breeding methods consist of population improvement and hybrid breeding. The most important step in any breeding programme is selection which is discrimination among individuals in the number of off-springs contributed to the next generation. Selection brings about changes in gene and genotype frequencies and results in improvement of genotypic value of trait(s) under consideration. Selection is effective only on already existing variability that has its origin in spontaneous mutations and that gets dispersed through natural hybridization, gene segregation and recombination. The naturally occurring genetic variability may be reinforced by induced mutations and planned hybridization. Common breeding procedures currently in use in maize improvement are listed in the following table (Ram 2011).

Table 2: Maize breeding procedures

1.	**Population Improvement**
a.	**Intra-population selection**
i)	Mass selection
ii)	Modified mass selection (Gardner method)
iii)	Half-sib selection with progeny test or ear-to-row selection (no replication-Hopkins method)
iv)	Modified ear-to-row selection (replicated test-Lonnquist method)
v)	Half-sib selection with test cross
vi)	Full-sib selection
vii)	S1 family selection
b.	**Inter-population Selection**
i)	Reciprocal recurrent selection (Comstock and colleagues)
ii)	Full-sib reciprocal recurrent selection (Hallauer, Lonnquist, Williams)
1.	**Synthetic Variety**
2.	**Composite Variety**
3.	**Hybrids**

Various breeding procedures as listed in the above Table 2 are briefly described as follows:

Mass Selection

This is the oldest, simplest and least expensive method of selection for cross-pollinated crops, particularly maize. This consists of selection of ears on the basis of plant and ear characteristics and bulking of seeds of harvested ears to grow the following generation in which again another cycle of selection can be initiated. Cycle of selection is completed in one season. This process can be repeated for several cycles till substantial gains are realized. This method although simplest in operation has certain limitations like inability to identify superior genotypes on the

basis of phenotypic performance alone and no control over pollen parent where inferior plants also contribute their pollen. Further, rigorous selection for specific plant trait often leads to inbreeding. Additional limitation is that selection is based on single plant, therefore the role of environment may affect the plant performance and one may select the plants which may be potentially not up-to mark. Therefore, this method has been subjected to some modifications. Incidentally, mass selection is the oldest breeding method for plant improvement and was employed by early farmers for the development of cultivated species from their ancestral forms.

Modified Mass Selection

This has been proposed by Gardner in the year 1961. In this method, the source population in which selection has to be done is grown in isolation at a low plant density to allow better phenotypic expression of each individual plant. The whole area is divided into sub-plots which are also called as grids. Selection for superior ears on superior plants is done in each grid. Selected ears across the grids are bulked. This method of selection is also known as stratified mass selection. This method of selection samples the entire field and therefore potentially good plants are selected even from poor environment. This can be easily adopted to sorghum, pearl-millet, cauliflower, cabbage, etc.

Half-sib Selection with Test Cross or Ear to Row Selection

In this method, phenotypically superior ears are selected from superior plants from a source or base population grown in isolation. Seeds from harvested ears (half-sib-seeds, one parent common) are kept separate unlike mass selection where the seeds from different ears are bulked. Seed from each selected ear is divided into two halves. In second year, half of seeds from each ear are used to grow progeny rows in isolation for evaluation. Remnant seeds are kept separately. In third year, a new population is reconstituted by bulking equal quantity of seeds either from harvested superior progenies or from the remnant seeds which gave rise to superior progenies. In this method, greater reliance is placed on selection of individual plants based on progeny performance rather than phenotypic performance as such.

Modified Ear to Row Selection (MER-Lonnquist Method)

This is a modification of ear to row, a type of half-sib selection in which half-sib families are planted in different environments for evaluation. Thus, more environments are sampled and selection is supposed to be more reliable. The experiment in one environment is sown as an isolated evaluation-cum-recombination block. In this block, ear-to-rows are inter-planted with a balanced male (BM) composite developed by mixing an equal number of seeds of all ears under evaluation. The ear-to-rows are de-tasseled and are thus pollinated by the

pollen of BM composites. Selection is carried out among and within families, selection among ear-to-rows being based on performance across the target environments and that within ear-to-rows being mass selection in isolated block. MER selection is a unique method of family selection as one cycle of selection is completed in one season but it provides no control over pollen parent. It has been used by many breeders including those at CIMMYT. Operational steps of this method are as follows (Mukherjee 1989a; 1989b).

i) 100 or more ears are selected from a base population based on grain and agronomic characters. Each ear is shelled separately (I season).

ii) Seeds of each selected ear are grown as progeny row in single replication at different locations of the environment for which the new variety is to be developed (II season).

iii) Replicate at the main station is handled as a crossing block. A balanced mixture of seed is used as pollen parent. For creating balanced mixture, equal number of seeds are taken from each progeny and mixed (II season).

iv) At the main location, five phenotypically superior plants in each progeny row are marked. Five cobs from the marked plants are kept in a bag after each row is harvested and weighed (II season).

v) Top 20% of the progenies are selected on the basis of average performance over locations and five ears/cobs from each of these 20% of selected progenies (100 cobs, if number of planted progenies is 100) are grown in next season in ear-to-row manner (III season) and above cycle may be repeated, till one gets good response to selection.

Half-sib Selection with Test Cross/Half-sib Progeny Selection

Individuals having one parent common are called as half-sibs. In half-sib breeding, the selected plants are always test crossed. Two different terms are used in this method depending upon as to how the next population is reconstituted. These terms are:

a. **Half-sib progeny test:** Under this scheme, male plants are selfed and crossed to females to produce half-sib families. Selfed seeds from selected males are composited to reconstitute the next population.

b. **Half-sib test:** It is similar to half-sib progeny test except that compositing of the seeds is done from open-pollinated seeds. The steps are as follows:

I Season: Selection of superior plants (50-100) in a source population, crossing to a tester, and selfing under half-sib progeny test and crossing only to a tester under half-sib test.

II Season: Evaluation of testcross progenies.

III Season: Compositing selfed seeds from plants with superior testcross progenies under half-sib progeny test and compositing open-pollinated seeds from plants with superior testcross progenies under half-sib test. Under half-sib progeny test if tester is broad based, the procedure is similar to recurrent selection for general combining ability and if the tester is an inbred line, then the procedure is equivalent to recurrent selection for specific combining ability.

Full-sib Selection

Individuals having both the parents common are known as full-sibs and are derived from crossing of two selected plants from the base population. The crosses are made between selected pairs of plants in the source/base population. Crossed seeds are used for progeny test and for reconstituting the improved new base population.

S1 Progeny Testing/Selfed-Progeny Selection

The selfed progeny is produced in a base/source population by one (S1), two (S2) or more selfings. The progenies are evaluated and selected ones are recombined using remnant seeds. Mostly S1 or S2 selection is carried out but there are instances where selection has been done even in S3. For some characters like disease and insect-pest resistance, selfed progeny (SP) selection has been found to more effective than other methods. However, the gains through SP selection are adversely affected when the contribution of non-additive variation is higher.

Reciprocal Recurrent Selection/Half-sib Reciprocal Recurrent Selection

This procedure proposed by R. E. Comstock, H. F. Robinson and P. H. Harvey in 1949 aims at simultaneous improvement of two heterozygous and heterogeneous populations (designated as A and B) where population A serves as tester for population B and vice-versa. This method is as effective as recurrent selection for general combining ability when additive gene action predominates and is as effective as recurrent selection for specific combining ability when non-additive gene action are of major importance in deciding the genetics of a particular trait. The operational steps involved are as follows:

I Season: Selected plants of population A are self-pollinated and crossed to plants of population B. Likewise, plants are selected and self-pollinated in population B and out-crossed to plants of population A.

II Season: Test cross progenies of both the populations are evaluated in replicated trial. Superior progenies are identified on the basis of performance in the trial.

III Season: Selfed seeds from plants with superior test cross progenies are grown population-wise separately and inter-crossed to reconstitute two new populations which will now be called as A′ and B′.

This completes one cycle of reciprocal recurrent selection and additional cycle(s) may be initiated depending upon the variability in the original population and the improvements achieved in the new populations.

Full-sib Reciprocal Recurrent Selection

Hallauer and Eberhart (1970) proposed full-sib reciprocal (FSR) recurrent selection for use in prolific maize. Two heterotic populations are taken and S0 plants in these populations are simultaneously selfed and crossed inter-population pairs to produce S1 and full-sib (FS) families, respectively. The FS families are evaluated and S1 seeds of S0 plants that have produced superior FS families are used to reconstitute the two populations separately. Hallauer (1973) proposed a modified FSR recurrent selection for use in single eared populations in which S0 plants are selfed to produce S1 lines which are crossed to develop FS families.

Synthetic Variety

The term synthetic variety has come to be used to designate a variety that is maintained from open-pollinated seed following its synthesis by hybridization in all possible combinations among a number of selected genotypes which have been subjected to combining ability test. The components of a synthetic variety could be inbreds (usually) or mass selected populations in context of maize. The components are maintained so that the synthetic variety could be reconstituted as and when required.

In maize first inbreds are developed. The inbreds to be used as component lines are selected on the basis of combining ability for which component inbreds are crossed in all possible combinations. The inter-crossed seed is called as S0 seed. Equal quantity of seed from all crosses is mixed and the mixture is allowed open-pollination in isolation and the open-pollinated seed is harvested. The harvested seed represents S1 generation. In absence of reconstitution of a synthetic variety at regular intervals, the synthetic variety becomes an open-pollinated variety.

Composite Varieties

The concept of composite varieties in India originated in maize. Composite varieties in maize are generally derived from varietal crosses in advanced generation. These are usually developed from open-pollinated varieties or other heterozygous populations or germplasm which have usually not been subjected to inbreeding or have not been elaborately evaluated for their combining ability. Usually they involve open-pollinated varieties, synthetics, double crosses, etc. selected for yield superiority, maturity, good grain characteristics, and resistance to diseases and pests. These composites often show a high degree of heterosis in F1's when widely diversed populations are crossed. Advanced generations of such heterotic crosses often show stabilized yields. General combining ability and additive gene action play predominant role in exploitation of these populations (Dhawan 1985; Singh 1985). Steps involved in development of maize composites are as follows (Singh 1980):

i) Screening of diverse germplasm by evaluation at multi-locations/years to identify the source populations having adaptability, desirable agronomic attributes and resistance to major diseases and serious insect-pests.

ii) Making of all possible crosses among selected superior genotypes or top crossing with a varietal complex of screened base varieties.

iii) Conducting multi-location test with the F1 and F2 of the varietal crosses and selection of F2's having desirable agronomic features along with least inbreeding depression in F2.

iv) Evaluation of selected F2 populations and identification of the best one as practically composites are constituted by compositing seeds of various populations and allowing the mixture to stabilize under open-pollination along with some mild selection in isolation. The constituent entries may not be maintained for reconstituting the composite. In addition to use of composites as direct cultivars, these can be used as base/source population to develop inbred lines. International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Hyderabad has adopted this procedure for developing promising composites in pearl-millet. It can be used in sorghum, sunflower, tropical cauliflower, carrot, radish, etc.

Hybrids

The term hybrid variety is used to designate F1 populations that are used for commercial plantings. One of the significant achievements in plant breeding has been the exploitation of heterosis through commercial cultivation of hybrid maize.

The earliest report on commercial exploitation of heterosis in maize has been by Beal (1980) who estimated heterosis and reported up-to 52% increase in yield of hybrids over OP parents and suggested commercial cultivation of F1 varietal hybrids. However, varietal hybrids could not make good impact. Large scale exploitation of heterosis in maize has to wait for the pioneering work of East (1908, 1909) on inbreeding, Shull (1908) on single crosses, Jones (1918) on double crosses. Shull and East conducted independent experiments on inbreeding and cross-breeding in maize and observed depression due to inbreeding and restoration of vigour on crossing. Shull (1909) gave a pure-line method of corn breeding which laid the foundation of present day hybrid breeding. The proposed method of Shull (1909) included three steps, namely, (i) large-scale inbreeding to obtain many homozygous or nearly homozygous inbred lines, (ii) testing the selected inbred lines in all possible crosses and (iii) practical utilization of inbred lines in seed production of single cross hybrids. Shull recognized that the hybrids produced from inbred lines which are homozygous and homogeneous, would be uniform and true to type. Historical perspectives and details of hybrid maize development have been given by Shull (1952) and Hayes (1963). Hybrids are uniform, generally higher in yield, offer opportunities for rapid deployment of dominant genes conferring resistance to diseases and insect-pests and the breeders/seed companies have exclusive control over the parental lines.

There were some problems associated with single cross hybrids due to which maize breeders were slow and reluctant in adopting pure-line method. These problems were as follows:

i) Lack of good inbred lines capable of producing single cross hybrids demonstrating significant yield advantage over the available best OP varieties.

ii) Poor germination and planting problems due to small and mis-shaped hybrid seed.

iii) Expensive hybrid seed due to low yield of female inbred parent and substantial proportion of the land devoted to the male inbred parent.

Jones (1918) suggested the use of double cross hybrids to overcome these difficulties associated with the single cross hybrids. The first double cross hybrids in 1920s yielded about 15% higher than the better OP varieties (Duvick 1999). Now, single cross and double cross hybrids pre-dominate the commercial hybrids world over. In vegetable crops, single cross hybrids are rule rather than exceptions.

Types of Hybrids

Conventional Hybrids

These are based on inbred lines. These are of following types:

i) **Single crosses:** A single cross is a hybrid progeny from a cross between two unrelated inbreds = (A x B).

ii) **Three-way crosses:** A three-way cross is the hybrid progeny from a cross between a single cross and an inbred = (A x B) x C.

iii) **Double crosses:** A double cross is the hybrid progeny from a cross between two single crosses = (A x B) x (C x D).

iv) **Modified single crosses:** A modified single cross is the hybrid progeny from a three-way cross which utilizes the progeny from two related inbreds as the seed parent and an unrelated inbred as the pollen parent = (A x A′) x B.

v) **Double modified single crosses:** A double modified single cross is the hybrid progeny from two single crosses, each developed by crossing two related inbreds = (A x A′) x (B x B′).

vi) **Modified three-way hybrids:** A modified three-way hybrid is the progeny of a single cross as female parent and another single cross between two related inbreds = (A x B) x (C x C′).

Non-conventional hybrids

The concept of non-conventional hybrids has been advocated by CIMMYT for the countries lacking an effective seed production industry. These hybrids are transitional stages between open-pollinated varieties and conventional hybrids. The major types are as follows (Vasal 1988).

i) **Inter-varietal hybrids:** These are formed by inter-crossing of two varieties. These are approximately equivalent to synthetics/composites.

ii) **Top cross hybrids:** These are inbred x variety hybrids. Following top cross hybrids may be constituted.

a. Inbred line x variety
b. Inbred line x experimental hybrid
c. Inbred line x synthetic variety
d. Inbred line x family

iii) **Inter-family hybrids:** These hybrids are progeny resulting from the crosses of two families originating from the same population or two different populations.

iv) **Double top cross hybrids:** A double top cross hybrid is the progeny of a single cross and a variety. Such hybrids have been commercialized mostly in India and China.

Single, double and three-way cross hybrids have mostly been commercialized world over. The most striking advantage of single cross hybrids over double and three-way cross hybrids is that single cross breeding is simpler and faster. The probability of identifying two superior inbred lines that combine well and are vigorous is higher than that for three or four inbred lines. It is expected that the best single cross has the highest yield followed by the best three-way cross and the best double cross. In presence of epistasis, it may however, be possible to find out a unique double/three-way cross which may be as good as the best single cross. Considering seed cost, the double cross hybrids are common in maize.

Single cross hybrids are homogeneous whereas all other kinds of hybrids are heterogeneous. Being uniform, single cross hybrids may lack somewhat in their adaptability as they have only individual buffering in comparison to other hybrids which are heterogeneous and have individual as well as population buffering (Allard and Bradshaw 1964). However, Troyer (1996) has demonstrated that stable single cross hybrids can be developed and identified. The uniformity of single cross hybrids has been an important factor in their wide-spread cultivation. The seed production of single cross hybrids has been rather simpler but at the same time, it is costlier. The number of seasons and isolations required for seed production of single cross hybrids is less than that in multi-parent hybrids. On the other hand, the hybrids from non-inbred parents are expected to be more variable but at the same time more stable.

Development of inbred lines

Inbred lines are developed through self-pollination which is the extreme form of inbreeding and leads to most rapid fixation of genes and attainment of homozygosity. One generation of selfing results in the same level of inbreeding as three generations of full-sib mating and six generations of half-sib mating. The rate of inbreeding in back-crossing with a homozygous parent is the same as in the selfing. Normally, five selfings are required to develop nearly homozygous inbred lines.

The pedigree method of breeding is the most widely used method to develop inbred lines. Initially the pedigree selection was initiated in the widely adapted OP landraces. Later on, the focus shifted to initiate this process in the F2's derived from

crossing the elite lines that complemented each other for different desirable traits. The base cross to initiate pedigree method of line development could be complex crosses and back-crosses but the use of single crosses for this purpose is most common. The pedigree method of inbred development is referred to as "standard method" when an open pollinated population is sampled (Sprague and Eberhart 1977).

In the pedigree method of inbred line development, the seeds of the selected plants are planted as ear-to-row. Phenotypic selection is carried out both among and within progenies. As selfing progresses, the differences among progenies increase and those within progenies decrease. In this process, some breeders prefer to have a smaller sample size and handle a large number of populations rather than sampling a large number of individuals in a smaller number of populations. Emphasis given to different traits during inbred line development in pedigree method has changed over the years. During the earlier years, the lines were selected primarily for hybrid performance but lately there has been increasing focus on vigour and grain yield *per se*.

Back-cross method is another important method of inbred line development. It is an easy and effective method to handle one or two genes and has been widely used to incorporate resistance to diseases and insect-pests. Normally, three to five back-crosses are made but the number may vary depending upon recovery of the recurrent parent, inheritance of the trait under transfer, selection during back-crossing, genetic similarities between recurrent and donor parent and linkage. With advent of genetically modified organisms, major emphasis is devoted to accelerate back-crosses to transfer the transgenes to elite inbreds. The use of DNA markers has facilitated both the speed and accurate recovery of the recurrent parent and the reduction of linkage drag. Modifications of back-cross method have also been suggested, for example, convergent improvement developed by Richey (Richey 1927), which involves back-crossing to both the lines by reciprocal transfer of dominant favourable genes present in parent and lacking in another parent. In this method, two inbreds A and B are crossed. The F1 is back-crossed with line A followed by selection of desirable traits of line B and F1 is also back-crossed with line B where selection for desirable traits of line A is made. After about three back-crosses and selection, selfing is done to fix the selected genes. This method is useful for improving such characters such as vigour, resistance to diseases, pests and lodging.

Stadler (1944) devised a scheme of gamete selection which is based on the premise that if superior zygotes occur with a frequency of p^2, superior gametes would occur with a frequency of p. The procedure involves crossing an elite line with a random sample of pollen of plants from a source population. The resulting F1 plants and the elite lines are test crossed to a common tester and F1 plants are also selfed. Test

cross progenies are evaluated in a replicated trial. The test crosses of F1 plants that exceed the elite line by tester are presumed to have obtained a superior gamete from the source population. Superior gametes are recovered as F2 selfs. Selection and selfing are continued till desirable homozygosity/uniformity is attained. The method of gamete selection is not used as extensively as pedigree and back-cross method, however it is used in some breeding programmes.

The technique of double haploid breeding is also used to develop inbred lines. Although maternal haploidy has been mainly exploited by private sector seed companies in a routine manner, it has not been widely used by public sector research institutions in India. One of the reasons for not using this on wider scale has been due to the fact that there is no scope of phenotypic evaluation and selection as a result of which large number of unselected inbreds are generated to be evaluated for combining ability.

Maintenance of inbred lines

Inbred lines are generally maintained by a system of self-pollination and growing progenies in ear-to-row in order to observe the changes for various traits. One purpose of developing inbred lines through selfing is to obtain genotypes whose genetic constitution will be maintained over the generations. However, significant changes have been reported over successive generations in long term inbred line maintenance. These points must be kept in mind while maintaining inbred lines.

Top-cross test and early testing of inbred lines

Initially, the lines were selected on the basis of their performance in single crosses produced generally in diallel matings and sometimes by factorial matings. However, with increase in number of inbred lines to be tested, it became difficult to make all possible crosses and evaluate the resultant large number of single crosses. Further, performance of inbred lines *per se* did not prove to be useful guide to select lines for combining ability for grain yield. Davis (1927) suggested the use of the top-cross method for preliminary evaluation of a large number of inbred-lines and this has been accepted as a method for preliminary evaluation of new lines for general combining ability followed by their evaluation in single crosses to identify the most productive specific combinations. It is generally accepted that GCA is more important than SCA in unselected lines whereas the reverse is true in previously selected lines.

In earlier days, top-cross evaluation was done in the lines which were in S5/S6 generations. However, Jenkins (1935) suggested early testing and presented data to show that lines acquired individuality as parents vary early during inbreeding and tend to remain fairly stable for combining ability in subsequent generations of

inbreeding. Therefore, early testing has been in use rather widely. Top-cross evaluations are done in S2 to S4 but evaluation at S3 is most common.

Nature of testers

On the basis of empirical results, testers from opposite heterotic populations are used. According to Hallauer (1975), the tester should be simple to use and should correctly classify the merits of the lines and maximize genetic gain. Mostly the choice of tester depends upon the breeding objective itself. If the objective is to identify good combiners for a given line or single cross, then appropriate tester is that line or the single cross itself. Initially top-cross tests depended on a broad based tester where the objective was to have preliminary evaluation of the GCA. There have been suggestions to use synthetic tester based on inbred lines in the current use. Narrow genetic base testers have been used to improve the SCA.

Combination of inbreds in hybrids and prediction of double cross performance

Single crosses: n (n-1)/2
Three-way crosses: n (n-1) (n-2)/2
Double crosses: n (n-1) (n-2) (n-3)/8
Where n is the number of inbred lines.
If there are four inbred lines, A, B, C and D, the performance of double cross hybrids (A x B) x (C x D) can be predicted as follows (Jenkins 1934).

i) Based on mean performance of all possible single crosses:

$$= \frac{(A \times B) + (A \times C) + (A \times D) + (B \times C) + (B \times D) + (C \times D)}{6}$$

ii) Based on mean performance of the four non-parental single crosses:

$$= \frac{(A \times C) + (A \times D) + (B \times C) + (B \times D)}{4}$$

iii) Based on mean performance of four lines over a series of single crosses:

$$= \frac{(A \times E) + (A \times F) + (A \times G) + (A \times H) + (B \times E) + (B \times F) + (B \times G) + (B \times H) \ldots (D \times H)}{\text{Number of hybrids}}$$

iv). Based on mean performance of top-crosses of the four inbreds:

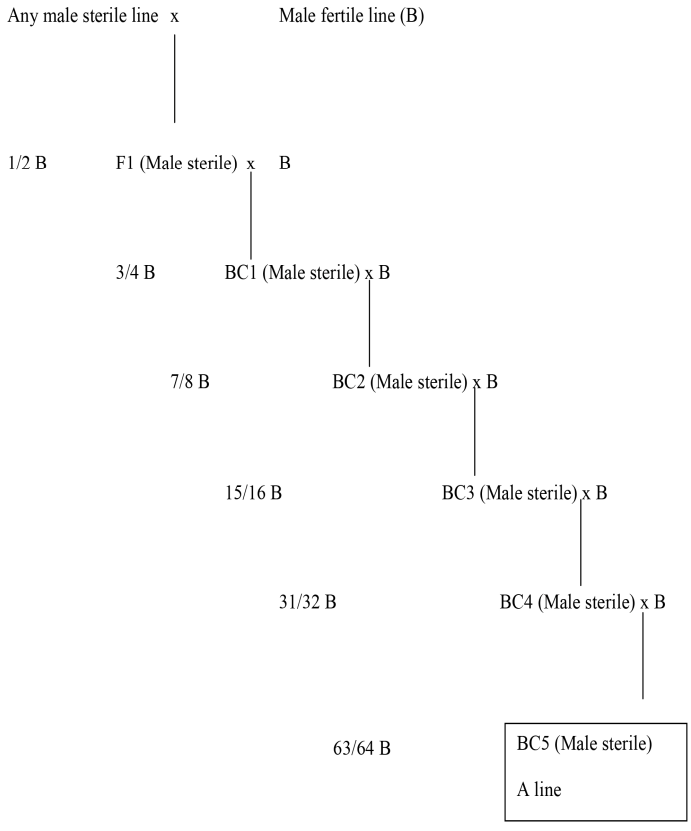
$$= \frac{(A \times \text{variety}) + (B \times \text{variety}) + (C \times \text{variety}) + (D \times \text{variety})}{4}$$

Jenkins evaluated different prediction methods and reported that the most accurate method for predicting the doubled cross was based on the mean performance of non-parental single crosses of the double cross. Since then, this has been most widely used method.

Use of cytoplasmic-genetic male sterility

This is a typical cytoplasmic male sterility for which a restorer gene is available. This is usually referred to as three line system where the three lines used in hybrid seed production are called as A, B, R or A, B, C lines. This approach originated in onion and is commonly followed on commercial scale in sorghum, pearl-millet, maize and lately rice. The male sterile parent is called as A line and its maintainer is called as B line. A and B lines are isogenic except that the A line is male sterile and B line is male fertile. The difference lies only in the cytoplasm where A line has sterile cytoplasm and B line has fertile cytoplasm. Any originally fertile line designated as B line is converted into A line by backcrossing provided the lines are devoid of the fertility restoring genes (Fig. 3).

Figure 3: Development of 'A' line



Maintenance of various lines and hybrid seed production scheme is as follows:

Maintenance of cytoplasmic male sterile line-A:

S-rfrf x F-rfrf

Male sterile line-A ⇩ Male fertile line-B

S-rfrf

Male fertile line-A

Single cross hybrid:

S-rfrf x F-RfRf

Male sterile line-A ⇩ Male fertile line-R

F-Rfrf

Male fertile

S = Sterile cytoplasm

F = Fertile cytoplasm

Rf = Fertility restorer gene

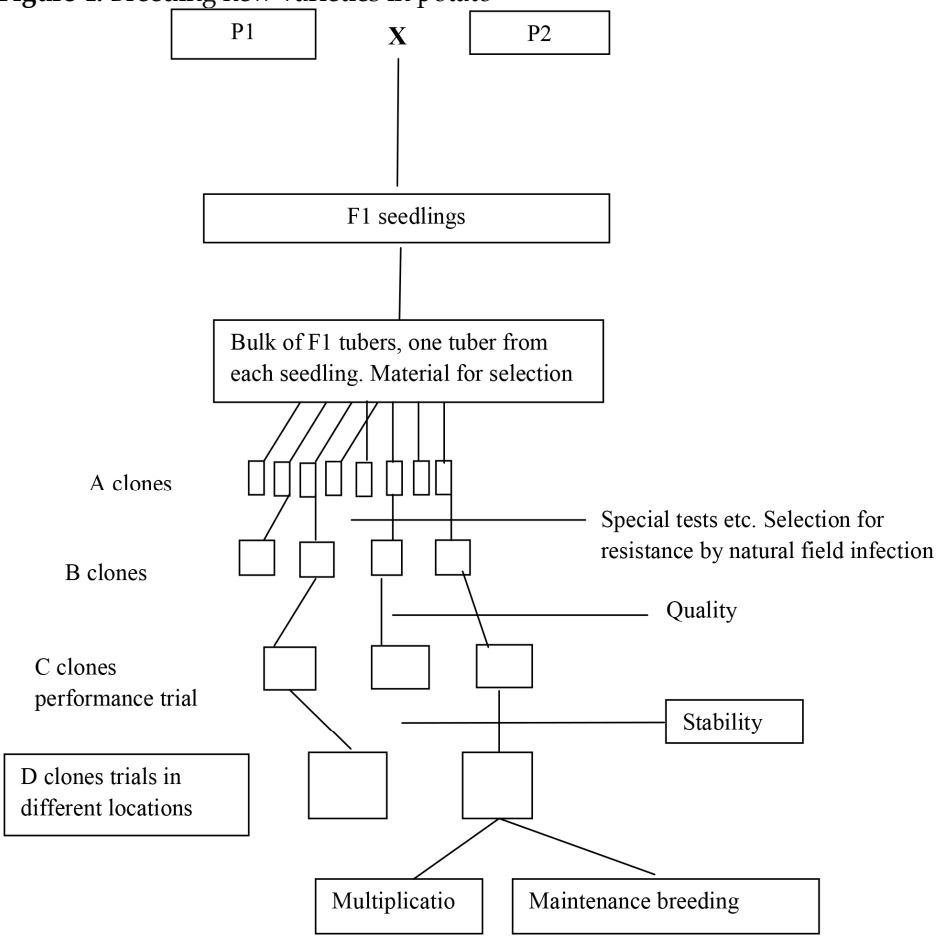
In the above programme for producing hybrid seed of A x R, A and R could be planted in alternate rows and seed set on A without detasseling will be hybrid seed in maize and will be male fertile due to restorer gene Rf. In maize three types of cytoplasm have been identified on the basis of fertility restoration when tested against a common set of inbred lines. These are cms-T, cms-C and cms-S. Cytoplasm cms-T has been found to be susceptible to race T of southern corn leaf blight. In commercial seed production, bulk production of A, B, and R lines is termed as foundation seed production and production of A x R hybrid seed is called as certified seed production.

Selection in Vegetatively Propagated Crops

Several commercially important crops like potato, sugarcane, many fruit species and ornamental plants are not multiplied by seed but are asexually/vegetatively propagated by cuttings, grafting of scions on root stocks, etc. All plants of a vegetatively propagated variety are identical genetically and they form a clone which is one individual divided into many parts with the same genetic constitution. Therefore, no genetic variation is to be expected in a clone variety unless mutation occurs spontaneously within the somatic tissue, resulting in change s of morphological traits or physiological behaviour. These mutants often called as sports have been important for development of new varieties especially in fruits and ornamental crops.

In potato, extensive genetic variation for selection can be generated by raising potatoes from seed. Since potato is strongly heterozygous, the first progeny from one female plant or from a cross of two genotypes is a segregating generation consisting of a large number of different genotypes. This can be compared with a typical F2 generation resulting from selfing of a cross between two dissimilar parents. Each genotypes that is selected from a segregating generation is constant in vegetative multiplication. With successful selection of a superior genotype, breeding success is immediately established. The breeding must begin with the selection of superior parents for producing seedlings and subsequent seedling selection. In potato, selection in inbred generation is generally not recommended as inbred generations (I1, etc) show inbreeding depression. Therefore, the most common method breeding in potato involves crossing of two superior parents followed by selection of superior and recombinant types in their progeny as shown in Fig. 4 (Kuckuck et al. 1991).

Figure 4: Breeding new varieties in potato



Molecular Assisted Selection (MAS)

Modern molecular genetics not only offers techniques for gene transfer but is also a very powerful tool in selection. DNA marker technology in the plant breeding process provides an opportunity for the breeder to select desirable lines based on genotypes rather than phenotypes. This approach has been very useful to plant breeders for analyzing plants at seedling stage, screening multiple traits that would be epistatic with one another, minimizing linkage drag and rapidly recovering the recurrent genotype. For MAS to succeed it is absolutely essential to identify closely linked markers which are flanking the gene of interest and validate them for use in regular breeding programme. The process of marker identification and validation is of crucial importance in any marker assisted selection programme. Steps in gene tagging and mapping are as follows (Sundaram and Rajendrakumar 2008):

1. Selection of target trait
2. Identification of parents differing in the trait of interest.
3. Development of Appropriate population segregating for the trait of interest.
4. Screening the population for the target trait (phenotyping).
5. Parental polymorphism survey with markers and identification of markers that co-segregate with gene(s) of interest in the individuals constituting the population.
6. Construction of linkage map and co-segregation analysis.
7. Test for reliability of the identical markers in predicting the trait in alternate population (marker validation).
8. Utilization of marker in breeding programme.

Selection of target trait is first step in gene tagging. The trait of interest may be qualitatively or quantitatively inherited. Qualitatively inherited traits are controlled mostly by single genes and follow dominant-recessive type of inheritance in a typical Mendelian fashion. These are easy to tag and map since they are not influenced by environment and genetic background. Examples of such traits include bacterial leaf blight resistance genes in rice where MAS is in use in India. Quantitatively inherited traits are controlled by many genes/loci where each locus typically has a small effect over the trait and the cumulative effect of alleles at the loci controlling the trait determines the trait expression. Quantitative traits show continuous variation in terms of expression of trait in segregating population and are usually difficult to tag and map since they are heavily influenced by the environment and the genetic background. Typical examples of such traits include yield and tolerance to abiotic stresses like drought, etc. Strategy of tagging and mapping for quantitatively inherited traits differs from that for qualitatively inherited traits. The quantitatively inherited traits need to be understood and deciphered into components before proceeding for tagging and mapping.

Next step is selection of parents differing for the trait of interest/target trait. This is easier for qualitatively inherited traits than that for quantitatively inherited traits. The mapping populations include segregating generations like F2, F3, BC1F1. However geneticists also prefer to use advanced generation materials like F6, F7 recombinant inbred lines (RILs), near isogenic lines (NILs), or double haploid lines (DHLs) if they are available since these are homozygous for all the loci involved. RILs are usually at F8/F9 generation onwards developed through single seed

descent, bulk or pedigree method. NILs are developed by repeated backcrossing of F1s, BC1F1s etc with the recipient line also called as recurrent parent while selecting for the trait of interest at each backcross generation. Usually backcross materials are called as NILs from BC3F1 generation onwards. DHLs are a special class of populations which are identical to RILs in their genetic constitution. Microspore culture of F1 anthers gives rise to haploid plants which on spontaneous or induced doubling yield DHLs.

Usually F2 populations consisting of 150-200 plants are used for tagging and mapping of single genes. If the trait under consideration is controlled by quantitative trait loci (QTLs), then population of higher sizes are required. The method of phenotyping differs significantly for qualitatively and quantitatively inherited traits. For most of the qualitatively inherited traits, like resistance to diseases and pests, phenotyping involves exposing the individuals of the population to a particular biotype/pathotype of the pest/disease and scoring the plants for resistance/susceptibility after a particular time interval. For example, if we expose F3 progeny rows to the pest/disease, then based on segregation pattern of the F3 lines, the genotype of each F2 plant can be inferred. The F2 plants whose progenies breed true for dominant trait in the F3, will be considered as homozygous dominant type for the target trait, the F2 plants whose progenies breed true for recessive trait in the F3, will be considered as homozygous recessive type for the target trait. The heterozygous F2 plant progenies in F3 will segregate in a ratio of 3 dominant to 1 recessive types. Success of tagging and mapping efforts depends mainly on precise phenotyping.

For quantitative traits, the process of phenotyping involves analysis of individual component characters that contribute towards the overall expression of the target trait, for example, when tagging and mapping QTLs for yield, it is necessary to phenotype individual components of yield like thousand grain weight, number of productive tillers, number of grains per panicle, etc for crop like rice. Performing experiments under replications helps to avoid the uncertainties induced by the environment.

After developing the population and phenotyping, the next task is to identify markers that co-segregate with trait of interest where the first step is to analyze the polymorphism among the parental lines with molecular markers. This section is somewhat elaborated to give clear picture about DNA or molecular markers in contrast to morphological and biochemical markers. Genetic markers represent genetic differences between individual organisms or species. Generally, they do not represent the target genes themselves but act as 'signs' or 'flags'. Genetic markers that are located in close proximity to genes (i.e. tightly linked) may be referred to as 'tags. Such markers themselves do not affect the phenotype of the trait of interest

because they are located only near or 'linked' to genes controlling the trait. All genetic markers occupy specific genomic positions within chromosomes (like genes) called (Collard et al. 2005).

There are three types of genetic markers: (1) morphological (also classical or visible) markers which themselves phenotypic traits or characters; (2) biochemical markers, which include allelic variants of enzymes called isozymes; and (3) DNA (or molecular) markers which reveals sites of variation in DNA. Morphological markers are usually visually characterized phenotypic characters such as flower colour, seed shape, growth habits or pigmentation. Isozyme markers are differences in enzymes that are detected by electrophoresis and specific staining. The major disadvantages of morphological and biochemical markers are that they may be limited in number and are influenced by environmental factors or the developmental stage of the plant. However, despite these limitations, morphological and biochemical markers have been extremely useful to plant breeders (Weeden et al. 1994).

DNA markers are most widely used type of markers predominantly due to their abundance. They arise from different classes of DNA mutations such as substitution mutations (point mutations (rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA. These markers are selectively neutral because they are usually located in non-coding regions of DNA. Unlike morphological and biochemical markers, DNA markers are practically unlimited in number and are not affected by environmental factors and/or developmental stage of the plant. Apart from use of DNA markers in the construction of linkage map, they have numerous applications in plant breeding such as assessing the level of genetic diversity within germplasm and cultivar identity. DNA markers are particularly useful if they reveal differences between individuals of the same or different species. These markers are called polymorphic markers, whereas markers that do not discriminate between genotypes are called monomorphic markers. Polymorphic markers may be described as co-dominant or dominant. This description is based on whether markers can discriminate between homozygotes and heterzygotes. Co-dominant markers indicate differences in size whereas dominant markers are either present or absent. Strictly speaking, the different forms of a DNA marker (e.g. different sized bands on gels) are called marker 'allele'. Co-dominant markers may have many different alleles whereas a dominant marker has only two alleles.

Usually, if mapped and co-dominant markers like SSRs are used, then it is necessary to scan parental lines with a set of uniformly spaced SSR markers (12-16 per chromosome) and identify at least 6-8 polymorphic markers per chromosome.

Care should be taken to ensure that the polymorphic markers on a chromosome are uniformly distributed. In rice, more than 20,000 SSR markers spread evenly across the rice genome are available and it is expected that the process of tagging and mapping of agronomically important genes will become much easier due to the availability of large number of markers. Once a set of markers polymorphic between the parental lines has been identified, the next step is to carry out co-segregation analysis for these markers. A simple strategy called 'bulk segregant analysis-BSA' can be employed to quickly identify markers which co-segregate with trait of interest. Bulk segregant analysis can be easily explained taking the example of an insect resistant gene in rice as explained by Sundaram and Rajendrakumar (2008).

A set of resistant and susceptible F2 lines (usually 10-15 lines in each case) are bulked separately and analyzed with parental polymorphic markers. If a fragment (hereafter called as marker) is present in the resistant donor, absent in the susceptible recipient, present in the resistant bulk and absent in the susceptible bulk, then marker is most probably associated with the resistance. The marker is then analyzed individually in all the lines constituting the resistant and susceptible bulks. If the marker is present in a majority (more than 70 % of individuals constituting resistant bulk and absent in majority of individuals constituting the susceptible bulk, then it can be assumed that the marker is linked with the trait of insect resistance. The next step is to perform co-segregation analysis with all the individuals constituting the population and then determine linkage distance based on extent of resistant individuals showing amplification of the resistance linked marker. In a similar way, markers co-segregating with susceptibility can also be identified.

Once a fragment/band (marker) is observed to co-segregate with resistance or susceptibility in the individuals of the population, then the next step is to undertake co-segregation analysis in all the individuals constituting the population (i.e. to check for presence of marker in the resistant or susceptible individuals of the population). Once a marker is confirmed to clearly co-segregate with the trait phenotype in a majority of individuals of the population, the next step is to identify the chromosomal location. A majority of rice SSR markers have been genetically and physically mapped on the rice genome (Sundaram and Rajendrakumar 2008). Thus, if an SSR marker is identified to tightly co-segregate with trait phenotype, then the tentative chromosomal location of the gene controlling the trait can be identified and more SSR markers in the vicinity of the co-segregating marker can be used for locating the exact position of the gene. Sundaram and Rajendrakumar (2008) in efforts towards tagging and mapping gall midge resistance gene (*Gm1*), identified an SSR marker RM219 to clearly co-segregate with trait phenotype in the population. Since RM219 is near the centromere region of chromosome 9, it was

logically assumed that *Gm1* is located in its vicinity and they looked for more SSR markers in the region. Out of 7 SSR markers near the centromeric region tested, two markers (RM444 and RM316) showed parental polymorphism and also exhibited clear co-segregation with trait phenotype. With the co-segregation data they constructed a linkage map of *Gm1* using co-segregation markers.

Construction of Linkage Map Using Markers

A linkage map may be thought of as a 'road map' of chromosomes derived from two different parents. Linkage map indicates the position and the relative genetic distance between markers along chromosomes, which is analogous to sign or landmarks along a highway. The most important use for linkage maps is to identify chromosomal locations containing genes and QTLs associated with traits of interest. Such maps may then be referred to as QTL or genetic maps. QTL mapping is based on the principle that the genes and the markers segregate via chromosome recombination (called cross over) during meiosis, thus allowing their analysis in the progeny. Genes or markers that are closely together or tightly linked will be transmitted together to the progeny more frequently than genes or markers that are located further apart. In a segregating population, there is mixture of parental and recombinant genotypes. The frequency of recombinant genotypes can be used to calculate recombinant fraction, which may be used to infer the genetic distance between markers. By analyzing the segregation of markers, the relative order and distance between markers can be determined-the lower the frequency of recombination between two markers, the closer they are situated on a chromosome (conversely, the higher the frequency of recombination between two markers, the further away they are situated on a chromosome (Collard et al. 2005). The markers that have a recombination frequency of 50 % are described as 'unlinked' and are assumed to be located far apart on the same chromosome or on different chromosomes. Mapping functions are used to convert recombination fractions into map units called centi-Morgans (cM). The three steps of linkage map construction are:

- Production of a mapping population
- Identification of polymorphism
- Linkage analysis of markers

Sundaram and Rajendrakumar (2008) have illustrated the procedure of construction of linkage map using 4 markers (E, F, G and H) and involving co-segregation analysis of RILs using co-dominant marker like SSR in rice. From co-segregation pattern of the 4 markers, it was quite clear that the marker E and F were quite close

to each other, while F and G, and G and H were not so close to each other. Similarly the co-segregation pattern of markers E and H showed that they were very far from each other. Based on co-segregation patter, percentage recombination is calculated for each pair of markers in terms of cM which is unit of linkage distance. Statistical soft-wares like 'Mapmaker', 'MapManager', 'JointMap', 'Cartographer' and 'Linkage' can be used for construction of linkage map. These are freely available from the internet. The co-segregation data has to be fed to the computer in excel format and soft-ware automatically constructs linkage map after calculating 'LOD' score for each pair of marker. In this process, linkage between markers is usually calculated using odds ratios (i.e. the ratio of linkage vs no linkage). This ratio is more conveniently expressed as the Logarithm of the ratio, and is called as a Logarithm of odds (LOd) value or LOD score (Collard et al. 2005). LOD values of more than 3 are typically used to construct linkage maps. A LOD value of 3 between two markers indicates that linkage is 1000 times more likely (i.e. 1000:1) than no linkage (null hypothesis). LOD values may be lowered in order to detect a greater level of linkage or to place additional markers within maps constructed at higher LOD values.

For constructing linkage maps for quantitative traits, usually marker intervals showing association with trait phenotype are identified and based on the extent to which these intervals are showing association, linkage distances are calculated using software 'MapMaker-QTL' after taking into consideration their LOD scores.

Once a marker or sets of markers are identified to be tightly linked with a particular gene, the next step is to validate the markers and their linkage distance in alternate populations. Alternate populations can be developed by selecting another donor line possessing the same resistance gene and crossing it with a susceptible parent. Once closely linked markers (which are less than 2 cM from gene of interest) and/or flanking markers (which are less than 5 cM on either side of the gene) are identified and validated in alternate populations, they are ready for use in marker-assisted breeding (MAS). Flanking markers have distinct advantage compared to single marker since selection based on two flanking markers will eliminate all false positives. For example, if a single marker about 2 cM away from the gene is used, about 2 out of 100 segregating plants will be false positives. But if flanking markers which are about 5 cM apart from the gene are used conjunctively, their combined recombination values would be very minimum (since theor combined recombination will be 5/100 x 5/100 = 25/10000 = 0.0025cM).

New types of markers and high-throughput marker techniques should play an important role in the construction of second –generation maps, provided that these methods are not too expensive. Due to the abundance of single nucleotide polymorphism (SNPs) and development of sophisticated high-throughput SNP

detection system, it has been proposed that SNP markers will have a greater influence on future mapping research studies and MAS. At present, methods for detection and analysis of widely used markers are becoming faster and more sophisticated, and many of these methods are automated. The development of more high-density or saturated maps that incorporate SNPs, EST (expressed sequence tag)-derived markers and STSs will provide researchers with a greater arsenal of tools for QTL mapping and MAS.

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Emerging Paradigms in IPR and Bioresources with special emphasis in Plant Breeding

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IPR and bioresources

Agriculture has become subject to IPR only after the creation of World Trade Organization (WTO) as a result of the General Agreement on Tariffs and Trade (GAIT) Uruguay Round agreement. It was thought that Agreement on Agriculture (AoA) is the central focus of WTO negotiations, since agriculture is lifeline of development of most countries in the world. It plays a pivotal role in ensuring food security, providing livelihoods, generating foreign exchange and determining the allocation of natural resources. As the legal instruments available to invoke IPR are inadequate to protect the vast intellectual resources (IR) available in the country with the indigenous people, we need to be agile and alert in watching the IPR infringement by others and claiming the benefit sharing in proportion -to the commercialization of our ITK. TRIPs does not provide for a mechanism to ensure repatriation of benefits to those who have knowledge of properties of natural resources. Actual innovators not benefited. This agreement obliges member governments to provide for "the protection of plant varieties either by patents or by an effective *sui generis* system or by any combination thereof. "CBD speaks of the need of equitable shares of benefits and recognition of the knowledge of the indigenous communities. Thus, current IPR mechanisms for indigenous peoples seem to be inherently unsuitable. Different procedures should be developed to recognize and reward community contributions to plant resources. The local communities or individuals do not have the knowledge or the means to safeguard their property in a system which has its origin in very different cultural values and attitudes. The existing IPR systems are oriented around the concept of private ownership and individual innovation. The issue of protection of traditional knowledge needs to be looked at from two perspectives, the protection may be granted to exclude the unauthorized use by third parties of the protected information. While recognizing the market-based nature of IPRs, other non-market based rights could be useful in developing models for a right to protect traditional knowledge, innovation and practices. Giving legally recognized ownership of knowledge to communities through *sui generis* IPRs has several benefits. New experiments are beginning to emerge on benefit sharing models for indigenous innovation certain patents or other IPRs may cover aspects of Traditional Medicine.

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There is clearly a need for more research on free trade, intellectual property rights, and the equitable compensation of local communities. IPRs being applicable directly for all new inventions of products and processes having industrial possibilities New IPR Regime will bring in many new challenges and opportunities to the Indian S&T and industrial sectors. In order to patent the knowledge it must be novel. Biopiracy calls for an effective system of laws and standards in India to protect our traditional wealth. In other words, this is also an area where large-scale patentable inventions are being made and naturally this has significant implications. Protection of knowledge, innovations and practices associated with biological resources, these do not seem to meet the conditions required for grant of patents or other IPRs (e.g. copyrights, trademark, etc.) under the prevalent IPR regimes, i.e. novelty, inventiveness and industrial applicability. Many of the grass root innovators, however, do not have the capacity for value addition. Some of the laws enacted in India for IPR protection in agriculture are Protection of Plant Varieties and Farmers' Right Act (2001), Protection through the Biological Diversity Act (2002) and Geographical Indication of Goods (Registration and Protection) Act (1999). Traditional Knowledge Digital Library (TKDL) is prepared by (SIR to protect biopiracy). Sui generis systems are needed to protect traditional knowledge. IPRs contribute to value addition of the resources. By incorporating appropriate conditions in the IPR laws for sharing of benefits through terms and conditions, What is the impact of new patent regime on medicinal plants especially on traditional wisdom? Price fee for accessing and prospecting of the medicinal plants? Types of institutional arrangements to execute it? How to determine the percentage of benefits to various parties concerned? How to get economic benefit to the nation? How to prevent biopiracy? This presentation will try to answer some of these questions

IPR in plant breeding

Increased attention has been given in the past few years to strengthening intellectual property rights (IPRs) in plant breeding. The number of countries that grant such rights has grown, the types of inventions that can be protected have expanded, and the scope of protection offered by extent IPR systems in different countries has also broadened. The Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS 1993) of the World Trade Organization (WTO) requires all WTO members to introduce at least a minimum level of protection in their national laws for plant varieties and inventions in biotechnology. Plant breeding research and seed provision are vital industries that need to be fostered and stimulated. Plant breeding is important for food security at the local and global levels; the ability of adapted varieties to cope with environmental stresses

contributes to strategies for sustainable agriculture, and the provision of productive options for commercial farming is essential for wider economic development.

In order to provide for the establishment of an effective system for the protection of plant varieties, the rights of farmers and plant breeders and to encourage the development of new varieties of plants it has been considered necessary to recognize and to protect the rights of the farmers in respect of their contributions made at any time in conserving, improving and making available plant genetic resources for the development of new plant varieties. The Govt. of India enacted "The Protection of Plant Varieties and Farmers' Rights (PPV & FR) Act, 2001" adopting sui generis system. Indian legislation is not only in conformity with International Union for the Protection of New Varieties of Plants (UPOV), 1978, but also have sufficient provisions to protect the interests of public/private sector breeding institutions and the farmers. The legislation recognizes the contributions of both commercial plant breeders and farmers in plant breeding activity and also provides to implement TRIPs in a way that supports the specific socio-economic interests of all the stakeholders including private, public sectors and research institutions, as well as resource-constrained farmers.

Biodiversity conservation in the context of Access and Benefit sharing

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Biodiversity comprises of different forms of life along with their existing habitats and eco system. The conservation of biodiversity is of prime importance in the present day as it has been over exploited by man to fulfill the day to day needs. The present trends in agriculture show that there is a higher demand for cash crops compared to food crops which has led to the increased application of hazardous chemicals. This has in turn led to the polluting of the environment and the replacement of traditional varieties of various crops. Kerala is blessed with a rich and highly diversified forest ecosystem. These forests are presently facing the problems including the entry of invasive species into the forest, and the over exploitation of medicinally and economically important plant species. Similar challenges are faced by the highly diversified marine ecosystem evidenced by reduction in the fish stock. Several management practices need to be implemented to replenish the fish stock, control coastal erosion and land use changes. Mangrove ecosystem is of high value as they provide protection to some of the rare species. *Ex-situ* conservation efforts are equally important conserving the biodiversity, especially the threatened ones. The botanical gardens are field laboratories for facilitating environmental education.

India is one of the first countries to implement Access and Benefit Sharing (ABS) in accordance with Nagoya protocol. This protocol regulates the utilization of genetic resource and associated Traditional Knowledge (TK) for commercial and research purposes. The ABS follows a guideline to implement the sharing of benefits to the community or locality from where the resources are exploited. This includes both monetary and non-monetary modes. The Biodiversity Management Committees (BMC) at Local level has to be strengthened for the effective functioning of ABS system. At State level, many sectors like Ayurvedic Industries, Marine food exporters etc., should be involved and through a process of negotiation, brought under the ABS system .Awareness and capacity building on ABS and related legal aspects is an area to be addressed. In recent times, only plant resources are considered under ABS and there is an urgent need to bring our animal resources also under the ambit of ABS.

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Dependence of Copra yield on component characters in coconut (*Cocos nucifera* L.)

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ABSTRACT: *Coconut palm is known as 'Kalpa Vriksha' due to its myriad uses. Due to perennial nature, the crop improvement programmes in coconut are highly time consuming. In this crop, only a limited number of works had been conducted regarding copra yield. An experiment was conducted on twenty year old palms planted in RBD with three replications and three palms per plot. The experimental material consisted of nine genotypes viz., West Coast Tall, Laccadive Ordinary, Philippines, Jawa, Cochin China, New Guinea, West Coast Tall x Choughat Yellow Dwarf, West Coast Tall x Gangabondam (Keraganga) and Laccadive Ordinary x Gangabondam (Lakshaganga). For copra yield (kg/palm), simple and regression models were prepared using seven component characters viz., trunk height, trunk girth, petiole length, leaf length, spadices/year, female flowers/spadix and fruit set. Final multiple regression model included leaf length along with height and girth of trunk. No reproductive character was included in the final selection model indicating that none had significant influence on the yield of copra.*

Key words*: Coconut, Copra yield, Regression model.*

Introduction

Coconut palm is known as 'Kalpa Vriksha' due to its myriad uses. Due to perennial nature, the crop improvement programmes in coconut are highly time consuming. Besides, selection of elite genetic material is of much importance in this crop. Composite and low heritability of yield characters highlights the importance of understanding their dependence on component characters. Regression analysis is one approach which helps to achieve this objective. In coconut, only a limited number of works are available in this area especially regarding content or yield of copra. Abeywardena and Mathes (1980) evolved an index for selecting mother palms in coconut based on trunk girth, number of opened inflorescences including mature bunches, number of nuts per bunch and number of fronds present at a given time. Balakrishnan et al. (1991) formulated selection indices by discriminant function analysis technique for fifteen hybrids and West Coast Tall using the component characters leaves/year, nuts/year and cumulative nut production since first flowering.

Discriminant function was adopted to formulate selection indices for copra content against seventeen component characters (Louis and Chopra 1989). Both indices had three common characters with positive role and two common characters with negative role. In a study conducted by Vanaja (1993) to assess the direct and indirect effects of various nut characters on copra content, maximum direct effect (positive) was by weight of split nut.

Materials and Methods

An experiment was conducted at the Instructional Farm, College of Horticulture, Vellanikkara on twenty year old palms planted in Randomised Block Design with three replications and three palms per plot. The experimental material consisted of nine genotypes *viz*., West Coast Tall, Laccadive Ordinary, Philippines, Jawa, Cochin China, New Guinea, West Coast Tall x Choughat Yellow Dwarf, West Coast Tall x Gangabondam (Keraganga) and Laccadive Ordinary x Gangabondam (Lakshaganga). For copra yield (kg/palm), simple regression coefficients were estimated using seven component chatracters *viz*., trunk girth, trunk height, petiole length, leaf length, spadices/year, female flowers/spadix, and fruit set as per Singh and Choudhary (1985). Step-down regression was performed for these traits using SPAR-1 computer programme.

Results

Simple regression coefficients (b) and coefficient of determination (R^2) of component characters on copra yield are presented in Table 1.

Table 1: Simple regression coefficients (b) and Coefficient of determination (R^2) of component characters on copra yield

Sl. No.	Character	Simple regression coefficient (b)	Coefficient of determination (R^2)
	Vegetative characters		
1	Trunk height (m)	-0.890	0.042
2	Trunk girth (m)	-28.281*	0.181
3	Petiole length (m)	-5.562	0.014
4	Leaf length (m)	-1.601	0.019
	Reproductive characters		
5	Spadices/year	0.630	0.019
6	Female flowers/spadix	0.146	0.093
7	Fruit set (%)	0.087	0.037

Copra yield was dependent only on vegetative characters especially trunk girth. An enhancement of trunk girth by 1m would be associated with a reduction of copra yield by 28.281 kg per palm with coefficient of determination being 0.181.

Determination of a complex character by a set of interrelated component characters using multiple regressions is of importance in crop improvement programmes.

Step-down regression employs gradual elimination of less important characters in a multiple regression model. Liyanage (1962) proposed the inclusion of earliness in bearing, nut yield and nut weight in the index for mother palm selection for copra production. Dependence of copra yield on vegetative and reproductive characters was examined using step-down multiple regression and the results are presented in Table 2.

Table 2: Multiple regression models for copra yield based on component characters

Component characters								
Intercept constant (a)	**Trunk height (m)**	**Trunk girth (m)**	**Petiole length (m)**	**Leaf length (m)**	**Spadices/ year**	**Female flowers/ spadix**	**Fruit set (%)**	**Coefficient of determination (R^2)**
71.78	3.40	-44.74	-10.67	-5.47	-0.66	0.16	0.09	0.66
65.75	3.41	-45.82	-10.37	-5.14		0.14	0.05	0.65
69.17	3.41	-47.22	-10.74	-5.03		0.14		0.64
57.42	3.18	-46.65		-5.12		0.13		0.59
70.17	2.91	-52.20		-5.45				0.54

Variability in the yield of copra could be explained up to 66 per cent when all the seven characters were included in the multiple regression model. During the step-down process spadices/year, fruit set, petiole length and female flowers/year had been eliminated successively in that order. Final model which included leaf length along with height and girth of trunk gave a coefficient of determination of 54%. No reproductive character was included in the final selection model indicating that none had significant influence on the yield of copra.

This result deviated from the logical expectation. Economic characters recorded during the current year would be influenced directly by the floral characters of previous year. In this experiment both copra yield and reproductive characters were recorded during the same year. Hence low association between them might be due to lack of correlation for floral characters between years. This suggests the existence of genotype x environment (year) interaction.

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Leaf morphology and stomatal studies of *Plumbago* L. species from India

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ABSTRACT: *Plumbago L., characterized with 18 species, belonging to the family Plumbaginaceae disseminated along the tropical and subtropical belt of the world. Three species such as Plumbago auriculata Lam., P. indica L. and P. zeylanica L. have so far been reported to be occurring in India. Some of the species are highly significant for their medicinal property. One of the bioactive compounds, plumbagin reported from Plumbago species has high value in pharmaceutical industry. Plumbagin has remarkable medicinal effect on immune-suppressive problems and also has anti-tumor, anti-fertility, anti-microbial and anti-protozoal properties. Interestingly, it is found that no detailed studies on foliar epidermal variations have so far been conducted on Indian specimens of the genus Plumbago. Leaf morphological and anatomical characterization studies carried out among various accessions of the three species occurring in India has revealed remarkable variability in stomatal index, nature of epidermal cell and anticlinal wall. The present paper deals with comparative studies on leaf morphology and stomatal characters on three species of Plumbago in detail.*

Key words: *Plumbago auriculata, P. indica, P. zeylanica, Plumbagin, Stomatal index.*

Introduction

The family Plumbaginaceae sometimes referred to as the Leadwort family or the Plumbago family, constitutes with 280 species beneath 10 genera distributed among the semi arid regions of the Old World, especially along the Mediterranean and Central Asiatic regions (Lawrence 1951). According to Kew Database, the genus *Plumbago* L. is represented by 18 species (http://www.theplantlist.org/tpl1.1/search?q=Plumbago). The Indian Subcontinent is known to host three species including one introduced taxon such as *P. auriculata* Lam. (= *P. capensis*), *P. indica* L. (= *P. rosea*) and *P. zeylanica* L. *P. auriculata* is a native of South Africa is under cultivation in India mostly for ornamental value while the other two species are indigenous to the country (Nayar et al. 2014).

From economic and utilization points of view, *Plumbago* species have much relevance in Ayurvedic system of treatment for various ailments such as intestinal disorders, skin diseases, bronchitis, liver diseases and rheumatism (Sharma et al. 2000). One of the bioactive compounds, plumbagin reported from *Plumbago* species has high value in pharmaceutical industry. Plumbagin has remarkable medicinal effect on immune-supressive problems, anti-tumor, anti-fertility, anti-microbial and anti-protozoal properties (Kini et al. 1997; Krishnaswamy and Purushothaman 1980). According to Gullett et al. (2010), plumbagin has been recognized as a cancer chemo-preventive agent. The developmental patterns of the stomatal complex shows absolute constancy in the majority of families and hence stomatal ontogeny

and structure are good systematic tools for characterization (Van Cotthem 1970). It also has systematic evidence in both living and fossil plants with a key role in framing hypotheses about early Angiosperm evolution (Abdel-Hameed et al. 2013). According to Van Cotthem (1970), stomatal types, ontogeny and distribution are of great importance in taxonomy and phylogenetic interpretations, since they are homologous structures among plant species. The comprehensive monographs on the application of cuticular analysis in plant taxonomy by Stace (1965) also support the importance of stomatal studies in phylogenetical and plant taxonomical characterization.

It has been found that no detailed studies have so far been conducted on foliar epidermal variations of Indian specimens among the three species under the genus *Plumbago*. In this context, the present investigations were taken up analyzing qualitative and quantitative characters such as foliar shape, laminar area, foliar length, foliar width, stomatal type, stomatal density, stomatal index and other epidermal traits such as epidermal cell shape, cell orientation, cell arrangement and anticlinal wall.

Materials and Methods

The *Plumbago* leaves have been collected during morning hours (8.00 to 10.00 am) from various accessions from the Field Gene Bank of medicinal plants at JNTBGRI. The leaf samples then cut into small pieces were slightly heated with 5% of Sodium hydroxide solution and kept overnight for conducting experiments (Singh and Viswanathan 2000). The very next day morning the epidermal peeling was taken from both abaxial and adaxial surfaces of leaf pieces by using fine brush and he peels were stained with 2% safranin for 60 to 120 seconds. Then the epidermal peels were transferred into distilled water for about 180 seconds and washed out the over stain. Later temporary slides have been prepared and relevant photographic documentations were made by using Leica DM 2500 trinocular microscope (20x and 40x). The experiments on stomatal replications such as stomatal morphology, epidermal morphology, stomatal length, stomatal width, epidermal cell arrangement, orientation, anticlinal wall and epidermal cell surface were carried out according to the classification by Singh et al. (2000) and Prabhakar (2004). Leica software was utilized for the measurements on stomatal dimensions. Stomatal index (SI) was calculated according to the formula by Salisbury (1927); SI = S/E + S × 100, where, S = number of stomata per unit leaf area, E = number of epidermis per unit leaf area. Later results were statistically analyzed by mean values and determined standard deviation.

Results

The qualitative characterization on leaf morphology revealed remarkable difference in foliar shape. The leaves of *Plumbago auriculata* possess with oblong shape, *P. indica* were ovate- elliptic while *P. zeylanica* demonstrated ovate shape. Chalk glands are important characteristic of the genus *Plumbago* and have been observed in all species. In *P. zeylanica* it was visible with nacked eyes, scattered on both surfaces. In *P. indica* as well as *P. auriculata* the glands are microscopic. The mean laminar area of *Plumbago indica* represents high value (61.75 cm^2) followed by *P. zeylanica* (55.88 cm^2) and *P. auriculata* (8.0 cm^2). The leaf length and width were also sequentially reducing from *Plumbago indica* (13.15 x 6.92 cm) to *P. zeylanica* (9.92 x 5.92cm) and then *P. auriculata* (5.3 x 2.08 cm) (Table 1). Glandular four celled trichomes were also been observed among all species.

All the three species have been demonstrated with amphistomatic and anisocytic stomata (Fig. 1). Stomatal density analysis exposed high density on adaxial surface in *Plumbago indica* (9.00±3.74) followed by *P. auriculata* (3.25±5.86) and *P. zeylanica* (2.75±1.70) respectively (Table 1). As regards to abaxial surface high stomatal density observed in *Plumbago auriculata* (66.75±10.88) followed by *P. indica* (31.25±6.30) and *P. zeylanica* (18.5±4.50) (Table 1). Remarkable variations have been observed in stomatal index analysis. High stomatal index on both adaxial and abaxial foliar surfaces was observed in *Plumbago indica* as 4.30% and 20.56% respectively. As regards to *Plumbago zeylanica* and *P. auriculata,* stomatal index on adaxial surface was recorded as 1.97% and 0.52% respectively, while on abaxial surface, *P. auriculata* characterized with 18.37% and *P. zeylanica* with 12.06% (Table 1).

Qualitative analysis of epidermal cells illustrated with two different shapes such as polygonal and irregular. *Plumbago auriculata* and *P. indica* have been displayed with polygonal shapes, while *P. zeylanica* has been characterized with irregular shape. Similarly, the anticlinal wall of epidermal cells also demonstrates significant variations, such as straight in *P. auriculata*, straight-curved in *P. indica* and wavy-sinuate in *P. zeylanica* (Fig. 1). As regards to epidermal cell orientation, all the three species displayed with different alignment with irregular arrangements. All the three species are unique in epidermal surfaces with glabrous nature on both sides (Fig. 1); nevertheless, quantitative difference has been observed in epidermal cells among the species. *Plumbago auriculata* was characterized with maximum epidermal cells on both surfaces, as 495.5±67.00 on adaxial surface and 299.5±44.56 on abaxial surface. *Plumbago indica* has been illustrated with 189.75±21.29 and 126.00±26.84 epidermal cells on adaxial and abaxial surface while in *P. zeylanica* it was 131.75±15.52 and 134±11.43 respectively (Table 1).

Table 1: Leaf morphology and Stomatal studies on the genus *Plumbago* L.

Characters	***P. auriculata***		***P. indica***		***P. zeylanica***	
Leaf shape	Oblong		Ovate – elliptic		Ovate	
Leaf length (cm)	5.3±0.62		13.15±1.23		9.92±2.58	
Leaf breadth (cm)	2.08±0.18		6.92±0.88		5.92±1.60	
Leaf area (cm^2)	8.0±1.59		61.75±5.54		55.88±4.25	
Trichome	Glandular, four celled		Glandular, four celled		Glandular, four celled	
	Adaxial	**Abaxial**	**Adaxial**	**Abaxial**	**Adaxial**	**Abaxial**
Stomatal type	Anisocytic	Anisocytic	Anisocytic	Anisocytic	Anisocytic	Anisocytic
No. of. Stomata/mm^2	3.25±5.86	66.75±10.88	9.00±3.74	31.25±6.30	2.75±1.70	18.5±4.50
Stomatal index (%)	0.52	18.37	4.30	20.56	1.97	12.06
No. of subsidiaries cells surrounded stomata	3	3	3	3	3	3
Stomatal length (µm)	9.70±11.20	19.61±3.16	18.74±1.80	18.04±2.27	26.30±7.49	29.78±7.02
Stomatal breadth (µm)	6.63±7.66	14.37±2.92	14.12±1.80	15.27±1.72	20.36±5.66	19.09±4.84
Epidermis cell shape	Polygonal	Polygonal	Polygonal	Polygonal	Irregular	Irregular
Epidermal cell wall	Straight	Straight	Straight – curved	Straight – curved	Wavy – Sinuate	Wavy – Sinuate
Epidermis cell orientation	Variously oriented	Variously oriented	Variously oriented	Variously oriented	Variously oriented	Variously oriented
Arrangement of epidermal cells	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular
Epidermal surface	Glabrous		Glabrous		Glabrous	
No. of Epidermal cells/mm^2	495.5±67.00	299.5±44.56	189.75±21.29	126.00±26.84	131.75±15.52	134±11.43

Discussion

Stomatal type is unique in all the three species (anisocytic) as reported by Galal et al. (2013). Metcalfe and Chalk (1950) also reported anisocytic stomatal type in *P. zeylanica*. In this context, it is also relevant to mention that the family Plumbaginaceae has mostly displayed with anisocytic type of stomata. The studies on other genera like *Armeria* Willd. and *Limonium* Mill. of the concerned family also

have been characterized with anisocytic stomata. Stomatal index has much relevance in regulation of transpiration rate among species and is determined by stomatal density and number of epidermal cells. As regards to *Plumbago indica* high stomatal index demonstrated with more number of stomata and lesser number of epidermal cells whereas, *P. auriculata* and *P. zeylanica* have been illustrated with lesser number of stomata with more epidermal cells on adaxial surface. The above results showed *Plumbago indica* has high transpiration rate, followed by *P. auriculata* and *P. zeylanica*. In abaxial surface, high stomatal index was observed in *Plumbago indica* followed by *P. auriculata* and *P. zeylanica* with lesser numbers of stomata. *Plumbago auriculata* has demonstrated maximum number of stomata in minimum foliar unit area followed by *P. zeylanica*; nevertheless, the latter has been characterized with larger size stomata than the former. Ayodele (2000), worked out on Nigerian *Plumbago* species such as *P. auriculata* and *P. zeylanica* also reported alternative changes in stomatal index on adaxial and abaxial surfaces. The characteristic chalk glands beneath the leaves are well distinguishing character found all the three species of *Plumbago* in India. According to Cutler (1978) and Sakai (1974) chalk secreting glands are important characteristic of the *Plumbago* species. *Plumbago auriculata* and *P. indica* demonstrate polygonal shape of epidermal cells whereas in *P. zeylanica* irregular shape. The anticlinal walls have also the same alternative variations. The present investigation on stomatal studies, leaf morphology and epidermal morphology are reliable tools for characterization and analysis on range in interspecific variations.

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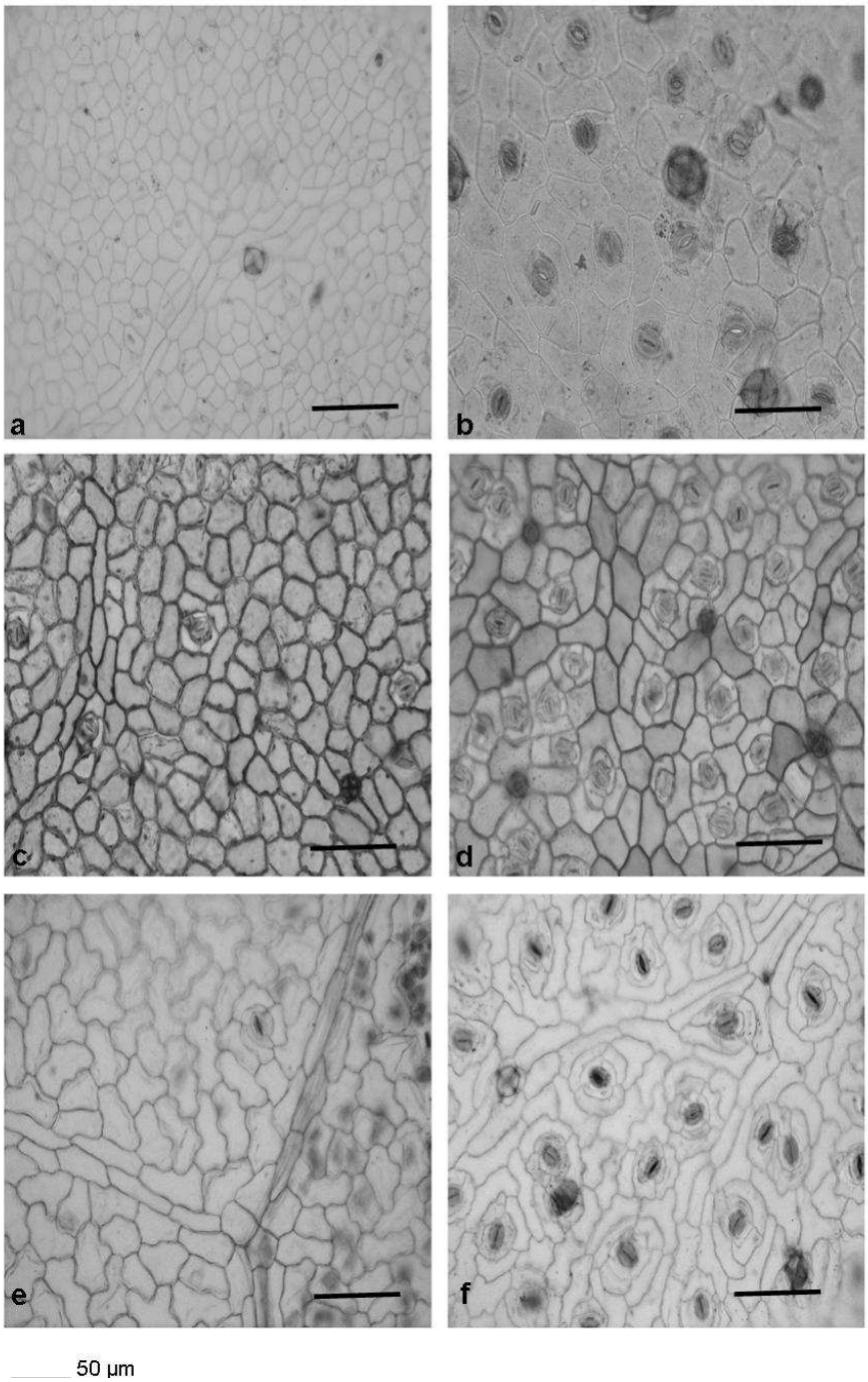
Figure 1: Stomatal morphology of the genus *Plumbago* L. (a). adaxial surface of *P. auriculata*, (b). abaxial surface of *P. auriculata*, (c). adaxial surface of *P. indica*, (d). abaxial surface of *P. indica*, (e). adaxial surface of *P. zeylanica* and (f). abaxial surface of *P. zeylanica*.



Variation in rhizome yield in different accessions of *Curcuma aeuruginosa* roxb. of northern districts of Kerala, India

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ABSTRACT: *Crop yield is defined as the quantity of the economic product of a crop harvested per unit of land area. Estimation of the variability among the yield parameters of a crop will help us to identify the best genotype showing the highest crop yield. This subsequently leads to the development of high yielding varieties along with other growth parameters. Curcuma aeruginosa Roxb. is not a new comer in flowering plants but it still remains an underutilized species though this has valuable medicinal properties. It comes under the family Zingiberaceae and is popularly known as "pink and blue ginger". It is also known as Travancore starch plant, arrowroot wild and East Indian arrowroot. Curcuma aeruginosa is a traditional herbal medicine prescribed for the treatment of many human ailments ranging from constipation to skin diseases.There are no recorded studies regarding the yield parameters and crop improvement of this species so far. The present experiment aims to assess the variability of yield among sixty eight accessions of Curcuma aeruginosa Roxb. collected from the Northern districts of Kerala state of India and to isolate the superior accessions from them.*

Key Words: *Curcuma aeruginosa; Travancore starch plant; Zingiberaceae; Genetic variability*

Introduction

Curcuma aeruginosa Roxb. is an under utilized starchy tuber crop with immense medicinal properties. It belongs to the family Zingiberaceae. The plant is a triploid with 2n=63 (Joseph et al. 1999). It is a perennial, semi erect rhizomatous herb. Rhizome is the useful part having pink tips and greyish blue/blue centres; hence the plant is popularly known as pink and blue ginger. In South India, the rhizomes are widely used for the extraction of East Indian arrowroot or Travancore starch (Sabu 2006). Therefore the plant also gains the name Travancore starch plant, arrowroot wild or East Indian arrowroot. The plant is a native of Myanmar and is widely cultivated in Malaysia (Sabu 2006). Common occurrence is found throughout South East Asia (Srivilai et al. 2011). In India it grows in West Bengal, Bihar, Coromandal coast, South Karnataka and is fairly common in Kerala (Srivastava et al. 2006). It grows wild in South India and is very common throughout the coastal areas and riverine alluvial soils extending up to midlands in Kerala and South Karnataka. During monsoon it grows as common undergrowth in coconut and arecanut groves and also as a weed in waste lands (Sabu 2006). It is a shade loving plant and grows well in partially shaded regions. The medicinal

properties of *Curcuma aeruginosa* is attributed to the various phytochemical constituents contained in it. The rhizome shows a yield of 1.25-1.50 kg/plant and the dry matter of the tuber has been reported to be 29.30%. Rhizome contains starch 14.10%, sugar 1.41% and lipids 0.47% (Sujatha and Renuga 2013; Angel et al. 2013).

The starch extracted from the rhizome is commonly used as a substitute for arrowroot (Ranjini and Vijayan 2006; Sabu 2006) and is an efficacious remedy for infantile diarrhoea as well as recommended for children and invalids (Ranjini and Vijayan 2005). It is used as an alternative food source to replace cassava and corn (Anonymus 2012) and is believed that the medicinal value of the starch increases, as the powder become aged (Sabu and Skornickova 2003). The starch of *Curcuma aeruginosa* is a highly amylose starch with lesser amount of amylopectin (Ranjini and Vijayan 2006). Starch granules are 6.66-33.30 µm in size (Sujatha and Renuga 2013).The rhizomes of *Curcuma aeruginosa* have been used in traditional medicine for gastrointestinal and uterine remedies. Various biological activities shown by this plant includes postcoital contraceptive effect, anti HIV, hepatoprotective, antimicrobial, antioxidant and anti-platelet activating effects and antinociceptive effects (Otake et al. 1995; Trakoontivakorn et al. 2000; Thaina et al. 2009). It is also used as a digestive aid and for the treatment of fever, asthma, wounds, infections, dysentery, arthritis, jaundice and other liver problems (Ibrahim and Rahman 1989; Gul et al. 2004).

Even though the genetic diversity of this species has not been assessed scientifically so far, there is no doubt that the species density is declining due to various anthropogenic activities such as habitat destruction, urbanization, industrialization, etc. Also there are no improved high yielding varieties developed so far and no scientific publications on its cultivation practices. The release of high yielding varieties of *Curcuma aeruginosa* will be thus beneficial for the large scale production and conservation of this valuable medicinal plant. The superior genotypes of this species can be produced through various plant breeding programmes and the study of variability is the initial step towards it. The present study aims to analyze the yield parameter of the species so as to identify superior accessions.

Materials and Methods

The present experiment was laid out in RBD in the experimental plot of the Genetics and Plant Breeding Division of the Department of Botany, University of Calicut, Kerala, India. Sixty eight accessions of *Curcuma aeruginosa* collected from the Northern districts of Kerala (Kasaragod, Kannnur, Wayanad, Kozhikode, Malappuram, Palakkad and Thrissur) formed the experimental material (Table 1 & Fig. 1).

Table 1: Variation of yield in the case of the *Curcuma aeruginosa* Roxb. accessions studied

Accession No. and Place of Collection	Mean (g) ± SE	Range (g)	Rank	Overall range (g)	CD (5%)
CUA 1 (Kuniyil, Malappuram)	504.16 ± 28.39	350-675	31	50-1875	201.97
CUA 2 (Pathiriyal, Malappuram)	505.55 ± 89.38	250-750	30		
CUA 3 (Kavanoor, Malappuram)	536.11 ± 7.35	325-725	22		
CUA 4 (Kondotty, Malappuram)	388.88 ± 80.65	100-700	47		
CUA 5 (Vazhayur, Malappuram)	463.88 ± 77.26	50-750	36		
CUA 6 (Cherukode, Malappuram)	549.99 ± 108.89	175-1050	18		
CUA 7 (Mundakkulam, Malappuram)	583.33 ± 38.53	350-800	17		
CUA 8 (Irumbhuzhi, Malappuram)	788.88 ± 104.90	350-1125	5		
CUA 9 (Alinchode, Malappuram)	866.66 ± 50.97	650-1125	2		
CUA 10 (Pananthara, Thrissur)	411.10 ± 16.91	250-600	44		
CUA 11 (Edappal, Malappuram)	544.44 ± 26.52	350-950	20		
CUA 12 (Karipur, Malappuram)	455.55 ± 36.79	350-675	37		
CUA 13 (Panakkad, Malappuram)	594.44 ± 51.95	300-800	13		
CUA 14 (Kooriyad, Malappuram)	599.99 ± 87.10	350-975	12		
CUA 15 (Venniyur, Malappuram)	508.33 ± 22.07	350-700	29		
CUA 16 (Valiyora, Malappuram)	541.66 ± 70.95	50-800	21		
CUA 17 (Kainod, Malappuram)	605.55 ± 26.52	325-900	11		
CUA 18 (Cheruvannur, Kozhikode)	702.77 ± 100.27	350-850	7		

CUA 19 (Kunnamangalam, Kozhikode)	552.77 ± 51.50	350-750	17
CUA 20 (Manassery, Kozhikode)	655.55 ± 48.25	375-1125	9
CUA 21 (Karassery, Kozhikode)	455.55 ± 69.86	200-700	37
CUA 22 (Feroke, Kozhikode)	388.88 ± 21.72	175-500	47
CUA 23 (Valluvambram, Malappuram)	490.27 ± 39.15	325-950	33
CUA 24 (Alukkal, Malappuram)	416.66 ± 61.49	125-575	43
CUA 25 (Pallikkal bazaar, Malappuram)	533.33 ± 19.26	400-650	23
CUA 26 (Karikkad, Malappuram)	533.33 ± 164.34	250-850	23
CUA 27 (Pallippadi, Malappuram)	541.66 ± 142.56	275-1125	21
CUA 28 (Old Vythiri, Wayanad)	352.77 ± 29.43	225-550	51
CUA 29 (Lakkidi, Wayanad)	447.22 ± 50.59	175-650	38
CUA 30 (Ramanattukara, Kozhikode)	530.55 ± 78.31	275-775	24
CUA 31 (Arappuzha, Kozhikode)	280.55 ± 18.23	150-400	55
CUA 32 (Mampuzha, Kozhikode)	336.10 ± 43.44	175-550	53
CUA 33(Chevarambalam, Kozhikode)	494.44 ± 22.76	275-775	32
CUA 34 (Muzhikkal, Kozhikode)	377.77 ± 12.12	150-525	49
CUA 35 (Pantheerpadam, Kozhikode)	347.69 ± 38.70	200-650	52
CUA 36 (Koduvalli, Kozhikode)	380.55 ± 27.39	300-450	48
CUA 37 (Puthur, Kozhikode)	622.22 ± 78.31	325-1125	10
CUA 38(Kakkayam, Kozhikode)	486.10 ± 99.22	250-875	34

CUA 39(Thalayad, Kozhikode)	594.44 ± 44.75	375-850	13
CUA 40 (Kariyathumpara, Kozhikode)	605.55 ± 155.81	50-1000	11
CUA 41 (Puthukkode, Malappuram)	802.77 ± 61.37	450-1050	4
CUA 42 (Mannarkkad, Palakkad)	966.66 ± 205.55	450-1875	1
CUA 43 (Thachampara, Palakkad)	811.10 ± 85.67	450-1125	3
CUA 44 (Kalladikkode, Palakkad)	511.10 ± 106.66	200-1050	28
CUA 45 (Kottekkad, Palakkad)	430.55 ± 59.45	225-650	42
CUA 46 (Mankkara, Palakkad)	327.77 ± 69.19	125-725	54
CUA 47 (Kallidukku, Thrissur)	688.88 ± 45.78	275-1425	8
CUA 48 (Kandanassery, Thrissur)	530.55 ± 65.04	350-950	24
CUA 49 (Agastyamuzhi, Kozhikode)	744.44 ± 51.05	475-1125	6
CUA 50 (Pulppalli, Wayanad)	566.66 ± 122.90	100-1175	16
CUA 51 (Chakkunthara, Thrissur)	436.11 ± 27.39	300-775	40
CUA 52 (Chittannur, Thrissur)	468.05 ± 78.57	150-675	35
CUA 53 (Guruvayur, Thrissur)	402.77 ± 107.74	50-850	45
CUA 54 (Arthat, Thrissur)	362.49 ± 12.04	200-625	50
CUA 55 (Pathakkara, Thrissur)	522.22 ± 80.93	250-850	26
CUA 56 (Muzhappilangadi,	545.83 ± 27.77	350-800	19

Kannur)			
CUA 57 (Nadal gate, Edakkad, Kannur)	588.88 ± 10.02	400-875	14
CUA 58 (Bakkalam, Kannur)	527.77 ± 21.72	375-825	25
CUA 59 (Thalangara, Kasaragod)	380.55 ± 40.10	175-650	48
CUA 60 (R.D Nagar, Kasaragod)	269.44 ± 41.53	100-450	56
CUA 61 (Anangur, Kasaragod)	352.77 ± 32.07	200-500	51
CUA 62 (Naimarmoola, Kasaragod)	508.33 ± 80.45	150-1000	29
CUA 63 (Chemnad, Kasaragod)	444.44 ± 41.53	125-600	39
CUA 64 (Perumbala, Kasaragod)	436.10 ± 62.68	150-725	41
CUA 65 (ThazheCherur, Kasaragod)	416.66 ± 96.21	75-675	43
CUA 66 (Thekkil ferry, Kasaragod)	505.55 ± 83.05	175-1050	30
CUA 67 (Mangad, Kannur)	511.11 ± 46.28	350-850	27
CUA 68 (Valapattanam, Kannur)	397.22 ± 77.27	125-525	46

The experimental material

Curcuma aeruginosa Roxb. is an underutilized species showing perennial, semierect, rhizomatous herbaceous nature. The whole plant is about 70-100 cm tall and has weak aromatic odour (Fig. 1). Rhizome is large, strongly aromatic, 5-10 cm in size, has pink tips and greyish blue or blue centres. The rhizome possesses camphoraceaous aroma and bitter taste. Depending on the nature of the soil and age of the rhizome, it shows variation in blue colour. Pseudostem is 30-35 cm tall with green sheaths. Leaves are distichous, 30-40 cm x 10-12 cm with oblong to lanceolate lamina. Leaves are characterized by a purple or reddish-brown patch along the sides on the distal half of the midrib on the upper side which fades at maturity. Inflorescence is lateral, 25-30 cm long, peduncle 12-18 cm with large coma bracts, pink to violet, lower ones streaked green. Fertile bracts are 18-20 in number; each bract subtends a circinnus of 8-10 flowers. Flowers are 4.5-5 cm long, equal to

or slightly shorter than the bracts. Calyx is 3-lobed at the apex and split on one side. Corolla tubes are pink in colour and its lobes are unequal. Labellum is yellow with a deep yellow median band. Anther is 7 mm long and yellowish green in colour. Ovary is trilocular with many ovules but fruiting is not common (Sabu 2006; Sujatha and Renuga 2013; Anonymous 2015a).

Growth requirements

Randomized block design (RBD) with 3 replications was used for the experimental programme. A crop of 68 accessions was raised using fresh healthy rhizomes. Seed rhizome fingers each of approximately 3-5 cm length and 25-30g weight were used as the planting material. The rhizomes were sown in 38 cm x 35 cm poly bags filled with garden soil, sand and cowdung in 3:1:1 ratio before the onset of south west monsoon during the first week of May 2013. Weeding was done as and when required. Plants were irrigated regularly and standard agronomic practices recommended for *Curcuma* (Sabu et al. 2011) were adopted. Two gram of N:P:K (18:18:18) was applied to each plant at the end of the first, second and third months of growth.

Observations

The plants were allowed to grow for six months and harvested simultaneously. Observations on rhizome yield were recorded immediately after harvest. The data were analyzed statistically to assess the genetic variability.

Results and Discussion

The yield data were analyzed statistically so as to assess its variability and also to identify the high yielding genotypes. Crop yield/agricultural output is the measurement of the quantity of a crop harvested per unit of land area (Anonymus 2015b). Rhizome yield per plant varied from 50g to 1875g in the case of the different accessions of *Curcuma aeruginosa* studied presently. Analysis of variance showed that this variation was statistically significant (Table 1). Among the different accessions studied, Accession No. CUA 42 showed the highest yield followed by CUA 9, CUA 43, CUA 41 and CUA 8 in that order (Table 1 & Fig. 2.) In the case of the best five accessions yield varied from 350g to 1875 g. This observation indicates the high level of variation in yield in the case of the *Curcuma aeruginosa* populations available in North Kerala thus highlighting the existence of potential genetic variability which could be exploited effectively for the commercial exploitation of the same through selection so that better planting material is made available for the organized farming of the species. Further analysis of this variation

in association with other agronomic characters will lead to the selection of superior accessions and release of superior varieties subsequently.

Similar works have been carried out in different crops by earlier workers. Jayasree et al. (2012a) and Jayasree et al. (2012b) conducted variability studies in *Curcuma amada* Roxb. and *Kaempferia galanga* L. and observed that yield per plant showed very high coefficient of variation indicating high level of variability. Radhakrishnan et al. (2004) and Radhakrishnan et al. (2005) studied the variability and performance of Cardamom genotypes and observed the importance of yield per plant and other yield related traits in cardamom improvement. Such an approach can be adopted in the case of *Curcuma aeruginosa* also so as to conduct detailed studies that may lead to useful information on the genetic structure, variability and scope for improvement in the species.

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Figure 1: (A). *Curcuma aeruginosa* in the experimental plot, (B). single plant, (C). plant with flowers and (E). inflorescence

Figure 2: Total rhizome yield per plant in the case of Accession No. CUA 42 (highest yielding) and CUA 60 (lowest yielding).

Variability in physical and yield related parameters in Coconut genotypes

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ABSTRACT: *Coconut products provide food, shelter and energy to farm households and can be made into various commercial and industrial products. Fully developed and strategically used, it would increase food production, improve nutrition, create employment opportunities, enhance equity and help conserve the environment; hence it is aptly called Kalpavriksha or tree of heaven. Much of the earlier grouping of Indian coconut germplasm has been based on morphological characteristics. The study was conducted at RARS, Pilicode, Kerala during 2013-2015 using 245 individuals representing 49 accessions. The normalized Shannon and Weaver (1949) estimates have been worked. Correlation between the characters was also studied. Highest variability among recorded traits was observed for the number of female flowers and male flowers. Since these are productivity determining factors, the great variability (Range 4.50 – 398.50, CV% 211.29% for number of female flowers, Range 11.00 – 98.00, CV% 100.28% for number of male flowers) that exists can be made use of in crossing programmes to enhance the number of female flowers. Least variability was noticed for the trait, number of leaflets per leaf (CV 10.26%). Variability of remaining characteristics ranged from 11.93 – 50.46%. The high diversity index of 0.841 indicates that high variability that exists in the germplasm maintained at RARS Pilicode. The diversity index varied from 0.695 to 0.916 with highest diversity index for the length of rachis and the least diversity index for the length of the stalk. Female flower number, the critical factor determining productivity was not associated with any of the traits under consideration indicating the importance using this character as an important selection tool. The male flowers also showed the same trend. The number of bunches on the crown had a significant positive correlation with all the traits considered under study except the number of female flowers. Our findings based on the morphological trait confirm the large diversity observed in the coconut germplasm. In our study attempts have been made to understand the diversity in the existing germplasm collections based on the morphological characters. The study has practical implications as information derived from this work would serve as complementary information for developing the core collection and other genebank management activities.*

Key words: *Variability, diversity index, Coconut*

Introduction

Coconut (*Cocos nucifera*) belongs to a monotypic genus. It is presumed that the generic name Cocos as well as the popular name coconut is derived from Spanish word *Coco,* meaning monkey face probably from the three scars on the base of the shell. Coconut belongs to the family Arecaceae (Palmae) (Heatubun et al. 2009). Coconut palm is called Kalpavriksha - tree of life - as all parts of the palm can be used to produce items of value for community. Coconut palm is one of the major perennial oil crops of the tropics providing the basis in many developing countries

for food products as well as serving industrial purposes (Jones 1991; Persely 1992). Coconut products provide food, shelter and energy to farm households and can be made into various commercial and industrial products. Fully developed and strategically used, it would increase food production, improve nutrition, create employment opportunities, enhance equity and help conserve the environment; hence it is aptly called Kalpavriksha or tree of heaven.

Narayana and John (1949) attempted the first systematic classification of Indian germplasm. Menon and Pandalai (1958) identified twelve forms of Tall and five of Dwarf coconut in India. Since then, germplasm prospectors have documented various coconut types in different agro-ecological regions in India, such as Orissa (Panda 1982), Andaman and Nicobar Islands (Balakrishnan and Nair 1979) and Lakshadweep Islands (Jacob and Krishnamoorthy 1981). There are also reports on distinct cultivars identified in a particular location, such as Spicata (Jacob 1941) and Ayiramkachi (Ramachandran et al. 1977). All the different forms of Coconut collected from different agro-ecological zones of India have been categorized by location of collection or cultivation, or by specific traits, such as small- fruited nuts (e.g. Laccadive Micro Tall), large quantity of nut water (Kappadam Tall), spikeless character with a large number of female flowers (Spicata) and Dwarfs for their distinct nut colour (e.g. green, yellow or orange-red). Thus much of the earlier grouping of Indian coconut germplasm has been based on morphological characteristics.

It is essential to understand the genetic diversity within Indian coconut cultivars in order to utilize effectively them for breeding purposes. Knowledge of relatedness among different coconut cultivars will also help in identifying cultivars and thus avoiding duplication in the continuing screening programme for diseases (Jacob et al. 1998). Knowing relationships among Indian cultivars and other major cultivars will also help when formulating future breeding strategies involving exotic cultivars.

Materials and Methods

The study was conducted at RARS, Pilicode, Kerala during 2013-2015. The phenotypic observations taken from the coconut collections maintained at coconut germplasm block, RARS, Pilicode. The 49 accessions were considered under study and 22 phenotypic characteristics were recorded from each accession in two years, average of which was considered in the study. A total of 245 individuals representing 49 accessions were used in the study. Proportion (Pi) of occurrence of each character state was used in each trait (i) and the Shannon and Weaver (1949) estimates have been worked out using the formula H'= - $\sum_{i=1}^{n}(Pi)\log 2Pi$ where n is

the total number of character states, Pi proportion of individuals in the i^{th} state of character. Calculations were performed using the example of studies in Sorghum (Grenier et al. 2001). Each H' estimate is normalized by dividing $\log_2$ n. Correlation between the characters were also studied.

Results

Highest variability among recorded traits was noted for the number of female and male flowers (Table 1). Since these are productivity determining factors, the great variability (Range 4.50 - 398.50, CV% 211.29% for number of Female flowers, Range 11.00 – 98.00, CV% 100.28% for number of Male flowers) that exists can be made use of in crossing programmes to enhance the number of female flowers. Least variability was noticed for the trait, number of leaflets per leaf (CV 10.26%). For the all other characters under study, variability ranged from 11.93 – 50.46%. The average diversity index is 0.841 indicating that high variability that exists in the germplasm maintained at RARS Pilicode (Table 2) indicating high diversity among the population. The diversity index varied from 0.695 to 0.916. Highest diversity index was observed for the length of rachis. Least diversity index was recorded for the length of the stalk among the observed traits.

Stem characteristics were found to be highly positively correlated with each other indicating selection for plant height will result in selection of plantlets with vigourous growth habit. The girth of the stem had a high bearing on leaf characteristics emphasing the implication of collar girth as the selection criteria in the nursery stage itself to have plant with desirable leaf characteristics. Female flower number, the critical factor determining productivity was not associated with any of the traits under consideration indicating the importance using this character as a specific selection tool. The number male flowers also showed the same trend. The number of bunches on the crown had a significant positive correlation with all the traits considered under study except the number of female flowers.

Table 1: Variability of traits in coconut germplasm collection at RARS Pilicode

Trait		Range	Mean	SD	CV%
Plant height		4.37 - 11.12	9.60	1.96	20.53
Girth	Upper	47.00 - 99.00	75.17	11.91	15.85
	Lower	61.50 - 138.75	108.46	23.70	21.85
Mean distance between internodes		0.90 - 8.75	5.61	2.18	38.89
Width of leaf scars		2.10 - 10.52	4.52	1.16	25.71
Height of 10 leaf scars		22.00 - 94.00	58.68	17.75	30.26
No. of green leaves		21.00 - 35.33	28.19	3.36	11.93
Petiole length		45.00 - 124.20	95.73	19.84	20.73
Petiole thickness		16.75- 30.67	26.00	3.93	15.11

Petiole width	6.00 - 13.20	10.57	1.90	18.01
Rachis length	228.25- 434.67	337.73	51.62	15.28
No. of leaf lets	80.00 - 130.00	105.77	10.85	10.26
Leaflet length	75.00 - 141.33	107.58	15.18	14.11
Leaflet width	2.80 - 6.32	4.85	0.76	15.75
Number	0.50 - 4.67	3.18	1.61	50.46
Female flower no.	4.50 - 398.50	26.28	55.54	211.29
Male flower no.	11.00– 98.00	42.51	42.62	100.28
Length of central axis	30.50 - 88.00	65.61	13.36	20.36
Length of stalk	21.25- 48.50	39.60	7.48	18.89
Stalk girth	2.25- 5.00	3.74	0.56	15.02
No. of spike	1.50 - 3.67	3.08	0.55	17.70
Bunches on the crown	6.00 - 16.67	13.06	2.97	22.75

Table 2: Shannon Weaver index of diversity in coconut germplasm collection at RARS Pilicode

Trait		SWD
Plant height		0.846
Girth	Upper	0.903
	Lower	0.883
Mean distance between internodes		0.864
Width of leaf scars		0.777
Height of 10 leaf scars		0.907
No. of green leaves		0.869
Petiole length		0.866
Petiole thickness		0.850
Petiole width		0.854
Rachis length		0.916
No. of leaf lets		0.871
Leaflet length		0.844
Leaflet width		0.867
Number		0.713
Female flower no.		0.881
Male flower no.		0.864
Length of central axis		0.861
Length of stalk		0.695
Stalk girth		0.839
No. of spike		0.760
Bunches on the crown		0.767
Average		0.841

Discussion

Our findings based on the morphological traits confirm the large diversity observed in the coconut germplasm. One of the ways of carefully selecting parents for improved traits of the offspring and expansion of crop genetic base is to understand their genetic diversity. The degree of success achieved in the genetic improvement of crops depends to a large extent on the amount of available variation as represented by the genetic diversity in the species. The available germplasm is a valuable source of parents for hybridization and subsequent development of improved materials (Ataga 2009). The primary classification of coconut is based on stature and breeding habit with two main categories: tall and dwarf (Menon and Pandalai 1958). Tall coconuts (talls) grow to a height of about 20–30 m and are allogamous, late flowering and their nuts are medium to large in size. They are hardy and thrive in a wide range of environmental conditions. Dwarf coconuts grow to a height of about 10–15m and are autogamous, early flowering, and generally produce a large number of small nuts with distinctive colour forms. Harries (1978) proposed two main types of talls: Niekafa, which evolved naturally and was disseminated by ocean currents and Nievai, which evolved as a result of selection from Niekafa under cultivation and was disseminated by man. Introgression of these two types and further selection and dissemination by man produced the wide range of varieties and pan-tropical distribution of coconut seen today (Harries, 1978). Despite the higher degree of autogamy, dwarfs cross pollinate with one another and also with talls. Assessment of the genetic diversity present within a species is a prerequisite for future sustainable breeding efforts. To date there are over 300 recorded ecotypes of coconut (Coconut Genetic Resources Network Database v. 2.2 COGENT/IPGRI), with evaluation and characterization being mainly carried out on morphological and reproductive traits (Fernando et al. 1995). In our study attempts have been made to understand the diversity in the existing germplasm collections based on the morphological characters. The present study has practical implications as information derived from this work would serve as complementary information for developing the core collection and other Genebank management activities.

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Table 3: Correlation between the biometric characters of Coconut germplasm at RARS, Pilicode

		Plant height	Stem girth		Mean distance between internodes	Width of leaf scars	Height of 10 leaf scars	No. of green leaves	Petiole length	Petiole thickness	Petiole width	Rachis length	No. of leaf lets	Leaflet length	Leaflet Width	Number of inflorescence	Female flower no	Male flower no	Length of central axis	Length of stalk	Stalk girth	No. of spikes	Bunches on the crown
			Upper	Lower																			
Plant height		1.00	0.23	0.59**	0.55**	0.16	0.62**	0.19	0.20	0.32*	0.30*	0.20	0.32*	0.18	0.19	-0.20	-0.10	0.21	0.28	0.33*	0.28	0.16	0.27
Stem morphology	Upper		1.00	0.75**	0.72**	0.26	0.69**	0.62**	0.77**	0.84**	0.77**	0.76**	0.67**	0.69**	0.55**	0.50**	-0.09	0.02	0.65**	0.61**	0.47**	0.49**	0.56**
girth	Lower			1.00	0.84**	0.41**	0.82**	0.47**	0.63**	0.72**	0.70**	0.64**	0.73**	0.55**	0.65**	0.25	-0.09	0.10	0.49**	0.52**	0.29*	0.51**	0.53**
Mean distance between internodes					1.00	0.37**	0.95**	0.57**	0.70**	0.76**	0.77**	0.67**	0.73**	0.61**	0.58**	0.20	-0.16	0.16	0.57**	0.52**	0.34*	0.45**	0.64**
Width of leaf scars						1.00	0.42**	0.00	0.25	0.30*	0.49**	0.28	0.29*	0.31*	0.37**	0.21	0.09	0.24	0.32*	0.30*	-0.07	0.32*	0.24
Height of 10 leaf scars							1.00	0.52**	0.63**	0.73**	0.77**	0.61**	0.73**	0.53**	0.51**	0.24	-0.08	0.20	0.53**	0.54**	0.30	0.48**	0.58**
No. of green leaves								1.00	0.69**	0.62**	0.57**	0.57**	0.59**	0.45**	0.27	0.28	0.02	0.19	0.55**	0.57**	0.54**	0.35*	0.61**
Petiole length									1.00	0.84**	0.76**	0.82**	0.68**	0.68**	0.53**	0.40**	-0.01	0.19	0.69**	0.74**	0.50**	0.32*	0.66**
Petiole thickness										1.00	0.88**	0.80**	0.70**	0.63**	0.50**	0.45**	-0.07	0.05	0.68**	0.74**	0.57**	0.47**	0.62**
Petiole width											1.00	0.75**	0.75**	0.64**	0.52**	0.40**	-0.07	0.18	0.70**	0.71**	0.47**	0.44**	0.62**
Rachis length												1.00	0.77**	0.79**	0.67**	0.50**	-0.03	0.27	0.64**	0.70**	0.49**	0.35*	0.61**
No. of leaf lets													1.00	0.58**	0.62**	0.43**	0.05	0.29	0.59**	0.58**	0.40**	0.45**	0.58**
Leaflet length														1.00	0.66**	0.31*	-0.04	0.17	0.62**	0.50**	0.46**	0.27	0.50**
Leaflet width															1.00	0.42**	0.04	0.20	0.48**	0.42**	0.25	0.42**	0.39**
Number of inflorescence																1.00	0.14	0.17	0.33*	0.34*	0.18	0.55**	0.35*
Female flower no																	1.00	0.11	0.01	0.06	0.02	0.13	0.02
Male flower no																		1.00	0.33*	0.36*	0.11	-0.07	0.36*
Length of central axis																			1.00	0.79**	0.67**	0.36*	0.60**
Length of stalk																				1.00	0.52**	0.28	0.60
Stalk girth																					1.00	0.28	0.30*
No. of spikes																						1.00	0.37**
Bunches on the crown																							1.00

Data on grade percent & bean parameters of cxr & s.274, coffee at higher & lower elevations of Kerala

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ABSTRACT: *Coffee is one of the popular beverages in South India and its unique aroma and flavours are responsible for its popularity. In the present study, 26 different coffee samples were collected from the three coffee growing districts across Kerala such as Wayanad, Palakkad and Idukki and the data on the grade percent & bean parameters were studied. Out of twenty six samples eleven samples are from variety S.274 and the remaining fifteen samples are from the variety CxR and are grown at different elevations ranging from 710-1350m above the sea level. The S.274 and CxR accessions are categorized into two, based on elevation such as thousand meters and above as higher and those below thousand meters as lower elevation. The higher elevation improves coffee bean quality by offering a moister surrounding, coming from the cloudy attributes that the atmosphere provides at these elevated levels. This also minimizes the need for excessive sunlight rays that could potentially harm the beans. Because the air is thinner, coffee matures more slowly, which can be a major element in the coffee's fine quality and superior taste. Present study also revealed higher 'A' grade percent when compared to the Sln.IR (S.274) selections. Observations indicated that the coffee Sln.IR (S.274) and Sln.3R (CxR) has recorded higher bean grade, bean volume, bean density and cup quality in higher elevation.*

Key words: *Coffee, CxR, S.274, Elevation, Grade percent, Bean parameters.*

Introduction

Coffee is one of the most important agricultural export product and is grown almost exclusively in developing countries. Many of these countries depend heavily on coffee for their foreign exchange (Graff 1986). Coffee is one of the popular beverages in south India and its unique aroma and flavors are responsible for its popularity (Anonymous 2004). Coffee is a well-loved drink and an important part of the dietary plan of many civilized people. Several characters of coffee make it good to man. Because of its delightful fragrance rich golden brown colour and taste coffee has come to fill a most important place as beverage.

Location with well distributed rain fall and good stand of evergreen trees are ideal for coffee cultivation. Coffee requires a humid climate with a well distributed rain above 200cm and a temperature ranging from 20^{o}-40°C. In choosing the site for coffee plantation, due consideration should be given to altitude, aspects, rainfall, exposure to winds and approach. A perennial source of water supply is an essential requirement. Soil rich in humus and with gentle slopes providing good drainage are to be preferred. It cannot withstand water-logging during the monsoon months.

Objectives of breeding program in Coffee are (1) high yield and improved vigour (2) adaptation to local environment (3) drought tolerance (4) short inter nodes (5) improved cup quality (6) less caffeine content, etc. Coffee production economics depend on the effort of controlling costs, increasing productivity and improving beverage quality (Sera et al. 2000). Quality comprises the totality of all characteristics that determine the acceptability of product by the consumers. Quality in coffee is a summative index of raw, roast and liquor characteristics of beans. The assessment of raw beans is made based on the parameters like moisture percentage, colour, size, shape, odour of sample beans (Jones 1964). Presence or absence of defective beans is also considered as one of the important parameters in the raw quality assessment of the coffee beans (Wormer and Njuguna 1966). Attempts have been made by different scientists to determine the factors affecting quality of coffee. According to Gialluly (1959), the quality of green coffee is affected by two types of factors, (1) genetic (2) environmental and physiological factors.

Materials and Methods

The experiment is conducted at Regional Coffee Research Station Chundale, Wayanad, Kerala. In order to study 'bean quality at different elevations', twenty six different samples are collected mainly from the three districts across Kerala such as Wayanad, Palakkad and Idukki. The quality study in bean grades of these districts is important because the zones among these three districts posses different altitude, climate, manuring practices, processing techniques, etc. depend on these overall criteria's the bean grades may be different in these samples. Among twenty six quality samples, two robusta accessions were selected for the present study. Robusta accessions were selected for the present study, from different zones of Kerala are Sln.1R (S.274) and Sln.3R (CxR). Sln.1R (S.274) is a robusta selection released by CCRI and is a high yielding variety. Beans are medium and round sized. Liquor is rich and the cup quality is neutral. Sln.3R (CxR) is a hybrid between *Coffea congenesis* X *Coffea canephora.* F_1 progenies thus established were back crossed with *Coffea canephora* and CxR were evolved. Liqour is of neutral with light to fair acidity. Cup rating is fair to good.

Preparation of sample

Wet processed and sun dried 26 coffee samples from different zones of Kerala were collected to assess the variability in bean grades and cup quality by considering factors such as Bean size and shape, Bean volume, Bean density and Cup testing. The green coffee beans of 26 samples were processed by following procedure to get quality coffee and to assess the quality of coffee and it involves the steps like harvesting and drying, hulling, grading and sorting, etc.

Results

In the present study variability of coffee bean grades and cup quality was observed in Robusta selections at different elevations. Sln.IR (S.274) and Sln.3R (CxR) are the two main Robusta selections growing all over Kerala. Both these Robusta selections show considerable variation in their bean grades and cup quality. Out of twenty six samples eleven samples are from variety S.274 and the remaining fifteen samples are from the variety CxR and are grown at different elevations ranging from 710-1350m above the sea level. The S.274 and CxR accessions are categorized into two, based on elevation such as thousand meters and above as higher and those below thousand meters as lower elevation.

At higher elevation the percentage of 'A' grade bean in S.274 ranged from 50.16% to 51.76% and in lower elevation ranged from 21.64% to 69.73%. In CxR cultivated at higher elevation the percentage of 'A' grade ranged from 45.29% to 57.50% whereas at lower elevation it ranged from 40.59% to 60.48 %. When mean value was computed the data indicated that Sln.IR (S.274) had 41.89 % 'A' grade and Sln.3R (CxR) had 50.87 % of 'A' grade at lower elevation. At higher elevation the mean percentage of 'A' grade was 50.96% in Sln.IR (S.274) and 51.09 % of 'A' grade in Sln.3R (CxR). This clearly indicates that the percentage of 'A' grade in both S.274 and CxR was high at higher elevation and low at lower elevation.

Bean volume of 100 beans of S.274 at higher elevation ranged from 14 cm^3 to 14.5 cm^3 whereas at lower elevation it ranged from 13 cm^3 to 15 cm^3. In CxR cultivated at higher elevation, bean volume ranged from 14 cm^3 to 17 cm^3 whereas at lower elevation it ranged from 13 cm^3 to 16 cm^3. It shows that Sln.1R (S.274) and Sln.3R (CxR) cultivated at higher elevation recorded higher bean volume of 14.25 cm^3 and 18.17 cm^3 respectively. At lower elevation Sln.IR and Sln.3R recorded low bean volume of 13.89 cm^3 and 14.18 cm^3 respectively. This data revealed that the bean volume in both S.274 and CxR was high at higher elevation and low at lower elevation.

Weight of 100 beans Sln.IR (S.274) and Sln.3R (CxR) also were recorded. At higher elevation weight of 100 beans of S. 274 ranged from 17gm to 17.5 gm, whereas at lower elevation it ranged from 15.5 gm to 18gm. In CxR, at higher elevation weight of 100 beans ranged from 17gm to 18.5 gm and at lower elevation it ranged from 16 gm to 17.5 gm. Mean weight of 100 beans at higher elevation in S.274 was 17.25 gm and in CxR was 18.17 gm, whereas at lower elevation mean weight of 100 beans in S.274 was 16.28 gm and in CXR it was 16.61 gm. In both the varieties mean weight of beans was higher at higher elevation compared to lower elevation.

At higher elevation the density of S.274 bean found to be 1.2 gm/cm^3 whereas in lower elevation ranged from 1.07 gm/cm^3 to1.23gm/cm^3. In CxR bean density at higher elevation ranged from 1.09gm/cm^3 to 1.34 gm/cm^3 and in lower elevation it ranged from 1.05gm/cm^3 to 1.32gm/cm^3. When mean was computed the data indicated that in both S.274 and CxR the bean density was higher in higher elevation (1.21gm/cm^3 and 1.22 gm/cm^3 respectively) compared to the same variety cultivated at lower elevation (1.17 gm/cm^3 and 1.18gm/cm^3 respectively).

Cup tasting is a very important and most reliable method of quality, evaluation by a cup taster which can reveal the true value of coffee in terms of strength, acidity and aroma. Cup quality rating of S.274 at higher and lower elevation ranged from above average to average. Whereas in CxR, the cup quality rating at higher elevation ranged from good to average and in lower elevation it ranged from above average to average. Data indicates that the cup quality rating of two samples of CxR was found to be good at higher elevation.

Discussion

The results obtained clearly indicates that Robusta selections Sln.IR (S.274) and Sln. 3R (CxR) cultivated at higher elevation has higher 'A' grade percent, higher bean volume, higher bean weight, higher bean density compared to same selection cultivated at lower region. Data indicate that the environment factor like elevation plays a major role in determining the quality of coffee. Among the two robusta selections, CxR selection is the most preferred variety than the S.274 selection due to its superior quality. In coffee generally higher percent of 'A' grade bean and lower percent of 'C' grade, BBB grade and PB has higher price in the market. Pea berry alone is a preferred grade for coffee lovers. It is observed that the environment factor like elevation greatly influences the quality of coffee because there is less oxygen at higher altitude, takes longer to mature than lower grown plants. Higher grown coffee beans usually have a higher density than the lower grown beans, which enhances the flavour profile. The higher elevation improves coffee bean quality by offering a moister surrounding, coming from the cloudy attributes that the atmosphere provides at these elevated levels. This also minimizes the need for excessive sunlight rays that could potentially harm the beans. Because the air is thinner, coffee matures more slowly, which can be a major element in the coffee's fine quality and superior taste (Anonymous 2007).

Present study also revealed that Sln.3R (CxR) is comparatively better selection which has the higher 'A' grade percent when compared to the Sln.IR (S.274) selections. Observations indicated that the coffee Sln.IR (S.274) and Sln.3R (CxR) has recorded higher bean grade, bean volume, bean density and cup quality in higher elevation. Coffee grown in higher altitudes is superior in quality than the coffee

grown in lower altitudes. This may be due to the delayed ripening of berries at higher elevation when compared to lower elevations. The present results are in confirmity with the earlier studies conducted at other coffee growing region.

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Table1: C x R at higher elevations

Name of the estate	Elevation (metres)	Variety	Pb %	A%	B%	C%	BBB %	Bean parameters: Bean volume	Bean parameters: Wt of 100 beans	Bean parameters: Bean density
Plakkad estate, Vandiperiyar	1001	C x R	9.93	46.47	31.31	5.28	7.01	15	17	1.13
AVT, Manalaroo estate, Nelliampathy	1000	C x R	8.24	57.50	25.07	6.51	10.23	15	18	1.2
Poabs plantations, Nelliampathy	1100	C x R, CHERRY	6.41	49.76	24.67	11.42	22.11	16	17.5	1.09
Poabs plantations, Nelliampathy	1100	C x R, PARCHMENT	8.89	54.30	33.8	12.22	10.47	16	17	1.06
Gudampara estate, Santhanpara (P.O), Adimali	1000	C x R, ROB CHERRY	13.73	45.29	30.32	12.72	11.61	14	15.5	1.11
Mannarghat coffee plantation, Silent Valley	1100	C x R	9.97	52.44	28.9	9.94	8.92	16	17.5	1.09
Mean			9.53	50.96	29.01	9.68	11.73	15.33	17.08	1.22

Table 2: CxR at Lower elevations

Name of the estate	Elevation (metres)	Variety	Bean parameters							
			Pb%	A%	B%	C%	BBB %	Bean volume	Wt of 100 beans	Bean density
Saroja estate, Panamaram	850	C x R	14.85	59.29	7.66	1.6	16.59	16	19.5	1.2
Jayan , Edakunna estate , Vaduvanchal	920	C x R	19.11	60.48	13.69	1.08	5.63	16	21	1.31
Vincent's estate, Padichira	750	C x R	9.51	56.89	19.74	3.98	9.87	13	16	1.23
V.B . Vijayakumar , Harshanilayam,Venniyodu	800	C x R	14.18	50.09	12.48	1.74	21.51	17	20	1.18
Malabar estate , Chappath, Idukki	750	C x R	13.15	40.59	18.46	6.36	21.44	15	17.5	1.17
Malabar estate , Chappath, Idukki	750	C x R PARCHMENT	14.87	54.32	10.98	1.89	17.93	15	18	1.2
K.V. Sebastian , Karimpanal , Idukki	900	C x R	12.94	59.79	9.63	2.96	14.68	19	20	1.05
A.C. Raveendran , Naduparampath , Meenangadi	850	C x R	4.97	41.74	38.19	6.89	8.2	16	18	1.13
Alathur estate , Kartikulam	850	C x R	12.44	54.67	17.68	5.24	9.97	16	17.5	1.09
Mean			12.89	53.1	16.50	3.53	13.98	15.89	18.61	1.18

Table 3: S.274 at higher elevations

Name of the estate	Elevation (metres)	Variety	Bean parameters							
			Pb%	A%	B%	C%	BBB %	Bean volume	Wt of 100 beans	Bean density
TAS Plantation, Meppadi	1350	S.274	4.02	51.76	30.13	18.84	20.25	14.5	17.5	1.21
AVT , Manalroo estate , Nelliampathy	1000	S.274	11.76	50.16	18.89	4.76	14.42	14	17	1.21
Mean			7.89	50.96	24.51	11.8	17.34	14.25	17.25	1.21

Table 4: S.274 at lower elevation

Name of the estate	Elevation (metres)	Variety	Pb %	A%	B%	C%	BBB%	Bean parameters		
								Bean volume	Wt of 100 beans	Bean density
Malabar estate, Chappath, Idukki	750	S.274	8.05	36.85	30.82	10.42	13.86	14	16.5	1.18
Mohanan, Parappurath , Muniyara	825	S.274 ROB CHERRY	18.77	44.36	19.54	3.89	13.45	15	16.5	1.1
Vijayakumar, Eranallur	850	S.274	19.49	21.64	24.29	11.5	23.08	16	16.5	1.03
K.V. Balakrishnan, Meenangadi	870	S.274	11.27	45.29	25.58	8.27	9.57	15	18	1.2
Mariamma George, Pallikkal estate, Ambalavayal	950	S.274	1.82	69.73	20.18	1.22	7.05	16	18.5	1.16
Alathur estate , Kartikulam	850	S.274	12.92	30.37	26.43	10.65	19.63	13	15.5	1.19
Mallappas estate, Perikallur	750	S.274	11.76	50.16	18.89	4.76	14.42	15	17.5	1.17
Sebastian, Karimpanal, Idukki	900	S.274	8.06	44.19	29.77	9.68	8.29	13	15.5	1.19
CDF, Mananthavady	710	S.274	7.01	34.45	34.01	12.24	12.28	13	16.5	1.27
Mean			11.02	41.89	25.50	8.07	13.51	14.44	16.78	1.17

Table 5: Data on mean grade percent and bean parameters of Robusta selection at higher and lower elevation

Variety	Elevation	Grade percent					Bean parameters		
		PB%	A %	B %	C %	BBB %	100 bean volume (cm^3)	Wt. of 100 beans (g)	Bean density (g/cm^3)
S.274	High elevation	7.89	50.96	24.51	11.8	17.34	14.25	17.25	1.21
	Lower elevation	11.02	41.89	25.5	8.07	13.51	13.89	16.28	1.17
CXR	High elevation	9.53	51.09	29.02	9.68	11.73	14.93	18.12	1.22
	Lower elevation	12.89	50.87	16.5	3.53	13.98	14.18	16.61	1.18

Performance of certain new hybrid clones of *Hevea brasiliensis* in preliminary yield trails

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ABSTRACT: *The performance of 21 hybrid clones of rubber, Hevea brasiliensis evolved from 1986 hand pollination programme, was evaluated in two small-scale trials over a period of 18 years after planting. RRII 105 was the check clone in both trials. RRIM 600 was included as a second check clone in Trial 2. Annual growth during immature and mature phase of tapping, dry rubber yield over a period of 10 years and secondary attributes including bark structural traits, incidence of diseases and bole volume were recorded. In trial 1, mean yield of clones ranged from 17.7 to 79.6 gram tree^{-1}tap^{-1}. The clone 86/772 recorded the highest dry rubber yield in the BO-1panel (78.6 gramtree $^{-1}$ tap $^{-1}$) as well as over 10 years of tapping (79.6gram tree $^{-1}$ tap $^{-1}$) which was significantly superior to the yield of RRII 105 (40.8 and 50gram tree^{-1}tap^{-1} respectively). Clone 86/380 was the second best performer with 56 gram tree^{-1}tap^{-1}. Girth at opening ranged from 37.7 (86/789) to 62cm (86/772). In trial 2, Clone 86/575 recorded significantly superior yield to RRII 105 and comparable yield with RRIM 600. The range in yield was 23.2 (86/894) to 64 gram tree^{-1}tap^{-1}(86/575) and girth at opening ranged from 37.8 (86/916) to 58.7cm (86/575). Bark structural traits showed significant clonal variation in both the trials. Clones 86/772 and 86/756 registered superior clear bole volume of 0.2m^3 as against 0.1m^3 for RRII 105. Based on overall performance, four clones viz. 86/772, and 86/575 with high yield and 86/380 and 86/ 756 with moderate yield combining desirable secondary attributes could be identified as clones in the pipeline for the final phase of evaluation.*

Key words: *Hevea brasiliensis, rubber yield, clones.*

Introduction

Genetic improvement in the para rubber tree *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. is mainly through hybridization and clonal selection. Parent clones for hybridization are selected so as to combine high yield with desirable secondary characters like high vigour, smooth and thick bark, high number of latex vessel rows, good bark renewal, high growth rate and tolerance to diseases and environmental stresses (Varghese and Mydin 2000). Indigenous development of the high yielding hybrid clone RRII 105 (Nair and George 1969) largely contributed to the success story of rubber in the country in terms of production and productivity. The adoption of recent hybrids of RRII 400 series clones (Licy et al. 2003) is on the increase. Several newer clones in the pipeline are under various stages of evaluation (Mydin 2014). Conventionally, the development and release of a clone, starting from nursery selection passes through 3 stages of field evaluation i.e. the small scale (SST), large scale (LST) and onfarm trials (OFTs). The present study reports the long term growth and yield performance of a set of 21 clones

derived from crosses involving 14 parental clones in two small scale trials with the objective of estimating the extent of genetic variability for yield and major yield components and to identify clones showing high yield and desirable secondary attributes in comparison with popular check clones.

Materials and Methods

A total of 1394 seedlings belonging to 56 cross combinations were obtained in the 1986 hybridisation programme. Based on test tap yield in the nursery, 189 and 131 selections were cloned and evaluated in SSTs 1989 and 1990 respectively. The final set of 21 clones was laid out in two SSTs in 1992 at the Central Experiment Station of RRII at Chethackal, Ranni in Central Kerala. These clones were derived from crosses involving four indigenous clones *viz*. RRII 105, RRII 118 and RRII 203 and RRII 208 and 11exotic clones *viz*. PR 107, IAN 45-873, PB 5/51, PB 86, PB 217, PB 242, PB 235, RRIM 612, RRIM 600, Tjir 1 and Gl 1. Details of clones and their parentage are given in Table 1.

A randomized block design with three replications and plot size of four (trial 1) and three trees (trial 2) was adopted for planting with a spacing of 4.9 x 4.9m. The trees were opened for tapping during the eighth year. Tapping system followed was S/2 d3 6d/7. Yield recording was done by cup coagulation and data over 10 years was collected. Girth of the trees was recorded annually from the third year onwards and used to determine the girth increment rate during pre-tapping and tapping phases. Bark samples were collected at the time of opening (virgin bark) and from the renewed panel, at 150 cm height. Bark thickness and number of latex vessel rows was determined under a bright field microscope. Number of trees showing incidence of powdery mildew and pink disease was noted. Clonal variability for all the traits was statistically analysed. Clear bole volume of the high yielding clones was determined at 22 years following Chathurvedi and Khanna (1982). In order to assess the overall performance of clones, a performance index was worked out based on eight characters following Mydin et al. (1996).

Table 1: Hybrid clones and their parentage

Clone	Trial 1 Parentage	Clone	Trial 2
86/337	RRII 105 x PR 107		Parentage
86/356	IAN 45-873 x RRII 105	86/264	RRIM 600 x PB 235
86/380	RRII 105 x PB 5/51	86/575	RRIM 600 x PB 235
86/424	RRII 105 x RRII 118	86/592	RRII 105 x PB 242
86/450	RRII 105 x PB 217	86/618	RRIM 600 x IAN 45-873
86/459	RRIM 600 x Gl1	86/756	IAN 45-873 x RRIM 600
86/583	RRII 203 x RRII 105	86/368	RRII 105 x PB 5/51

86/761	RRII 105 x PB 86	86/894	PB 5/51 x RRII 203
86/764	RRII 105 x PB 86	86/916	RRII 105 x RRII 208
86/772	RRII 105 x IAN 45-873	RRIM 600	Tjir 1 x PB 86
6/779	PB 5/51 x RRIM 612	RRII 105	Tjir 1 x Gl1
86/789	PB 242 x RRII 105		
86/948	PB 242 x RRIM 600		
RRII 105	Tjir 1 x Gl1		
	Country of origin of parents		
RRII	Rubber Research Institute of India		
IAN	Institute Agronomico du Norte, Brazil		
PR	Profestation voorRubber, Indonesia		
PB	Prang Besar, Malaysia		
RRIM	Rubber Research Institute of Malaysia		
Tjir1	Tjirandji, Indonesia		
Gl 1	Glenshiel, Malaysia		

Results

Yield of clones

The performance of 21 hybrid clones in respect of yield is presented in Table 2. Highly significant clonal variation was observed in panel wise yield as well as over 10 years of tapping. In trial 1, mean yield over six years in the BO- 1 panel revealed three clones viz., 86/772, 86/380 and 86/424 to be significantly superior to the check clone. The hybrid clone 86/772 was the highest yielder with 78.6 gram tree^{-1}tap^{-1}. The control clone recorded 40.8 gram tree^{-1}tap^{-1}. In the BO-2 panel 86/772 and 86/380 showed a rising trend with 80.9 gram tree^{-1}tap^{-1} and 67.5 gram tree^{-1}tap^{-1} respectively whereas 86/424 showed a declining trend. RRII 105 also recorded a rising trend with 64 gram tree^{-1}tap^{-1}. Mean yield over 10 years of tapping ranged from 29.5 (86/789) to 79.6 gram tree^{-1}tap^{-1} (86/772). The yield of 86/772 was significantly superior to that of RRII 105 which recorded 50.4 gram tree^{-1}tap^{-1}. 86/380 was the second best clone with 56 gram tree^{-1}tap^{-1}. The average yield of clones in trial 2 was less than that in trial 1. In the BO-1 panel, among the check clones RRIM 600 was the top yielder with 51.5 gram tree^{-1}tap^{-1} compared to 35 gram tree^{-1}tap^{-1} for RRII 105. The yield of the best clone 86/575 was better than RRIM 600 with 55.3 gram tree^{-1}tap^{-1} and significantly superior to that of RRII 105.86/756 was the second best with 41.2 gram tree^{-1}tap^{-1}. These two clones showed a rising yield trend in the BO-2 panel also with 76.2 and 56.5 gram tree^{-1}tap^{-1} respectively. Over 10 years of tapping, mean yield of the top ranking clone 86/575 (64 gram tree^{-1}tap^{-1}) was comparable to that of RRIM 600 (62 gram tree^{-1}tap^{-1}) and significantly superior to that of RRII 105 (40 gram tree^{-1}tap^{-1}). The hybrid clone 86/756 recorded 47 gram tree^{-1}tap^{-1}.

Table 2: Dry rubber yield of clones in different panels

Trial 1 Mean yield (gramtree $^{-1}$ tap $^{-1}$)				Trail 2 Mean yield (gramtree $^{-1}$ tap $^{-1}$)			
Clone	BO -1 (over 6 years)	BO-2 (over 4 years)	Over 10 years	**Clone**	BO -1 (over 6 years)	BO-2 (over 4 years)	Over 10 years
86/337	15.5	21.0	17.7	86/264	20.9	29.6	24.4
86/356	30.5	59.2	42.0	86/575	55.3	76.2	63.7
86/380	48.0	67.5	55.9	86/592	27.5	37.3	31.4
86/424	47.3	42.8	48.7	86/618	33.2	34.5	33.7
86/450	36.4	54.4	43.6	86/756	41.2	56.5	47.3
86/459	28.7	32.6	30.3	86/368	28.8	54.2	38.9
86/583	29.2	31.3	30.1	86/894	20.5	26.5	23.2
86/761	29.0	44.0	35.0	86/916	18.5	36.9	25.3
86/764	31.2	32.7	31.8	RRIM 600	51.5	77	62
86/772	78.6	80.9	79.6	RRII 105	35	45	40
86/779	32.1	35.5	33.5	**CD**	17.6	28	21
86/789	33.4	21.8	29.5	* **(P <0.05)**			
86/948	33.2	60.3	44.0				
RRII 105	40.9	64.4	50.4				
CD*	13.9	18.8	13.4				

Growth parameters

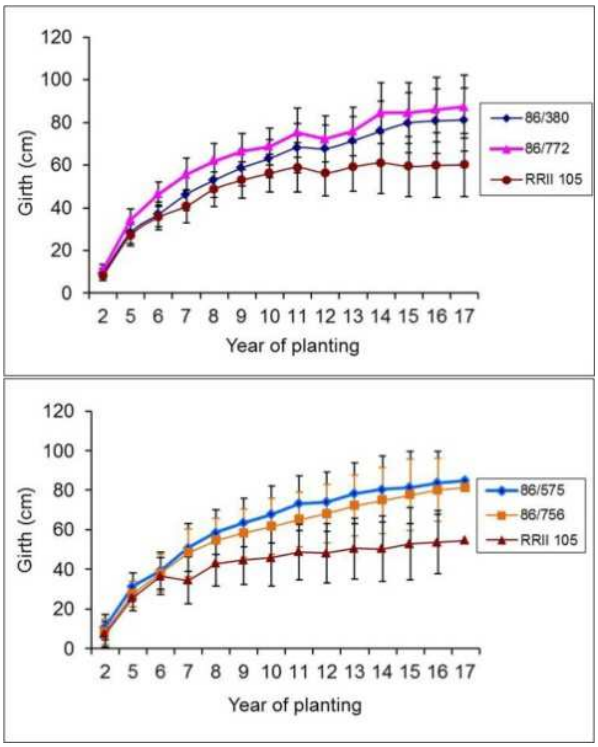
The growth performance of clones from both trials is presented in Table 3. In trial1, mean girth of the clones ranged from 37.7 (86/789) to 62 (86/772) cm. Girth at opening of 86/772 was significantly superior to that of RRII 105. The clones showed varied girth incrementrate in the pre tapping and tapping phases. At immaturity, maximum girth increment rate was exhibited by 86/772 (8.5 cm per year) followed by 86/948 (7.9 cm per year). Clone 86/948 also recorded the highest girth increment rate on tapping (4.4 cm per year). Girth increment rate under tapping of all the clones except 86/459 and 86/583 was better than the control. In trial 2, mean growth performance of clones was relatively poor compared to trial 1. The highest girth at opening was recorded by 86/575 (58.7 cm). The check clone RRIM 600 exhibited better growth (54 cm) than RRII 105. Clones 86/756 and 86/368 showed comparable girth with RRIM 600. During the immature phase, clones 86/575, 86/756 and 86/368 recorded a girth increment rate of more than 7 cm per year. The check clones recorded 7mm (RRIM 600) and 6 mm (RRII 105) increment per year. The girth increment rate on tapping also showed similar trend and the above clones

recorded 3mm girth increment rate on par with RRIM 600. Girth increment rate of RRII 105 on tapping was 1.3 mm. Growth curve plotted for the high yielding clones 86/772 and 86/380 in trial 1 and 86/575 and 86/756 in trial 2 showed consistently superior growth to RRII 105 during the entire study period (Fig. 1).

Table 3: Growth characters of clones

Trial 1				Trial 2			
Clone	Girth at opening (cm)	Girth increment rate/yr (cm)		Clone	Girth at opening (cm)	Girth increment rate/ yr (cm)	
		At immaturity	On tapping			At immaturity	On tapping
86/337	42.0	5.6	2.0	86/264	47.1	6.3	1.3
86/356	43.2	6.0	4.0	86/575	58.7	7.9	3
86/380	52.9	7.2	3.1	86/592	47.0	6.2	1.5
86/424	53.2	7.1	2.0	86/618	46.8	6.3	2.3
86/450	53.3	7.0	1.9	86/756	54.5	7.6	3
86/459	43.5	5.8	1.3	86/368	55.8	7.8	3.0
86/583	50.5	7.1	1.3	86/894	39.8	5.2	1.9
86/761	46.8	6.8	2.4	86/916	37.8	4.9	2.7
86/764	45.5	6.1	1.6	RRIM 600	54	6.9	3.1
86/772	62	8.5	2.8	RRII 105	48	5.9	1.2
86/779	51.7	7.3	2.5	**CD**	11.3	1.8	-
86/789	37.7	5.5	2.3				
86/948	57.1	7.9	4.3				
RRII 105	48.7	6.7	1.2				
CD	8.6	1.3	1.3				

Figure 1: Growth trend of selected high yielding clones**.** Vertical bars indicate CD values.



Bark characters

Bark thickness (BT) and number of latex vessel rows (LVR) in the clones are given in Table 4. Significant clonal variation was observed for both the traits in both the trials. In trial 1, the virgin bark thickness, ranged from 5-7 mm and LVR of the clones ranged from 5.7 to 12.6. Three clones recorded significantly superior LVR in the virgin bark than the check clone. Renewed bark thickness varied from 6.3 to 9.7mm and number of latex vessel rows in the renewed bark ranged from 18 to 31. The bark anatomical features of the top yielding clones 86/772 and 86/380 was comparable to RRII 105 which recorded high values for these traits. In trial 2, in the virgin bark, clones 86/575 and 86/756 showed the highest bark thickness above 6mm. Maximum LVR was observed in 86/618 (12). Renewed BT ranged from 6.1mm to 8.4mm and LVR in the renewed bark from 8.6 to 29.3. Among the controls, BT and LVR of RRII 105 were better than RRIM 600 in the virgin bark and vice versa in the renewed bark. The top yielding clones 86/575 and 86/756 showed more number of LVR in the renewed bark than the controls.

Table 4: Structural features of clones

Clone	Trial 1				Clone	Trail 2			
	Virgin bark		Renewed bark			Virgin bark		Renewed bark	
	BT†(mm)	LVR #	BT (mm)	LVR		BT (mm)	LVR	BT (mm)	LVR
86/337	4.9	6.8	6.9	18.4	86/264	5.8	6.7	6.1	16.0
86/356	6.0	5.7	9.1	18.6	86/575	6.0	7.1	8.5	29.3
86/380	6.1	8.2	8.1	28.4	86/592	5.3	6.7	6.4	17.0
86/424	5.3	8.4	7.7	23.6	86/618	5.7	12	6.5	20.8
86/450	5.8	12.6	6.3	21.9	86/756	6.3	6	7	23
86/459	5.5	9.9	6.3	23.5	86/368	4.8	4.6	6.7	16.1
86/583	6.3	7.5	7.5	20.5	86/894	5.7	6.6	6.8	16.9
86/761	4.8	7.3	8.0	22.8	86/916	5.6	7.7	4.8	8.6
					RRIM		6.8		
86/764	5.9	10.8	7.4	24.1	600	5.3		8.1	22.6
					RRII	6.1	8.8		
86/772	6.2	6.8	9.7	30.4	105			6.5	20
86/779	6.9	9.2	7.6	26.6	**CD**	0.7	1.8	1.8	9.3
					†bark thickness # number of latex				
86/789	-	-	7.4	26	vessel rows				
86/948	5.8	10.6	8.8	29.9					
RRII 105	6.0	6.9	8.4	30.9					
CD	0.6	2.1	1.8	6.5					

With respect to disease incidence, 86/772 and 86/756 showed the lowest incidence compared to the other clones. These two clones also registered superior clear bole volume of 0.2m^3 as against 0.1m^3 for RRII 105. The bole volume of the other two clones was comparable to that of RRII 105. The performance index of clones in trial 1 ranged from 23 to 83 (Table 5). The two high yielding selections 86/772 and 86/380 were in the 2nd and 4th position respectively, whereas RRII 105 occupied the 8th position. In trial 2, the index values were low, and ranged from 40 to 54. The clones 86/756 and 86/575 topped the ranking, against 4th and 5th position for the controls.

Discussion

Highly significant clonal variation for dry rubber yield, growth and girth increment rate, thickness of virgin and renewed bark and number of latex vessel rows in virgin and renewed bark was observed in the present study. Significant clonal variations for the above mentioned traits have been reported earlier (Licy et al.

2003). The variability for secondary traits could be exploited for component level selection of clones apart from identifying promising clones for rubber yield.

Growth is a major criterion in deciding the yielding capacity of a clone by way of length of the tapping panel. According to Simmonds (1989), yield and vigour in this crop are hardly separable. Girth increment rate in the initial years determine the tappability of the trees and early tappability combined with precocious yield could be ideal. Maintaining the girth increment rate over long years of exploitation is needed to sustain high yield levels (Mydin et al. 2007). On opening the trees for tapping, growth rate tends to decline due to partitioning of photosynthates (Templeton 1969). In the present study, the clones with high rubber yield combined high growth vigour in the immature as well as mature phase which implies the capacity of these clones to come to tapping phase early and also maintain the growth rate under tapping stress.

The laticifer system in *Hevea* is not only the region of storage of latex which is released during tapping but also the site of the final stages of rubber biosynthesis and hence the structure and orientation of laticifers has a direct bearing on the productivity of rubber (Gomez 1982). Bark thickness is a clonal character showing association with number of latex vessel rows and influencing the latex yield of different clones (Premakumari et al. 1985). In general, high correlation of girth with yield and bark thickness has been noticed in high yielding clones during early selection (Lavorintic et al. 1990). Results from the present study are in conformity with these findings.

Two high yielding hybrid clones viz., 86/772, 86/575, with significantly superior yield and two moderate yielders 86/380 and 86/ 756 with comparable yield to RRII 105 were identified from the study. Among the check clones, the growth and yield performance of RRII 105 was better in trial 1 whereas RRIM 600 performed better than RRII 105 in trial 2. This could be presumably due to the slope aspect, since trial 2 was laid out on top of the slope were soil depth was relatively low. In such areas RRIM 600 is a better performer than RRII 105. The parents of 21 hybrid clones tested were of diverse origin. The clone 86/772 (RRII 105 x IAN 45-873) emerged as the most promising high yielder combining high growth vigour, timber yield and better tolerance to diseases. Tolerance to various biotic and abiotic stresses is of great significance in assessing the performance of different clones. Disease assessment from small scale trials with a small plot size is however not conclusive. Nevertheless it gives an indication of the clonal response. IAN 45-873 is reported to be tolerant to *Oidium* (John et al. 2000; Varghese and Abraham 2007) and the clones with least incidence of disease had IAN 45-873 as one of the parents. 86/380 (RRII 105 x PB 5/51) combined high girth increment under tapping with a rising yield trend in the BO-2 panel. The clones 86/575 and 86/756 had RRIM 600 as one of its

parents. Both the clones showed vigorous growth coupled with good bark regeneration after tapping in a not so congenial terrain.

Among the ancillary products from rubber plantations rubber wood is the major by product in enhancing the net farm income (Viswanathan et al. 2002). Vigorous and fast growing trees not only attain early tappability but timber output will also be high (Meenakumari et al. 2013). Clones 86/772 and 86/756 indicated suitability as latex –timber clones by way of high clear bole volume. The performance index worked out for trials 1 and 2, indicate clearly the conditions under which both the trials were conducted. The index values in trial 1 were higher than that of trial 2. However the selections 86/772, 86/380, 86/575 and 86/756 adjudged for superior performance in both the trials showed a very high index confirming their overall superiority and potential to combine high yield with desirable secondary traits.

Table 5: Performance index (PI) of clones

Trial 1		Trial 2	
Clone	**PI**	**Clone**	**PI**
86/779	82.8	86/756	54.1
86/772	81.7	86/575	52.3
86/948	81.3	86/368	51.7
86/380	78.5	RRII 105	51.1
86/356	75.1	RRIM 600	50.8
86/583	74.5	86/592	49.6
86/450	74.0	86/618	45.8
RRII 105	72.9	86/264	45.2
86/764	72.2	86/916	44.4
86/424	69.8	86/894	40.6
86/761	65.3		
86/337	61.7		
86/789	23.5		
86/779	82.8		

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Growth and yield of *Hevea brasiliensis*: effect of juvenility of source bush nurseries

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ABSTRACT: *Natural rubber tree, Hevea brasiliensis (Willd. ex A.Juss.) Mull. Arg. is being propagated predominantly by asexual methods through patch budding. However, trees exhibit intra-clonal variation in growth and yield which reduces the uniformity of plantations. Commercially, buds were collected from budwood sticks regenerated every year from the same source bush nurseries. The age of source nurseries may vary from one to 20 years. Present study examines the effect of age and juvenility of buds from six source bush nurseries and mature trees on growth and yield of the most popular clone RRII 105. Bud patches were collected from healthy regenerated budwood aged one year, four year and 20 year old budwood nurseries and directly from 10 year old mature trees. These buds were grafted onto healthy stock seedlings raised in the nursery. The resultant plants were evaluated in a field trial during 2006 to 2014 following standard field practices. Girth of trees in the seven treatments varied during early growth phase. However, in the seventh and eighth year after planting, except the plants raised directly from 10 year old trees, growth of trees did not show any statistically significant difference. Trees raised from mature trees (34.0 cm) compared to those from regenerated budwood nurseries aged one year, four year and 20 years (girth ranged from 40.0 to 46.3 cm). The results indicated that trees originated from all rejuvenated bud sources viz., one, four and 20 years old budwood stocks registered significantly higher rubber yield (ranging from 31.0 to 37.0 gram/tree/tapping). However, trees originated from buds of mature trees registered significantly ($p<0.05$) inferior growth, tappability (8.0%) and dry rubber yield (28.0 grams per tree per tapping) compared to other treatments. Moreover, buds of mature tree branches recorded higher intraclonal variation in girth than that of trees raised from budwood nurseries. Results of this study demonstrate that the age of budwood sources ranging from one to twenty years does not appear to have any significant role in determining intraclonal variability in growth and yield of rubber and strongly suggest avoiding use of buds directly from mature trees for commercial propagation.*

***Key words*:** *Hevea brasiliensis*, Propagation, Juvenility, Age of budwood, Source bush nurseries, Budding

Introduction

Natural rubber tree, *Hevea brasiliensis* is being propagated predominantly by asexual methods through patch budding. However, this tree crop exhibit intra-clonal variation in growth and yield which reduces the uniformity of plantations (Jayasekera and Senanayake 1971; Senanayake 1975; Senanayake et al. 1975). There were many attempts to investigate this issue by establishing the role of different factors like stock-scion interaction (Gireesh et al. 2012) and graft incompatibility (Mercykutty and Gireesh 2015). Source and juvenility of vegetative propagules plays an important role in the success of vegetative propagation. Seneviratne (2000) and Senevirathna et al. (2007) studied quality of planting materials with respect to

growth variation of the trees and reasons for not achieving the potential rubber yield. This could be circumvented to a limited extent by adopting standard management practices but most of the growers are doubtful about the role of age of budwood source bush nurseries. Many commercial nurseries now generate budwood repeatedly from same stock for over 20 years and in certain cases buds are harvested directly from the mature trees for bud grafting. Reestablishment of source bush nurseries has been practised (Seneviratne et al. 2000) but causing huge spending on man power and time. Juvenility of source budwood stocks from which buds are harvested for multiplication, is as important as any other factors which contributes to the success of bud grafting as well as uniformity and growth of plants in later stages. Present study examines the effect of age and juvenility of buds sourced from different source bush nursery plants and mature trees on long term growth and yield of the most popular clone RRII 105.

Materials and methods

Well grown one year old budwood of the clone RRII 105 were collected from young and mature budwood nurseries. Different age of budwood nurseries viz., one, four and twenty year old from three different locations (A, B & C) were chosen for the study. The appearance of three types of budwood is illustrated schematically in the Fig. 1. Buds were collected from budwood sticks and grafted onto stock seedlings of one year old seedlings raised in the ground of Central Nursery, Karikkattoor. Buds collected from the terminal branches of 10 year old mature trees of RRII 105 were also bud grafted on to the stock seedlings.The seven treatments are detailed in the Table 1. Successful bud grafts were raised in top soil filled black low density poly bags (55 x 25 cm size lay flat size with 400 gauge thicknesses) and later when the plants attained 2-3 whorls stage, field planting was done. The trial was laid out in randomised block design with 3 replications with nine plants per plot in each treatment. Field experiment was conducted at the Central Experimental Station of Rubber Research Institute of India at Chethackal in Central Kerala. All recommended field management practices were followed throughout the experiment.

Girth at breast height of trees in the field was recorded annually from the third year of planting onwards and estimated the tappability of trees during 7th year after planting. Dry rubber content of latex from each tapping was determined at monthly intervals and expressed as gram per tree per tapping (g/t/t). Tapping system followed was S/2 d/3 6d/7 (once in three days).

Figure 1: Progression of budwood nursery over years. Schematic representation of a budwood nursery, each branching point indicates cut back point where one year old branches were pruned for collecting new shoots every year.

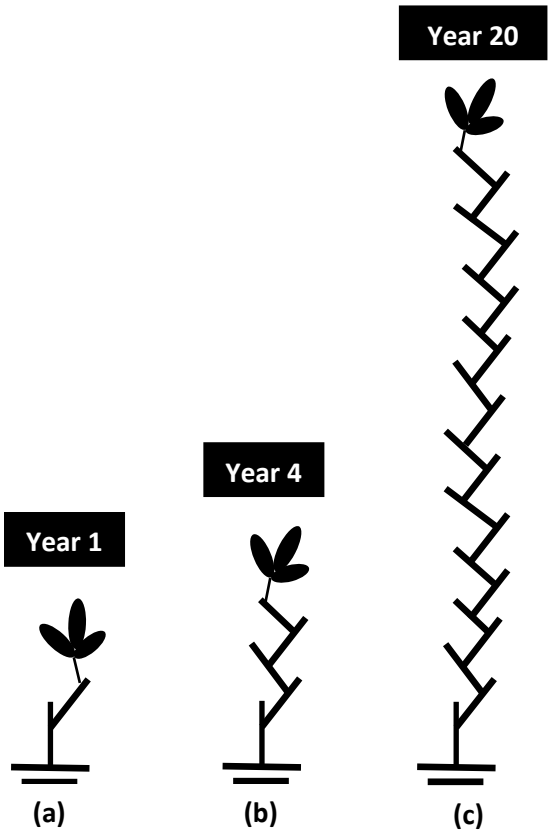

Table 1: Details of treatment combinations

Treatments	Age of bud source	Locations
T1	20 year old budwood plants	Nursery A
T2	4 years old budwood plants	Nursery A
T3	4 year old budwood plants	Nursery B
T4	4 year old budwood plants	Nursery C
T5	20 year old budwood plants	Nursery C
T6	10 year old trees	Plantations
T7	1 year old budwood plants	Nursery B

Results and discussion

Budwood plants are pruned in Sept-Oct every year and a length of 15 cm was retained for regeneration of new shoots. One or two shoots in each branch is allowed to grow for harvesting budwood. This practice was being continued for different time periods ranging from one year to 20 years. In certain cases, source bush will remain for more than 20 years (Fig. 1). Juvenility and vigour of buds and its potential to become successful plantation was tested in this long term study.

Growth variation in the trees reflected in the girth measurements from three years after field planting. Difference in the growth was significant until fifth year across the treatments. Table 2, shows that trees originated from buds of old trees without rejuvenation lagged in growth behind all other age groups significantly throughout the period under observation. Initially trees raised from young budwood nursery (T2, T3, T4 and T7) recorded a mean girth of 16.3, 16.8, 16.2 cm and 17.2 cm respectively and the treatments were on par, while better initial growth (17.2 cm) was registered by trees raised from young bud source (one year). However, the difference among other treatments narrowed down and no significant difference was noticed in 6th and 7th year after planting. Annual girth increment rate and uniformity of trees are also considered as an important growth parameter which determines the commercial success of plantations. This study illustrates that trees raised from young budwood stock plants showed better growth parameters in the initial growth phase and the differences were subsequently narrowed down. The results suggest that age factor in the budwood nurseries doesnot influence the growth of trees in later stages. General understanding is that when the harvesting point moves away from the base of the tree the plants may have lost juvenility and results in lack of vigour of progenies. But it is true in the case of buds collected from the tree top (un- rejuvenated) as the resultant trees showed significantly less vigour.

Table 2: Growth of trees over seven years

	Treatments	Mean girth (cm)				
		yr. 3	yr. 4	yr. 5	yr. 6	yr. 7
T1	20yr. nursery A	15	22.7	30.8	35.5	42.0
T2	4yr. nursery A	16.3	24.9	33.8	37.0	46.3
T3	4yr. nursery B	16.8	25.6	34.1	38.5	46.2
T4	4 yr. nursery C	16.2	24.1	32.4	38.8	44.5
T5	20 yr. nursery C	16.1	24.4	30.3	35.2	42.5
T6	10 yr.trees	11.03	16.4	23.0	26.2	34.0
T7	1yr.nursery B	17.2	25.2	32.7	36.8	45.1
	CV	6.4	5.3	7.4	8.2	6.9
	CD($p<0.05$)	1.4	1.8	1.9	4.2	4.3

Early tapping and high precocious yield from trees is important when discounted cash flow and return on the investment is considered (Wycherley 1969; Mydin and Mercykutty 2007). Tappability is an important parameter which determines the commencement of tapping in plantations. Opening of trees for tapping require a minimum girth of 50 cm at breast height. Earlier attainment of tappability ensures higher initial returns to growers.The tappability percentage of trees among different treatments (Table 3) illustrates that highest score (70.7%) was registered by trees originated from young budwood source (T3) while others are also observed to fall in an acceptable range of 51 to 60%. Only 14% of the trees attained tappability in T6, where the trees were raised from the un-rejuvenated buds collected from 10 year old trees. This result clearly indicates that the buds from old trees should never be used for raising planting materials. Earlier reports by Mercykutty et al. (2014) also support these findings.

Rubber yield is the most important economic trait of rubber trees. Table 3 illustrate the average yield potential of trees originated from different categories of bud wood. Young budwood originated trees (1 year old, T7) registered highest mean yield of 37 gram/tree/tapping compared to 20 year old trees (T1 and T5) which recorded 31 to 32 gram/tree/tapping respectively. Lowest yield (34 gram/tree/tapping) was recorded by the trees originated from the buds of 10 year old trees. To minimise the immaturity period and increase the uniformity of stand, raising quality planting material from a young healthy budwood source is very crucial. The study suggests that there was no wide difference between the age group of budwood nurseries. Results of this study demonstrate that the age of budwood sources ranging from one to twenty years was not significant in determining intraclonal variability in the growth and yield of rubber. The study also strongly suggests that use of buds directly from mature trees for commercial propagation could not be advocated.

Table 3: Tappability and yield over two years

	Treatment description	Tappability (%)	Mean yield over two yrs. (gram/tree/tapping)
T1	20yrs. nursery A	59.5	31.8
T2	4yrs. nursery A	59.3	33.1
T3	4yrs. nursery B	70.7	34.7
T4	4 yrs. nursery C	51.3	34.7
T5	20 yrs. nursery C	38.5	30.6
T6	10 yrs.trees	8.0	28.2
T7	1yr.nursery B	59.3	37.0
	CV	--	8.7
	CD($p<0.05$)	--	4.2

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Growth and yield performance of half-sib progenies of para rubber (*Hevea brasiliensis*) generated through poly-cross breeding in a polyclonal seed garden

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ABSTRACT: *As a part of polycross breeding, half-sibs were collected from nine clones in a polyclonal seed garden in Karnataka (India) and evaluated in mature trial. More trees of polyclonal seedling origin (71%) attained tappable girth compared to check clone, RRII 105 (11 %). Families of PB 217 followed by RRII 203 showed high CV for girth indicating wide variability and scope for selection. Family of Ch 26 attained maximum mean girth (71.5 cm) compared to other families and check clone (51.3 cm). Family PB 28-83 showed a minimum girth of 62.1 cm and low CV. Check clone showed lowest girth of 51.3 cm. Families RRII 105, AVT 73 followed by PB 28-83 showed high CV for yield indicating scope for selection. Family Ch 26 followed by PB 242 and RRII 203 showed maximum yield of more than 38 $gt^{-1}t^{-1}$. CH 26 produced a polycross progeny with highest yield of 72.9 $gt^{-1}t^{-1}$. Family PB 28-83 showed minimum yield (26.6 $gt^{-1}t^{-1}$). Progenies of Ch 26 indicated high potential for selection for growth as shown by high proportion of superior performers. Families PB 217, AVT 73, PB 215 and RRII 203 also offered scope for selection for growth. Family Ch 26 produced higher proportion of superior yielders. Based on rank sum method, progenies of clone Ch 26 was ranked as top performer followed by RRII 203 and PB 217. The above study showed high rate of recovery of superior genotypes could be achieved through poly-cross breeding.*

Key words: *Hevea brasiliensis,* Co-efficient of variation, grams per tree per tap.

Introduction

The Para rubber tree *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. (family, Euphorbiaceae; diploid, 2n=36), is a forest species native to the tropical rain forests of Central and South America, domesticated outside its natural range of distribution only in recent times (Narayanan and Mydin 2011). The tree, which is monoecious, entomophilic, and predominantly out-crossing (>60%), produces latex (essentially cytoplasmic fluid) in its specialized laticifer cells located in bark tissue. The latex is a major source of natural rubber in the entire plant kingdom attributed with only hypothetical roles in the host tree's life-cycle. Latex is manually extracted through systematic incision ('tapping') of bark tissue and forms significant raw material with manifold uses including those for medical, automotive and strategic purposes. Almost all of the cultivated clones of *Hevea* were derived either through selection or breeding among the few selected seedlings (or their progenies)

reportedly collected in 1876 from a site in Boim, near the Tapajos river in Brazil (Allen 1984; Baulkwill 1989; Schultes 1977; Wycherley 1968).

Crop improvement in *Hevea*, primarily aimed at breeding clones with potential to produce more dry rubber, has been achieved predominantly through introduction, ortet (plus tree) selection, or hybridization, ultimately followed by clonal selection (Mydin and Saraswathyamma 2005). The conventional method of cyclical 'generation-wise assortative mating' (GAM), where superior genotypes of one generation form parents for subsequent breeding programs has been the prime breeding strategy in *Hevea* (Simmonds 1989). While hybridization has been major method for forming recombinants with selected traits, the size of initial selection population has been limited due to various inherent as well as management limitations. Owing to its high-outcrossing behaviour, the species has capability of producing large number of natural hybrids. This predominantly out-breeding trait led to establishment of polyclonal seed gardens for production of genetically improved genotypes, or poly-cross progenies, through natural hybridization between superior clones (Simmonds 1989). Thus, the polyclonal gardens assisted in rapid crop improvement in *Hevea* through poly-cross breeding. This paper discusses the performance of a population of polycross progenies derived from nine clones in a polyclonal seed garden in the Karnataka State of India. The possibility of recovering several potentially superior clones from the above population is also highlighted.

Materials and methods

The experimental population of polycross progenies (half-sibs) was collected from a polyclonal seed garden established at the *Hevea* Breeding Sub-Station (Nettana, Dakshina Kannada Dt., Karnataka). Half-sibs were separately collected from nine clones (RRII 105, CH 26, AVT 73, PB 215, PB 217, PB 28/83, PB 242, PB 252 and PB 5/51) and planted in a trial in 2005 at the Central Experimental Station of Rubber Research Institute of India (Pathanamthitta Dt., Kerala). For comparative evaluation, clone RRII 105 was planted as check clone. Details of the half-sibs and their parental clones are given in Table 1. The trees were planted at a spacing of 4.9 x 4.9 m following CRD with each progeny constituting a single tree plot. In 2012 (seven years after planting), the trees were evaluated for their tappability and those trees which attained tappable girth (polycross progenies, ≥ 50 cm girth at 90 cm height; clonal population, ≥ 50 cm girth at 150 cm height) were tapped following standard tapping system (S/2 d3 6d/7). Growth performance of the polycross progenies and check clone was compared in terms of girth (at 90 cm for seedlings and 150 cm height for check clone) during the third year of tapping. Dry rubber yield (gram per tree per tap; $gt^{-1}t^{-1}$) was recorded in all the tress following cup-

coagulation method based on annual mean of monthly recordings (Mydin and Saraswathyamma 2005). The data was subjected to '*t*' test analysis for finding significance and variability among the families, which for the basis for selection, was assessed based on coefficient of variation (CV). Proportion of family-wise best performers (per cent recovery of superior clones) was computed based on number of progenies exhibiting better growth and yield than the population mean. Rank sum method was followed to identify top families for growth and yield performance.

Table 1: Details of half-sibs

Parent clone	Parentage (Country of origin)	Hal-sib family size
PB 242	PB 5/51 x PB 32/36 (Malaysia)	21
RRII 203	PB 86 x Mil 3/2 (India)	22
PB 217	PB 5/51 x PB 6/9 (Malaysia)	21
Ch 26	Primary clone (Malaysia)	22
RRII 105	Tjir 1 x Gl 1 (India)	18
AVT 73	Estate selection (India)	15
PB 5/51	PB 56 x PB 24 (Malaysia)	22
PB 215	Primary clone (Malaysia)	23
PB 28/83	Primary clone (Malaysia)	14

Results and Discussion

The half-sib population exhibited high level of variability for growth and yield performance as supported by '*t*' test. Regarding growth performance in terms of girth attained by trees seven years after planting (Table 2), more trees of polyclonal seedling origin (71%) attained tappable girth when compared to check clone, RRII 105 (11%). Polyclonal seedlings are known to possess comparatively more vigorous growth trait compared to clonal populations which is also in agreement with present observation. Family of clone Ch 26 attained maximum mean girth (71.5 cm) compared to other families as well as check clone RRII 105 (51.3 cm). Families of clones PB 217 followed by RRII 203 comparatively higher *CV* indicating wide variability and scope for selection. Family of clone PB 28-83 showed a minimum girth of 62.1 cm and low level of *CV*. Check clone showed lowest girth of 51.3 cm reflected in its low tappability percentage in the initial years. Based on performance of individual polycross progenies, a progeny of clone RRII 203 showed highest girth (97 cm) followed by a progeny of Ch 26 (96 cm). It is may be noted that RRII 203 is a clone with high girth developed by RRII through hybridization and selection. Lower level of CV in this family also indicated uniform growth among the progenies.

Table 2: Variability for girth (cm) and family-wise recovery of superior performers in half-sib families

Family	Mean girth (range)	Co-efficient of variation (C.V. %)	Family-wise superior selections (%)
PB 242	62.7 (31.0-80.0)	18.2	38.1
RRII 203	67.3 (42.0-97.0)	19.1	50.0
PB 217	67.7 (39.0-85.0)	20.0	61.9
Ch 26	71.5 (48.0-96.0)	18.1	72.7
RRII 105	61.4 (39.5-83.0)	18.7	33.3
AVT 73	68.7 (52.5-83.0)	14.2	60.0
PB 5-51	67.6 (43.0-88.0)	18.9	54.6
PB 215	65.9 (52.0-83.0)	14.4	43.5
PB 28-83	62.1 (47.0-78.0)	13.0	21.4
RRII 105 (Check)	51.3 (24.0-73.0)	16.3	
Population mean	66.3		

*Based on half-sib population performance

Polycross progenies exhibited superior yield performance compared to check clone (Table 3). Families of RRII 105, AVT 73 followed by PB 28-83 showed high CV for yield indicating scope for selection for this trait. Among the half-sibs, family of clone Ch 26 followed by PB 242 and RRII 203 showed maximum yield performance of more than 38$gt^{-1}t^{-1}$. CH 26 also produced a polycross progeny with highest yield of 72.9 $gt^{-1}t^{-1}$. Half-sibs of family PB 28-83 showed minimum yield (26.6 $gt^{-1}t^{-1}$).

Polycross progenies generally exhibit very high variability for growth and yield traits due to their inherent heterozygosity caused by high outcrossing rate as shown in many studies using polycross progenies (Chandrasekhar et al. 2002; Deepthy et al. 2014; Mydin et al. 1996; 2002, 2010; Mydin 2014). The data on growth and yield from the present study also supported the above observation. The present study showed scope for selection of appreciable proportions of superior performers in terms of growth and yield. Regarding growth vigour, polycross progenies belonging to family Ch 26 indicated high potential for selection against this trait as shown by high proportion of superior performers (Table 2). Families of clones PB 217, AVT 73, PB 215 and RRII 203 also offered scope for selection as reflected by more than 50 % superior performers. With reference to yield, progenies of family Ch 26 carried higher proportion of superior yielders based on family mean yield as well as general mean yield (Table 3). Based on rank sum method, polycross progenies of clone Ch 26 was ranked as top performer followed by RRII 203 and PB 217 (Table 4).

Table 3: Variability for dry rubber yield ($gt^{-1}t^{-1}$) and family-wise recovery of superior performers in half-sib families

Family	Mean yield (range)	*CV* (%)	Family-wisesuperior selections (%)	
			Based on family mean	Based on general mean
PB 242	39.2 (16.1-69.6)	37.3	42.86	57.1
RRII 203	38.4 (19.1-57.6)	31.3	40.91	63.6
PB 217	35.6 (16.6-58.6)	34.7	42.86	47.6
Ch 26	39.6 (11.9-72.9)	38.9	50.00	59.1
RRII 105	28.7 (8.6-56.2)	46.6	33.33	38.9
AVT 73	34.9 (10.6-70.8)	46.4	33.33	46.7
PB 5-51	35.0 (14.4-49.3)	29.2	40.91	59.1
PB 215	37.7 (6.8-70.5)	42.9	34.78	47.8
PB 28-83	26.6 (12.9-49.1)	45.2	21.43	35.7
RRII 105 (Check)	29.9 (9.0-55.8)	32.5		
Population mean	35.6 (6.8-72.9)	39.2		
General mean	33.1			

Table 4: Ranking of clones

Clone	Rank*
Ch 26	1
RRII 203	2
PB 217	2
PB 242	4
AVT 73	4
PB 5-51	6
PB 215	6
RRII 105	8
PB 28-83	8

*Based on rank sum method using mean girth and yield

In an earlier study based on nursery evaluation using nine separate sets of half-sib progenies collected from the parental clones in the above polyclonal seed garden, an attempt was made to assess the drought tolerance potential of the half-sib families based on test-tap yield during summer (Mercy and Mydin 2012). The above study showed a high proportion of seedlings with high summer yield which indicated scope for selection for drought tolerance. Since drought tolerance is a complex trait dependent upon environmental factors, more long-term observation

in drought prone sites is required to derive such conclusion from the present mature tree population. However, the present study as well as the pervious study based on nursery evaluation indicated good scope for selection of many good performers from the polycross progenies of the above nine parental clones.

The present study is only indicative and requires further long-term monitoring of yield in the experimental clones in different tapping panels to confirm the superior yield potential of the top performers. Nevertheless, recovery of a high proportion of superior performers in terms of growth and yield in the initial years of tapping based on mature tree data, is highly encouraging, offering good scope for selection.

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Root anatomy of modified root trainer plants of *Hevea Brasiliensis*

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ABSTRACT: *Manipulation of root architecture in a perennial tree crop like Hevea brasiliensis assumes dual significance. Recently, polybag planting materials are being replaced with modern root trainer plants characterised by air pruned tap roots and numerous laterals. Air pruned tap root raises apprehension among the growers on further root development and establishment after field planting. More over, moisture stress and wind susceptibility due to changing climatic conditions are becoming severe in rubber growing areas. In this context attempts to modify the root architecture and detailed investigations on the structural organization of the tissues in the modified roots with respected to polarity was studied. Modification in root architecture could be achieved by allowing the air pruned roots to grow into a root elongation tube (RET) filled with potting medium, and attached tightly to the drainage hole of the root trainer cup. The root core developed in the RET after two months is characterized by 10-12 thick vertical roots, profuse laterals and fine roots. Average root elongation of the vertical roots within the RET was 1.8cm per day. Proportion of various tissues, number of pericycle layers, shape of stele, accumulation of starch and birefringent property of the cellulosic microfibrils in the pith are distinct for vertical and horizontal (lateral) roots, which assume taxonomic significance also.The root architecture thus modified can increase the functionally active surface area for better absorption of soil resources and can ensure better growth and anchorage.*

Key words*: Birefringence, Hevea, Planting material, Root anatomy, Root architecture, Root trainer plants*

Introduction

Shortage of water is a constraint to life that recurs more and more in many regions of the world due to global climate change (IPCC 2007). Increasing drought has an impact on the survival of plants in natural ecosystems, while it also results in reduced yields in crops. The prime factor that determines the ability of the plant to access water and nutrients from the soil is the size and architecture of the root system (Werner et al. 2010). Most studies on organ size control is confined to terrestrial organs (Li et al. 2008), whereas roots are only rarely considered. As the trait is governed by many genes, classical breeding approaches are difficult to achieve the goal (Werner et al. 2010). In this context, understanding and manipulation of root architecture becomes more important.

Hevea brasiliensis, the prime source of natural rubber, is a perennial subtropical tree crop with more than thirty years of economic life span. Among the rubber growing countries India rank first in productivity and fourth in production of natural rubber (NR). As the national production is not adequate to meet the domestic requirement,

cultivation is extended to non-conventional rubber growing tract where climatic conditions particularly drought is a serious matter of concern for plant growth. Summer irrigation together with mulching is the way for early plant establishment in these areas (Vijayakumar et al. 2000).

Among the planting materials of rubber, recently introduced root trainer plants have got wide acceptance due to its unique advantages including better root establishment after transplanting (Soman et al. 2013). Budded stumps or germinated seeds *in situ* are planted in a soilless potting media i.e., coir pith or elephant dung (Thomas and Soman 2014; Thomas and George 2014), in plastic containers. In this technique, hardening is a pre-planting cultural operation by which the plant is equipped for facing unexpected stress situations in the open field (Soman et al. 2010). During the course of hardening, tap roots undergoing air pruning near the drainage hole are in a state of starvation. Modification in root architecture could be achieved by allowing it to grow into a root elongation tube (RET) filled with potting medium (Fig. 1 A & B), attached tightly at the drainage hole of the root trainer (Thomas et al. 2015). The plants with modified root architecture is characterized by a lengthy root core comprising of 10-12 thick vertical roots, large number of laterals and fine roots (Fig. 1 C - E), with the expected advantages of mining soil resources from deep 'reservoir-levels' sheltered from evaporation and to provide required anchorage, particularly in non-traditional rubber growing tract of India where drought and cold prevails a major limiting factor for plant growth.

To address the issue of further growth of air pruned roots of root trainer plants, modified root trainer plants (MRTP) were planted in the soil and root samples collected were subjected to anatomical and histochemical studies to explore the tissue orientation of the roots with respect to polarity at different levels of its development.

Materials and Methods

MRTP were prepared following the procedure of Thomas et al. (2015). These plants were planted in the field at HBSS, Paraliar, Tamilnadu. One month after establishment, plants were dig out and both lateral and tap root were collected from three locus viz., the region where the roots originally developed within the root trainer cup, the region within the RET and roots grown into soil. The samples were sectioned with a thickness of 40µm and stained with Toluidine blue O (O'Brien et al. 1964) for general histology. Histochemical stains such as I_2KI and Periodic acid-Schiffs reagent for starch (Johansen 1940), Acid fuchsin (McCully1966; Pramod et al. 2008) and Mercuric chloride- bromophenol blue (Mazia et al. 1953) for protein, Oil red O for lipid (Lillie 1965) and latex vessels (Omman and Reghu 2003) were used

and observations under carried out both light and polarization microscopes. Measurements and photomicrographs were taken using a Leica Qwin image analysis system attached to Leitz Diaplan and Leica DM 2500P polarization microscope.

Results

The modified root trainer plant of rubber has a two part root system i.e., the one developed inside the root trainer cup characterized by a tap root and large number of laterals trained to grow by the vertical ridges present inside the cup, and the second part developed in the root elongation tube (RET). Callus tissue at the wounded area of the tap root of the root trainer plants are under severe stress due to temporary arrest in growth till field planting. Once they are allowed to grow in the RET profuse rooting occurs within the tube. A root trainer plant with a total root length of 70-75cm can be produced before the onset of monsoon so that planting of the MRTP can be done on the same season without delay.

Lateral root anatomy

Cross section of the lateral roots developed in the root trainers, RET and in the soil for one month revealed that they are structurally identical with single layered epiblema ensheathed with a thick and intruded cuticle, a regular cortex constituted of loosely organized parenchyma cells, endodermis, multilayered pericycle and the stele. The epiblema cells protruded out to form fine roots (Fig. 1E) which are functionally active in the uptake of nourishments from the medium (Jessy et al. 2005). Phenolic depositions are observed in some of the cortical cells but are devoid of starch grains. Vascular stele is quadrangular with endodermis, 2-3 layered pericycle, four xylem groups and a quandrangular pith with compactly arranged parenchyma cells is the structural orientation of tissue in the lateral root developed both in root trainers and in RET (Fig. 1 F & G). The vascular stele of the laterals collected from the soil is circular in outline with endodermis, 8-10 layered pericycle and the formation of secondary vascular tissue and a circular pith. Passage cells occur in four locations in the endodermis adjacent to the xylem elements (Fig. 2A).

Lateral roots when viewed under polarization microscopy revealed strong birefringence property for the xylem and pith cells (Fig. 1H & J). In addition, the multilayered pericycle and intruded cuticle over the epiblema also showed birefringence property for the laterals grown in the soil. To understand the relationship between structural organization of tissue and polarity the plants were retained in root trainers for a year. The laterals continued to exhibitd similar anatomical organization of tissue to the recently developed laterals except for the stele where the pericycle is 3-4 layered and an octagonal stele (Fig. 1H - J). Root

hairs, octagonal vascular cylinder, primary phloem fibers and pith showed high degree of birefringence (Fig. 1G & I) while it is feeble for phloem cells. Both transverse and radial walls the endodermis with more deposition of suberin, on the latter to form the casparian strip (Fig. 2B). Endodermis possess passage cells in seven sites where it faces to the protoxylem points. Cortical cells are devoid of starch but could localize in some of the pith cells. Lipid materials were found at the apical part of the root hair, as globules attached to the wall of the cortical and phloem cells. Phloem tissue is developed more than that of xylem elements organized in a file. Latex vessels of 10-15 were differentiated more or less in a row in each phloem group (Fig. 2A). Fascicular and interfascicular cambium join to form a continuous ring but the secondary growth is not yet started.

Tap root anatomy

The tap root inside the root trainers developed originally from the radical of the germinating seed. During the course of hardening this root growth is arrested at the drainage hole at the bottom. The callus tissue at the site of injury is instrumental in healing process and the development of new roots in the vertical file into the RET attached to the bottom of the root trainer. It has been noted that the roots originating from the callus tissue retain its polarity in the vertical file.

The vertical roots developed in the root trainer, RET and soil are stiff and structurally identical with a cork zone followed by cortex, secondary vascular tissue and circular pith (Fig. 2C). Starch grains were localized both in the xylem parenchyma and pith cells (Fig. 2 D & E). Within one month of growth in the soil the vertical roots showed quick secondary vascular tissue formation and make the root harder. A strong birefringence property is not noticed for pith and xylem cells of tap root as the case with lateral roots (Fig. 2E).

Irrespective of the growth medium and stages of development before planting, the birefringence property exhibited by the laterals is a unique characteristic and can be used as an anatomical marker for distinguishing laterals from tap root or the roots developed from the callus of the same plant at an early stage (Table 1). Lateral roots are characterized by its polarity (mostly horizontal), single layered epiblema with fine roots, multilayered pericycle, quadra or octangular stele and strong birefringence for xylem and pith cells. Polarity in the vertical file, early development of cork cells and vascular tissue, accumulation of starch in the xylem and pith, circular pith and feeble birefringence for the stelar tissue are the notable features for tap root.

Table 1: Distinctive characteristics of lateral and tap roots of root trainer plants

Characters	Lateral root	Tap root
Polarity	Horizontal to vertical	Vertical
Boundary of the root	Single layered epiblema	Cork layer
Fine roots	Present	Absent
Pericycle	Multilayered	Not distinct
Shape of stele	Quadra/octangular- circular	Circular
Secondary tissue formation	Delayed	Early
Starch in the xylem and pith	Absent	Present
Shape of the pith	Quadra or octangular	Circular
Birefringent xylem and pith	Strong	feeble

Secondary xylem and starch grains in the axial and ray parenchyma and in the pith showed birefringence. Xylem rays are multiseriate that become dilated in the secondary phloem in response to the increase in circumference of the axis, by radial anticlinal cell divisions and tangential cell enlargement. Widened connective tissue with tangentially oriented loosely arranged cells traverse through the secondary phloem, and that extends up to the cork layer. These tissues gave differential stainability and may have direct involvement in active translocation. The pericyclic cells undergoing periclinal and anticlinal divisions cause an increase of the number of pericyclic layers in the radial extent.

Early initiation of secondary growth in vertical roots compared to laterals resulting a higher proportion of stelar tissue than cortical cells in the vertical roots (Fig. 3 & 4). In lateral roots proportionately more area is occupied by the cortical cells. Laterals can remain in the root trainers without the initiation of secondary growth for a longer period of time. As soon as the transplanting in the field, laterals started to produce secondary tissue from the continuous cambium formed by the fascicular and inter- fascicular cambium. The area proportion of cortex and stele of laterals and vertical roots in the early stages of its development are different.

Birefringence revealed deposition of radial cellulose microfibril systems on their periclinal walls, as part of polarity, which means specific orientation of plant activity and morphogenesis in space, or the existence of functionally significant asymmetric structures that are formed in response to vectorial cues (Medvedev 2012). The birefringence observed in the cellulose-type cell wall is practically always pure intrinsic birefringence hence, it is important for diagnostic purpose (Ruch 1966). The colour difference observed under polarized light in the present study for the cells of pith, cuticle and root hairs indicate the varied orientation of the microfibril molecules in them. Birefringence is unique for laterals and not

observed for tap root or the roots developed from the callus tissue at the wounded end of the budded stump or the air pruned roots developed during the hardening process of the root trainer plants. Lateral roots took its origin from the pericycle and maintains its polarity in the horizontal file while those developed from the callus tissue are always in the vertical file without exhibiting birefringence.

There exists a general belief that the tap root of the rubber plant should not be injured as once damaged, one or two laterals take up the role of tap root and grow vertically downwards. The apprehension is about the anchorage ability of these roots. The cut end of the tap root develop a wound periderm which transforms into a callus from which further root development takes place in the vertical file under conducive conditions. The study conducted by Thomas et al. (2015); Thomas and Soman (2013) revealed that acute stress developed on the tap root is advantageous as the callus tissue developed at the cut end of the tap root/ air pruned roots produce more number of stiff roots that grow vertically downwards into deeper strata in the soil. These vertical roots are however not laterals as they do not exhibit birefringence property under polarization microscopy. The roots thus produced can increase the functionally active surface area for better absorption of soil resources and can ensure better growth and anchorage. Such 'replacement roots' developed following injury to the tap root either by pruning or during planting are common in many plantation and horticultural crops (Esau 1977).

Rubber planting materials with modified root architecture (MRTP) are expected to survive and establish better in regions where drought is a serious limitation for initial field survival of the plant. To meet the domestic demand for NR, rubber cultivation in India is being extended to non-traditional regions faced with adverse climatic conditions. Life saving irrigation and mulching to young rubber plants is the recommended practice for the drought prone north Konkan belt of western India (Vijayakumar et al. 2000). The improved root system of MRTP not only enhances the growth but also can provide substantial savings in irrigation.

Figure 1: (A). Root elongation tube (RET) attached to root trainer plants for the modification of root architecture, (B & C). Initiation and development of roots in the vertical file from the callus tissue of the wounded tap root of root trainer plants, (D). Modified root architecture in the RET, (E). Fine roots on the laterals, (F). Cross section of the lateral roots developed in the RET with large cortex and a quadrangular pith, (G). Both xylem and pith cells showing birefringence property and (H –I). Cross section of the lateral roots collected from one year old root trainer plants showing octagonal stele with birefringence property.

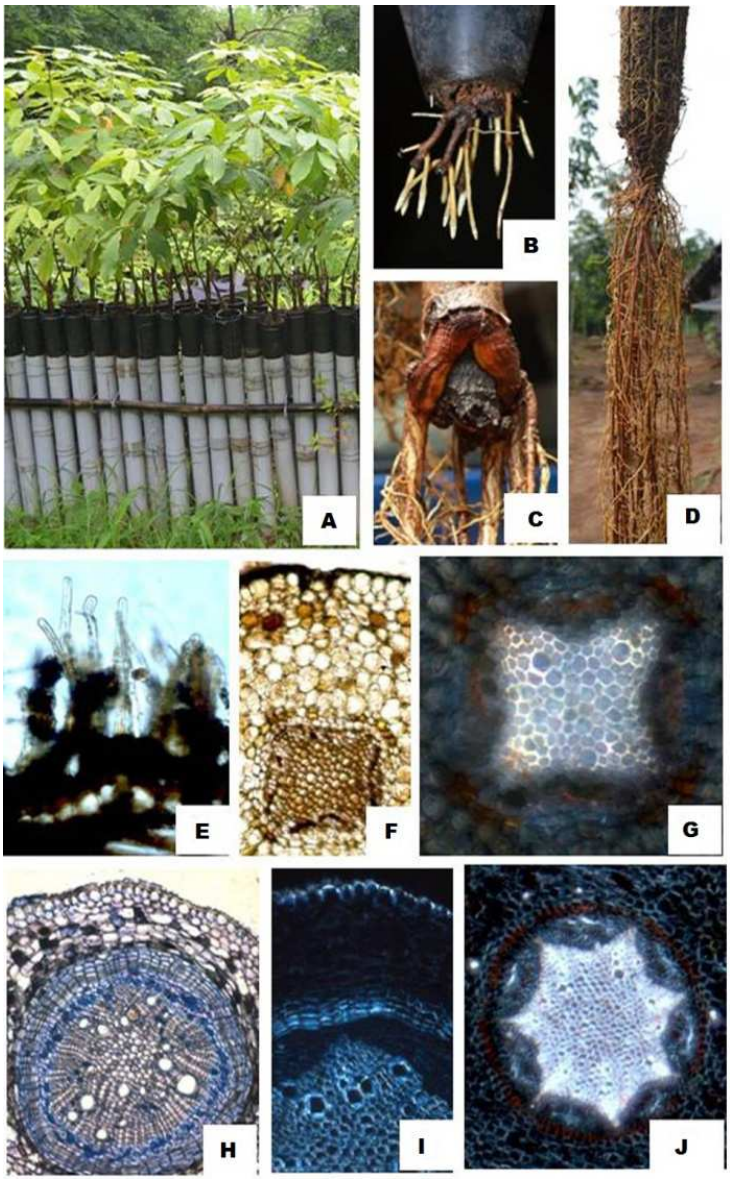


Figure 2: (A). Passage cell in the endodermis of laterals under polarization microscopy. Latex vessels are interspersed with phloem cells, (B). Casparian strip of endodermis and (C-E). Cross section of vertical root showing secondary tissues, starch accumulation in the xylem parenchyma and feeble birefringence for the pith cells.

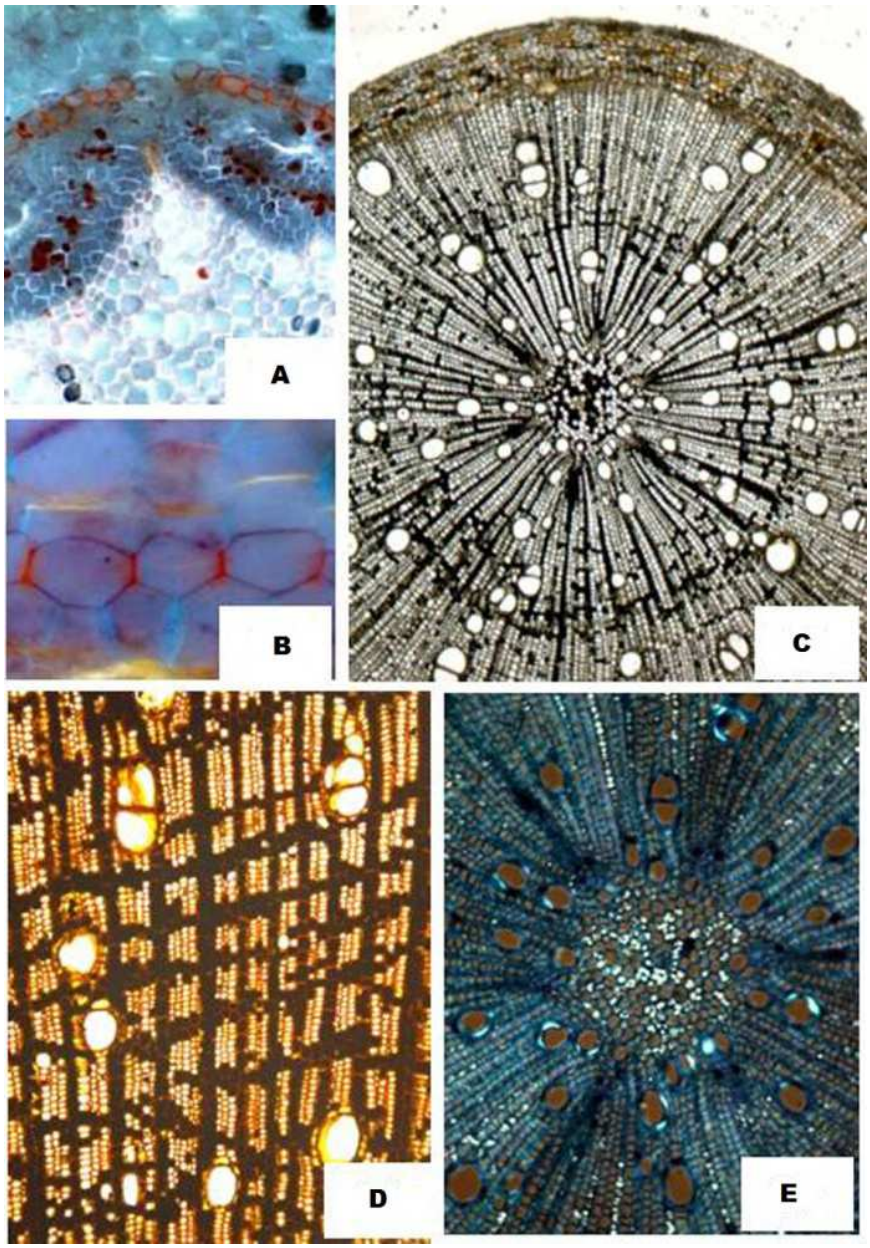


Figure 3: Proportion of different tissues in the lateral root

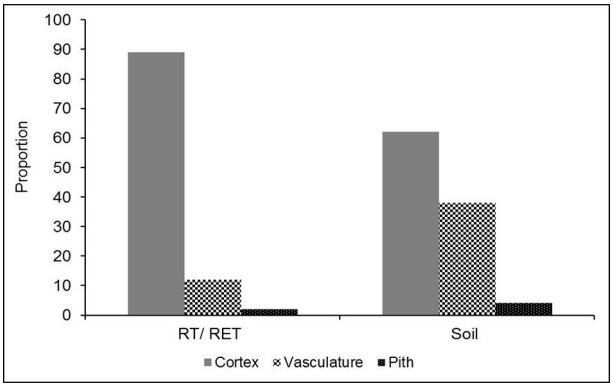
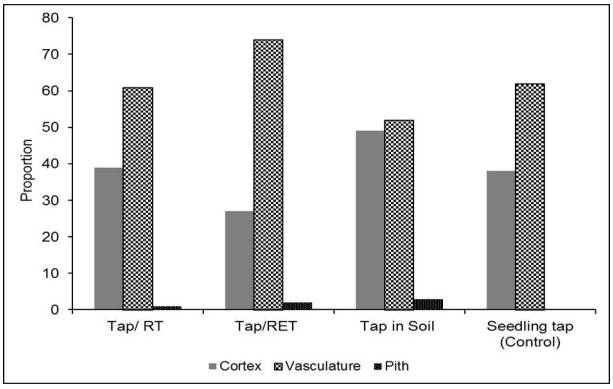


Figure 4: Proportion of different tissues in the tap root



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A study of the genetic variability of *Chryzopogon zizanioides* (L.) collected from Peninsular India

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ABSTRACT: *Chryzopogon zizanioides (L.) commonly known as vetiver grass, is a perennial species belonging to the family Gramineae. Because of the vast distribution of Chryzopogon zizanioides significant genetic variation may occur. The present study is an effort to analyse the variability of fourteen Chryzopogon zizanioides genotypes collected from different geographical and agro climatic regions of north and south parts of southern Western Ghats of India. The experiment was laid out in randomized block design with three replications and twelve plants per plot. Observations on twelve growth characters were recorded for three consecutive seasons and analyzed. All the characters studied showed statistically significant variation indicating the genetic difference between accessions. Phenotypic Coefficient of Variation (PCV) was higher than Genotypic Coefficient of Variation (GCV) in all cases indicating polygenic background of the characters and additive gene action. All the characters under study showed high percentage of heritability (H^2). Genetic Advance (GA) was also comparatively high in most of the cases. Among the characters, highest GCV and PCV were shown by fresh root weight followed by number of roots per tiller. Highest heritability (broad sense) was shown by root length and highest genetic advance by fresh root weight. Cluster analysis indicated the existence of considerable quantum of genetic divergence in the species under study.*

Key words: *Chryzopogon zizanioides*, Phenotypic coefficient of variation, Genotypic coefficient of variation, Genetic Advance, Heritability

Introduction

Chryzopogon zizanioides (L.) commonly known as vetiver grass is a perennial species belonging to the family Gramineae. Earlier it was known as *Vetiveria zizanoides*. It originated from India and the Africa continent (Xia et al. 1998). In India, only two species of vetiver are found: *Chryzopogon zizanioides* and *Vetiveria lawsoni* syn. *Vetiveria nemoralsis* and is found growing wild in almost all parts of the country (Ramanujam and Kumar 1964; Lal 2000, 2012). Vetiver is an economically, pharmaceutically and ecologically important plant. Roots of vetiver yield essential oil that is used as a basic material for perfumery and cosmetics (Champagnet et al. 2006; Maffei 2002; Massardo et al. 2006). It has strong ecological adaptability and resistance to drought, wetness, cold, heat, acidity and alkalinity. It has a strong root system, fast growth, easy plant set and high survival rate. It never turns into a weed, since it cannot be pollinated and fertilized. In the past, the utilization of the vetiver grass was limited to the extraction of the fragrant oil from the root (Cheng 1998). Generally it propagates by producing new shoots at the joints above the soil

surface and by branching at the joints below the soil surface. The seeds are very thin and have a short dormancy period. Because of the vast distribution of *Chryzopogon zizanioides* significant genetic variation may occur. Genetic diversity plays an important role in crop improvement, because the segregates between lines of diverse origin generally display an improved performance than those between closely related parental genotypes (Hariram and Appalaswamy 2014). The present study is an effort to analyse the variability of *Chryzopogon zizanioides* genotypes present in the different geographical and agro climatic regions of the southern part of Western Ghats of India.

Materials and Methods

The present experiment was designed to analyse the variability among different accessions of *Chryzopogon zizanioides* (L.). The experiment was carried out at Kerala Forest Research Institute, Peechi, Thrissur, Kerala, India during 2011- 2013. The study area is located at an altitude of 80 m above MSL at 10^{o} 31'51.4" N latitude and 07^{0}20'47.58" E longitude and it enjoys humid tropical climate. *Chryzopogon zizanioides* accessions collected from different geographical and agro climatic regions of north and south parts of southern Western Ghats of India were raised and observed critically. The experiment was laid out in Randomized Block Design with three replications and 60 plants per plot. Observations on twelve morphological characters such as plant height (cm), number of tillers, tiller girth, leaves per tiller, leaf length (cm), leaf breadth (cm), root length (cm), number of roots per tiller, fresh weight- shoot (g), fresh weight- root (g), dry weight- shoot (g) and dry weight- root (g) were recorded for three consecutive seasons and analysed.

Mean, standard deviation and critical difference (CD) were calculated in the case of the 12 characters studied. Analysis of Variance (ANOVA) was carried out to test the significance of variations between the accessions. Test of significance was done with reference to standard F table (Fisher and Yates 1963). Phenotypic and genotypic variance, coefficients of variation, heritability (broad sense) and genetic advance were calculated to find out the variation among the cultivars as per Singh and Choudhary (1985), Burton and Devane (1953) and Abraham (2000).

Phenotypic and genotypic variance

Phenotypic and genotypic variances for the different characters studied were estimated as per Singh and Choudhary (1985).

$$\text{Genotypic variance } (\sigma^2 g) = \frac{\text{MSS for treatment - MSS for error}}{\text{Number of replications}}$$

Phenotypic variance $(\sigma^2 p) = \sigma^2 g + \sigma^2 e$ where $\sigma^2 e$ is the error variance.

Coefficients of variation

Phenotypic and genotypic coefficients of variation were estimated following Burton and Devane (1953).

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sigma g \times 100}{X}$$

where σg= genotypic standard deviation and X = grand mean for the character.

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\sigma p \times 100}{X}$$

Where σp = the phenotypic standard deviation and X = grand mean of the character.

Heritability (broad sense)

Heritability (broad sense) is the fraction of the total variance that is heritable and is estimated as the percentage of genotypic variance over phenotypic variance (Jain 1982).

$$H^2 \frac{\sigma^2 \times 100}{\sigma^2 p}$$

Genetic advance

Genetic advance under selection was calculated using the following formula proposed by Abraham (2000).

$$GA = \frac{KH^2 \sigma p}{\overline{X}}$$

Where H^2 = heritability in the broad sense; σp = phenotypic standard deviation; K = selection differential which is 2.06 at 5% intensity of selection in large samples (Allard 1960); X = grand mean of the character.

Cluster analysis

The fourteen genotypes studied above have been subjected to cluster analysis using the software STATISTICA, following UPGMA procedure (Sneath and Sokal 1973) to find out the affinities between them, based on growth and yield characters.

Results and Discussion

All the twelve characters studied in the case of *Chryzopogon zizanioides* (L.) presently showed statistically significant variation indicating the genetic difference between the accessions (Table 1). Among the accessions, plant height varied from 81cm to 121.33 cm; number of tillers varied from 6.67 cm to 22 cm; tiller girth varied between 0.600 cm to 0.797cm; leaves per tiller between 4.467 to 6.580, leaf length from 50.033 cm to 78.940 cm; leaf breadth from 0.532 cm to 0.800cm; root length from 38.333 cm to 124.667 cm; number of roots per tiller from 10 to 51; fresh weight- shoot from 6.478g to 20.132g, fresh weight- root from 4.00 g to 21.794 g; dry weight- shoot from 3.524 g to 8.226 g and dry weight- root from 3.087 g to 7.422g.

Table 1: Variability of morphological characters in the vetiver cultivars studied

Sl. No	Accession	Plant height (cm)	No. of tillers	Tiller girth	Leaves per tiller	Leaf length (cm)	Leaf breadth (cm)	Root length (cm)	No. of roots per tiller	Fresh weight-shoot (g)	Fresh weight-root (g)	Dry weight-shoot (g)	Dry weight-root (g)
1	ODV-4	106.67	15.33	0.76	6.13	52.53	0.80	89.33	31.33	17.26	9.17	6.14	3.93
2	ODV-26	116.33	18.33	0.72	5.73	71.63	0.76	77.33	24.67	16.69	10.61	6.27	4.94
3	ODV-5	91.67	22.00	0.66	4.47	59.20	0.68	56.33	25.00	14.61	9.80	7.32	6.43
4	ODV-18	87.33	14.33	0.72	6.58	50.03	0.76	96.33	23.33	11.6	7.463	5.62	4.88
5	Periavura	109.67	11.00	0.71	5.67	78.90	0.79	117.00	45.00	13.35	21.79	4.62	7.42
6	Madapalli	99.67	13.67	0.61	4.85	59.46	0.78	124.67	25.33	9.68	11.67	3.52	3.93
7	Pandimedu	121.67	14.67	0.62	5.37	77.27	0.68	71.67	40.00	20.13	13.34	8.23	6.59
8	ODV-23	106.00	14.33	0.63	4.77	87.51	0.58	86.00	25.66	11.85	9.54	4.48	4.79
9	ODV-16	81.00	13.33	0.66	5.80	51.96	0.67	57.00	17.33	6.48	14.38	5.17	5.23
10	ODV-27	121.33	7.67	0.68	4.53	78.56	0.79	59.00	25.00	15.99	7.56	4.87	3.41
11	ODV-20	94.00	20.00	0.60	5.90	69.21	0.52	38.33	51.00	15.38	11.94	6.51	6.38
12	ODV-24	83.33	13.00	0.63	6.10	59.12	0.61	53.66	30.66	10.92	7.66	4.39	3.57
13	ODV-8	99.67	6.67	0.80	5.60	74.10	0.63	63.33	10.00	8.74	4.00	4.03	3.09
14	ODV-30	92.33	17.33	0.68	4.80	77.87	0.70	86.00	22.00	13.11	9.42	4.78	4.06
Mean		**100.76**	**14.41**	**0.68**	**5.45**	67.67	**0.07**	**76.86**	**28.31**	**13.27**	**10.58**	**5.43**	**4.90**
SD		**26.65**	**4.27**	**0.06**	**0.66**	12.07	**0.09**	**24.75**	**10.8**	**3.70**	**4.17**	**1.33**	**1.35**
CD		**4.81**	**2.58**	**0.53**	**0.63**	**6.67**	**0.52**	**14.1**	**4.68**	**1.51**	**1.49**	**1.38**	**0.94**

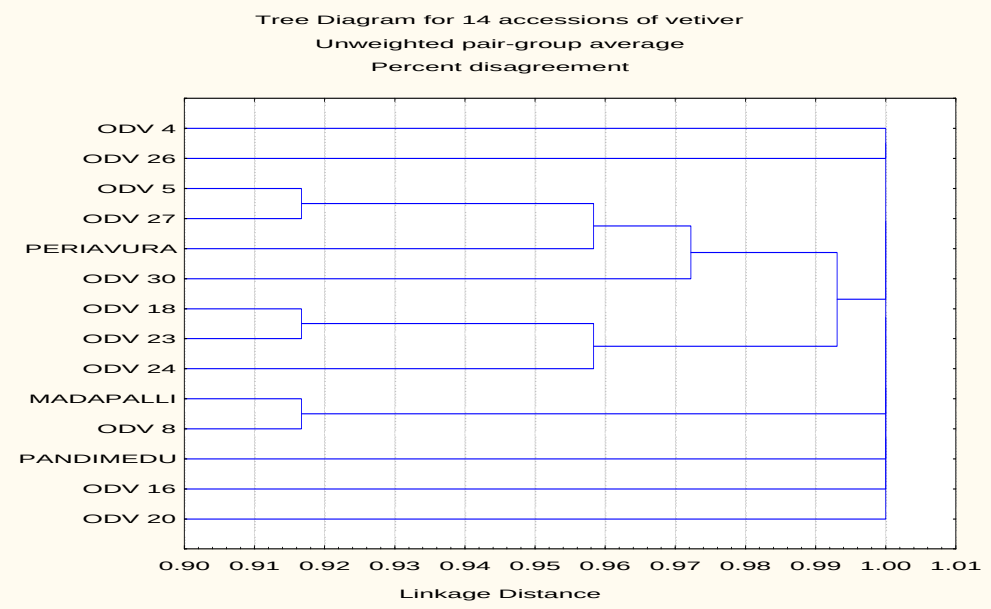
Phenotypic coefficients of variation were higher than genotypic coefficients of variation in all cases indicating polygenic background of characters and additive gene action. All the characters under study showed high percentage of heritability. Genetic advance was also comparatively high in most of the cases (Table 2). Among the characters, the highest GCV (38.98) and PCV (40.21) were shown by fresh weight of root followed by number of roots per tiller. The highest heritability (broad sense) was shown by root length (96.24). Study of variability of the genetic resources of a crop is the first step towards the understanding of the genetic diversity of the genetic stock available so as to use them in crop improvement programmes. Most of the agronomic characters of plants are polygenic and Vetiver is not an exemption. Polygenic characters show different levels of heritability based on their response to environmental factors. High heritability of characters indicates the limited influence of environment on these characters. Genetic advance of characters in percentage of mean is a very effective indicator of the characters that could be utilized in selection programmes. It is a measure derived from heritability. Statistically significant characters analysed presently showed genetic advance ranging from 14.31% to 75.18%. Dry weight of root showed the highest genetic advance.

Table 2: GCV, PCV, heritability (broad sense) and genetic advance of the characters in the case of the vetiver accessions studied.

SI. No	Characters	GCV	PCV	Heritability (broad sense) (%)	Genetic advance (%)
1	Plant height	13.16	13.46	95.51	26.50
2	Number of tillers	28.81	30.75	87.87	55.67
3	Tiller girth	8.05	9.30	75.00	14.31
4	Leaves/ tiller	11.38	13.39	73.58	20.30
5	Leaf length	5.84	6.16	94.81	12.03
6	Leaf breadth	12.09	12.81	87.5	23.08
7	Root length	31.62	32.64	96.24	64.67
8	Number of roots/ tiller	37.73	38.99	93.60	75.19
9	Fresh weight shoot	27.63	28.45	94.32	55.20
10	Fresh weight root	38.98	40.21	93.99	77.84
11	Dry weight shoot	22.78	27.39	68.78	38.88
12	Dry weight root	4.90	29.06	84.34	50.32

The statistically significant variability observed between different genotypes of a species can be utilized to group the genotypes into different clusters of genetically closer accessions based on genetic divergence studies. Cluster analysis carried out presently indicated the genetic identity of most of the accessions under study. ODV (Odakkali Vetiver) 4, ODV 16, ODV 20 and ODV 26 formed distinct clusters at a linkage distance of 1.0 when analysed based on the 12 characters under study (Fig. 1). Madapally and ODV 8 formed a distinct cluster and ODV 5, ODV27, Periavura, ODV 30, ODV 18, ODV 23 and ODV 24 formed another cluster. ODV 5 and ODV 27; ODV 18 and ODV 23 and Madapalli and ODV 8 were the closest genotypic pairs. Genotypes belonging to different clusters can be considered to be genetically divergent and such genotypes could be used for breeding programmes.

Figure 1: Cluster diagram for the 14 accessions of vetiver studied



Genetic variability and association analysis for yield and yield components in indigenous and exotic collections of vetiver was done by Lal (2000). He reported that plant height by itself may be a good direct selection criterion to obtain promising lines with longer roots in vetiver aiming at a better oil yielder and a good soil binder. Lal (2013) studied the genetic variation for seven traits of 40 Vetiver genetic stocks and found that all the traits expressed high heritability, variation and medium to high genetic advance.

Analysis of differential variability of quantitative characters has been carried out by earlier workers in other crops like cardamom (Radhakrishnan et al. 2006), coffee (Nikhila et al. 2002; Raghu et al. 2003) and medicinal and aromatic plants (Misra et

al. 1998; Jayasree et al. 2006) so as to utilize the findings in future improvement programmes. The present study unravels such a stock of genetic diversity in Vetiver which could be exploited potentially for the development of promising lines of the plant in future.

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Multiple users: Challenges and opportunities in sustainable management of landscapes – a case study from Wayanad, Kerala, India

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ABSTRACT: *The present work focuses on the factors attributing to the sustainable management of bio-resources being accessed by different ethnic communities from a same landscape and how the external pressures breaking the cultural values are associated with conservation and thus leading to un-sustainable resource management regime. The study was conducted among three different ethnic communities living adjacent to Wayanad wildlife Sanctuary, Wayanad, Kerala. The study relied on ethnographic methods and focus group discussions to elicit information about wild edibles forage practices adopted by different communities and conservation and sustainability issues pertaining to management of landscape. The uniqueness in cultural traditions, customs, consumption pattern, knowledge, socio-economic background and prevailing traditional institutions determine the extent of resource utilization by each community. The differences in these attributes helped to evolve sustainable management practices that embedded with certain traditional norms and values. However, opportunities for marketing of selected non timber forest produces like honey and gooseberry induced unsustainable harvest and paved way for breaking the conservation norms. The Kattunaikas, who are experts in collecting these resources gained more benefits and that led to long lasting changes in their coping mechanisms and preferences over other resources. The other two communities identified new resources potential for market. The study also reveals that the integration with market economy influenced the food habits of the tribes and found that the transfer of knowledge between generations has come down among all three ethnic communities.Traditional value systems and biodiversity management practices are closely interlinked. When external influences exceed the traditional values, it in turn perpetuates threats in biodiversity conservation. This necessitates formulation of strategies that accelerate traditional value principles in sustainable management of bio-resources.*

Key words: Sustinable management, Ethnic communities, Conservation

Introduction

Forest and forest products play a vital role in the life and livelihoods of people around the world from the time immemorial. An estimated 1.6 billion people around the world depend on forest for their livelihoods (World Bank 2004). In India, it is estimated that 50 million people live in and along the periphery of forests (NCHSE 1987). Forest and non-timber forest produces provide significant share of income and employment to people, living in and along the periphery of forests (Rasul 2008). India's tropical deciduous forests contribute significantly to the national economy by its wide range of Non Timber Forest Products (Chopra 1993).

In India over 3000 plant species provide commercially significant products (Tewari 1994).

A study conducted in the *Biligiri Rangan* hills found that 70% of the households extract on an average 1000kg of *Emblica officinalis* fruits realizing US $45 as cash income each year (Uma Shankar et al. 1996). In Nilgiri Biosphere Reserve, 50 to 75% of the households gather diverse forest products, which contributes up to 60% annual income to the dependent households (Narendran et al. 2001). NTFPs, in principle, contribute to the economic wellbeing of the forest depending communities (Uma Shankar et al. 1996; Hall and Bawa 1993) along the forest peripheries in India. Even though NTFPs play vital role in rural livelihoods, studies (Ganesanen 1993; Hegde et al. 1996; Gubbi and MacMillan 2008) showed that the current pattern of NTFPs extractions have threaten the integrity of forest ecosystems. Increasing population and pressure from market are important reasons attributed to the unsustainable harvest of resources from forest (Uma Shanker et al. 2004). Present paper highlights the factors affecting the pattern of harvest of NTFPs in Wayanad wild life sanctuary.

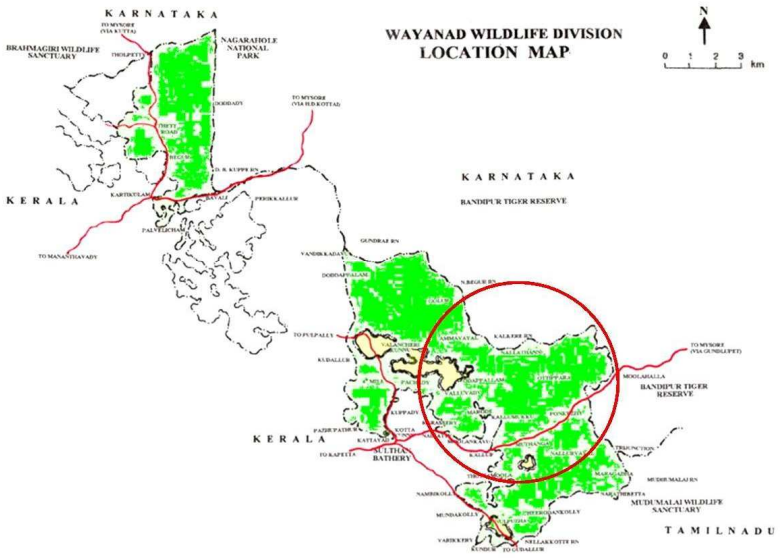
To ensure the best use of the forests, it is necessary for rural communities to share the responsibility of forest management with the forest department (Appasamy 1993). Changes in the agricultural system and a gradual decline in the importance of NTFPs in people's lives will reduce the incentive for maintaining NTFP species and lead to the eventual loss of the landscapes (Schreckenberg 1999). A study conducted in Wayanad Wildlife Sanctuary shows that alternative income sources would greatly reduce the dependence on forests and hence increase the conflict between people's interest and forest management in conservation activities (Shylajan and Mythili 2007). Therefore an effective governance system is essential for the conservation of forest resource and landscapes and it should be in a way to ensure the sustainability of resources. Studies regarding the natural regeneration of NTFP is needed for preparing effective management plan for forest landscapes (Gunatilake et al. 1993). In this paper we examine, (1) nature and extent of the use of forest resources by three tribal communities, (2) factors that influence the harvest of resources and (3) harvesting practices adopted by different forest communities.

Materials and Methods

Study area

Wayanad Wild Life Sanctuary (WWLS) is situated in contiguous protected network of Nagorhole and Bandipur of Karnataka in the North East and Mudumalai of Tamil Nadu in the South East. The sanctuary is divided into four ranges for administration. We carried our study in the Muthanga range of the sanctuary (Fig. 1).

Figure 1: Location map of the study area



The tribal communities

The major tribal communities in the study area are Paniya, Kuruma, Kattunaikka, Urali and Adiyan. For this study, we selected Kattunaikka, Paniya and Kuruma, the dominant tribal communities in terms of population size in the Muthanaga range. The Kattunaikkas are forest dwellers living inside the sanctuary area, who depend mainly on forests and its resources for their livelihood. The Paniya communities are the landless communities depending on agriculture wage labour for their survival. They also depend on forest for collecting resources for their own consumption and market. The Kurumas are tribal communities engaged in agriculture and cattle rearing. They are marginal landholders. Apart from agriculture, they occasionally depend on forest for NTFPs.

Data collection

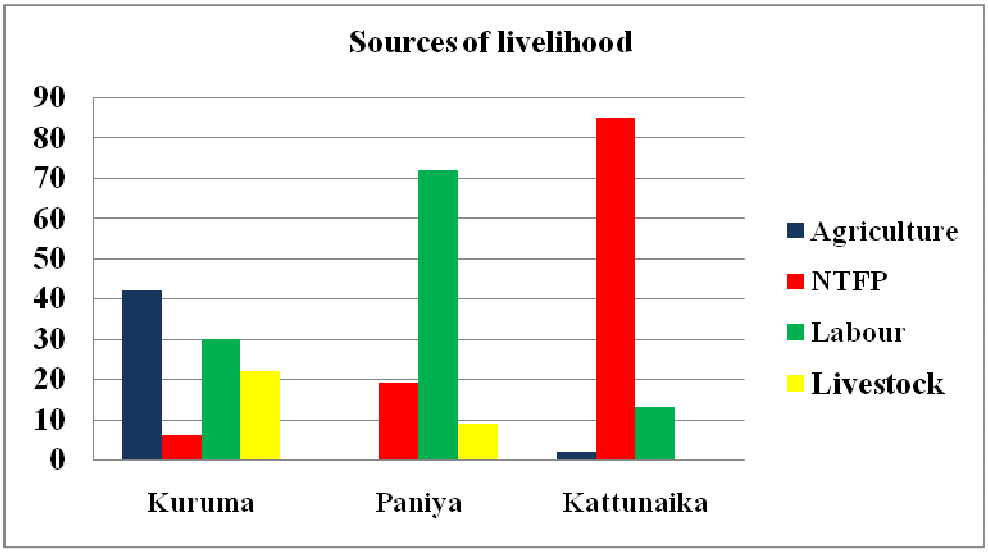
A combination of ethnographic and household survey methods has been used for the purpose of data collection. Both qualitative and quantitative data were collected by (1) exercising a questionnaire survey, (2) conducting focus group discussions and key informant interviews and (3) through transect walk. A total of 45 households were covered in the survey to ensure equal representation of three selected communities.

Results and Discussion

Dependency on forest by three communities

Among the Kurumas, 42% households depend on agriculture (crop production) as their principal source of income, while 30% of households depend on agriculture wage labour and rest of them depend a combination of crop production, wage labour and livestock rearing. Collection of NTFPs is an occasional activity for majority of them. Among the Paniyas, 72% of the total household depend on agricultural wage works and none of them are directly involved in crop production in their own field. NTFPs contribute to 19% of household income while livestock provides a lesser share (9%) of gross household income. The Kattuniakkas depend mainly on forest resources. NTFP collection contributes more than 85% of their household income (Fig. 2). They work for forest department in forest protection activities like fire line marking and weeding of exotic plants like *Lantana*. The study shows that Kattunaikkas depend more on NTFPs while the Kurumas depend the least.

Figure 2: Sources of livelihood in three communities



Economic and cultural background of communities has an important role in extraction of forest resources (Lozada 2001; Augustine et al. 2008). In Nilgiri Biosphere Reserve, the tribes obtain a larger proportion of their annual income from NTFPs as compared to the non tribes. On an average, NTFPs provide up to US $59 in cash per year per household (Narendran et al. 2001). The present study shows that Kattunaikka tribes depend more on forest and NTFPs when compared to other tribal communities in the study area.

Source of food

People depend on multiple sources like market, own farm, forest and its peripheries for their food purpose. Even though the Kurumas depend more on their own farm for food, the aggregate data showed that most of the households depend primarily on market for procuring food stuffs (Fig. 3). Leafy greens, mushrooms, honey, tubers, fruits and seeds, medicinal plants and firewood are the important resources being accessed by different households across three tribal communities in the study area. Even though there are differences across communities within the resources in terms of number of species, mushrooms collection is relatively high (32%) followed by leafy greens (28%), fruits and seeds (26%) and tubers (14%).

When analyzing the community wise dependency on different food items, the Kattunaikka tribes collect and consume large number of wild food species when compared to the other two communities. The study shows that the Kattunaikkas alone use 96 species of plants species as food from forest and peripheries while the Paniyas access 73 species (Table 1). The Kurumas depend on just 41 species including tubers, mushrooms, leafy greens, fruits and seeds from forest. Of the total 96 species accessed by the Kattunaikkas, 43 species are exclusively accessed only by them. The Paniya men and women exclusively collect 26 species. Relatively high incidence of dependency on exclusive species by the Kattunaikkas and the Paniyas indicates the exclusive domains of knowledge on collection, processing and consumption of these wild edible species. The Kattunaikkas depend more on tubers and mushrooms compared to other two tribal communities. Multiple reasons are attributed to the increased access on diverse species of mushrooms and tubers. Unique knowledge about processing/cooking is one of the attributes that help the Kattunaikkas access more species. For example, tuber called *kottunoora* (*Dioscoria hispida*) is considered as toxic by all communities other than Kattunaikkas. In order to avoid toxicity, the Kattunaikkas put the chopped tubers in a white cloth and keep it in running water for over 24 hours before being cooked (Narayanan et al. 2004). Similarly, Kattunaikkas are more adept at recognizing edible mushroom varieties on the basis of odour and colour (Narayanan et al. 2004). They are also familiar with the landscapes in which each species occur in abundance. The vernacular nomenclature used by the Kattunaikkas for different species of mushrooms or other wild edibles indicate the link with either landscapes or habitat in which they occur. For example, the folk name *puttanavai* (*Plutorous* sp.) indicates its occurrence in termite pits. The close association with landscapes, knowledge associated with harvest of resources, knowledge and skills in processing, willingness to forage deep inside the forest etc. give a cutting edge to the Kattunaikkas over other communities in accessing and consuming large number of wild edible species in general and exclusive species in particular.

Figure 3: Community dependency on different sources for food

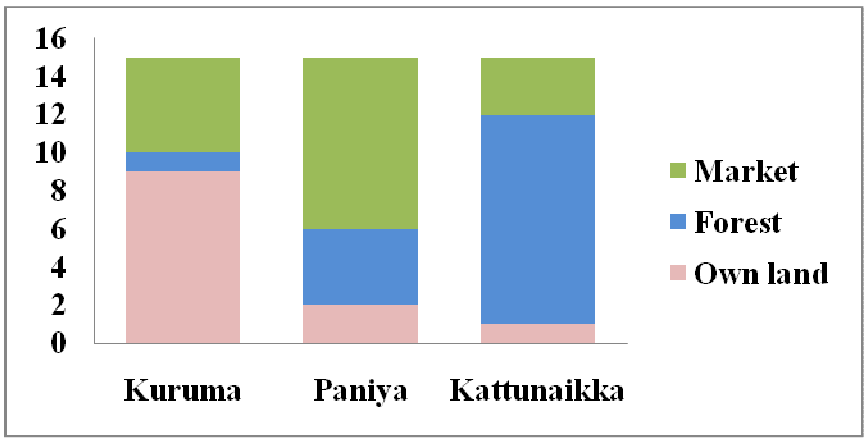


Table 1: Major plant genetic resources accessed by the three communities

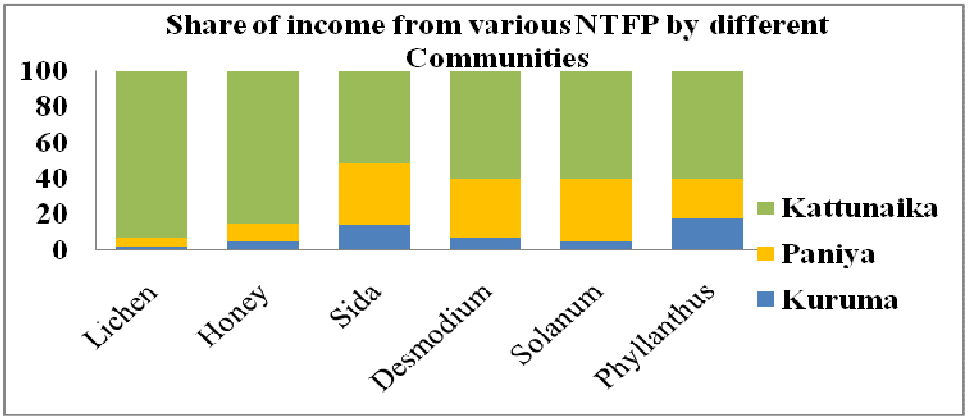
Si No	Wild-food category	*Kuruma*		*Paniya*		*Kattunaikka*	
		Total (Species)	Exclusive use (Species)	Total (Species)	Exclusive use (Species)	Total (Species)	Exclusive use (Species)
1	Leafy greens	9	0	28	12	15	9
2	Fruits& Seeds	13	0	18	10	22	7
3	Tubers	2	0	4	0	19	10
4	Mushrooms	17	0	23	4	40	17
	Total	41	0	73	26	96	43

Factors influencing the harvest of NTFPs

Gooseberry, diverse medicinal plants, honey and lichen are the important non-timber forest produces collected for marketing purpose by different tribal communities in WWLS. Market price is one of the crucial factors that determine the quantity of harvest of resources. The consistent market price of honey and lichens over a period of more than a decade helped them to gain the status of high value NTFPs. The price of lichen in the year 2001 was just Rs. 49/kg, which increased to Rs. 250/kg in 2013, marking more than fivefold increase. Similarly, the honey price was Rs. 50/kg in 2001, which increased to Rs. 160/kg in 2013. While the price of honey and lichen shows an increase in actual and relative terms, the price of other NTFPs like *Sida*, *Desmodium*, *Solanum* and Gooseberry shows a narrow increase in actual terms.

Acquisition of unique skill sets and knowledge to harvest honey and collection of lichens helped the Kattunaikkas to benefit more from NTFPs marketing, compared to other tribal communities (Fig. 4).The *Paniya* men and women are engaged mainly in collecting medicinal plants like *Sida, Desmodium* and S*olanum.* Most of these medicinal plants are occurring along the periphery of forests.The Kattunaikkas never hesitated to travel long distance inside the forest in search of NTFPs. Since they are familiar with forest and engage in harvesting NTFPs in groups, they used to spend more than a week at a stretch. Willingness to spend more days inside the forest gives them advantage over others in NTFPs harvest. The Paniyas and the Kurumas on the other hand spend up to seven hours at a stretch. They return before the sunset. All three tribal communities collect gooseberry for either market or for own consumption (Narayanan et al. 2004).

Figure 4: Income profile of the three communities



Seasonality of forest dependence

Even though, there is no specific institutional arrangement in place for controlling the over harvest of these resources, an invisible equilibrium of harvest is maintained for most of the NTFPs due to various reasons. The skill set and knowledge to harvest certain high value resources like lichen and honey are confined to the Kattunaikka tribal communities. They face little or no competition from other communities in harvesting these resources.

The study shows that different tribal communities depend on forest in different frequencies to collect diverse NTFPs in different seasons. Kattunaikkas depend on harvest of NTFPs throughout the year. At the same time, the Paniyas and the Kurumas depend on forest for NTFPs only when they face acute shortage of employment or financial insecurity. During peak agricultural season, the Kurumas

and the Paniyas abstain from collecting NTFPs. During Monsoon seasons, the Paniyas depend on forest for harvesting wild edibles including leafy greens. At the same time, due to competition from the Paniyas on wild edibles, the Kattunaikkas rely more on mushrooms and less on leafy greens during the same period. The Kattunaikkas enjoy dominance over the collection of mushrooms from forest, as they are well versed in identifying edible ones compared to others (Table 2). Kattunaikkas are more familiar with forest environment and wild life. They live inside the forest areas and engage in collecting NTFPs in small groups and stay inside the forest for many days at a stretch and harvest NTFPs. Collection of NTFPs in long stretch makes it profitable. Certain resources are historically assigned with an inferior status and thus collecting such resources is avoided by certain communities. For example, Kattunaikkas assign an inferior value to a type of mushroom known as *chevikkoon* and it is extensively collected and consumed by Paniyas. At the same time certain resources including Gooseberry and medicinal plants like *Sida* are facing acute competition, because they are easy to harvest and accessible to all communities.

Table 2: Frequency of access in an year by different community

MONTH	PANIYA	KURUMA	KATTUNAIKKA
JAN	Amla,Sida, Cheevakka	Tubers	Kattumanjal,Honey, Cheevakka,Amla, Lichen, Tubers
FEB	Amla, Cheevakka, Kaattumanjal	Amla, Cheevakka, Kaattumanjal	Honey, Amla, Lichen, Tubers
MAR	Cheevakka, Kattumanjal, Fruits	Amla, Cheevakka, Kattumanjal	Honey, Amla, Lichen, Tubers
APR	Fruits and Seeds		Fruits, Seeds, Lichen
MAY	Fruits and Seeds		Fruits, Seeds, Lichen
JUN	Leafy green, Mushrooms	Leafy green, Mushrooms	Leafy green, Mushrooms
JUL	Leafy green, Mushrooms	Leafy green, Mushrooms	Leafy green, Mushrooms
AUG			Moovila, Chunda, Sida, Lichen
SEP			Honey, Lichen
OCT	Honey, Amla, Lichen	Honey, Amla	Honey, Lichen
NOV	Honey, Amla, Lichen	Honey, Amla	Honey, Amla, Lichen
DEC	Amla, Sida		Honey, Lichen, Amla, Tubers
High Frequency		Medium Frequency	Low Frequency

Table 3: Mode of harvest for selected NTFP's

SI. No	Name of Species	Local Name	NTFP Collection Months	Phenology
1	*Acacia sinuata* (Lour.) Merr.	Cheevakai	Jan- Mar*	Feb - Mar
2	*Phyllanthus emblica* L.	Nelli	Nov- Jan***	Jul - Feb
3	*Pseudarthria viscida* L.	Moovila	Jul- Sep**	Nov - Mar
4	*Solanum violaceum* Ortega.	Chunda	Jul – Sept**	Aug - Dec
5	*Sida rhombifolia* L.	Kurumthotti	Jul – Sept**	Sept- Dec
6	*Curcuma aromatica* Salisb.	Kattumanjal	Jan – Mar*	May - Jun
7	*Curcuma zanthorrhiza* Roxb	Manjakkoova	Jan – Mar*	Apr - Jul
8	*Desmodium velutinum*	Orila	Jul – Dec**	Oct - Feb

* Harvest at the time of flowering ** Harvest before flowering *** Unscientific harvest

Narayanan et al. (2004) observed a sustainable harvesting practice of tubers followed by the Kattunaikka tribal community in Wayanad. After the tuber is dug out, the apical portion of it, along with the stem (vine) is put back in the pit and filled with soil so as to assure the regeneration for the next season. Another piece is placed in a small pit close by to confuse the wild boars that are in constant competition with the tribes for wild tubers. The present scenario is entirely different from the past, only the aged people among the Kattunaikkas follow this practice while others (including non-tribes) harvest the entire portion and they are little concerned about the conservation of forest resources.

Lack of knowledge about the importance of sustainable harvest, market induced competition for resources and unsustainable and destructive harvest of existing resources pose threats to diversity of NTFPs and consequent setbacks in the life and livelihoods of forest depending communities. *Kattunaikkas* are of the opinion that they used to walk more distances to collect NTFPs compared to past, which indicates the intensity of scarcity of resources compared to past. Participatory approach is one of the important tools for preventing destructive harvest of NTFP's. From the evaluation of participatory resource monitoring system for gooseberry (*Phyllanthus emblica*) in Biligiri Rangaswamy Temple Wildlife Sanctuary underlines it (Setty et al. 2008). By adopting participatory monitoring techniques they successfully implemented effective resource mapping, better productivity estimation and most importantly assurance of an improved and sustainable harvesting system. In WWLS, there prevails no community restriction or

institutional mechanism to govern the harvest of NTFPs, which in turn deplete the stock of biodiversity of NTFPs.

Conclusion

Tribal communities living inside and adjacent to forest depend on NTFPs for their multiple needs and income. Among them the Kattunaikkas depend exclusively on forest resources for their survival. Due to various reasons, diversity of NTFPs has come down drastically, which in turn increased the pressure on existing resources and hence led to unsustainable extraction. Lack of proper governance, absence of regulating mechanisms and consequent unsustainable harvest of resources are severe problems faced by the forest dwellers. Even though, the Kattunaikkas enjoy a cutting edge over other communities in terms of harvesting high value NTFPs, they also face hardships in harvesting resources from interior forest. They are of the opinion that if the present trend and pattern of harvest continues, the stock of resource base will deplete within short future. People's participation is vital in forest regeneration and conservation of biodiversity. Similarly, there is ample scope for value addition and market value chain development in order to benefit the forest gatherers. The study suggests effective institutional mechanism for regulating the harvest of resources in an optimal way and maximising the benefits from NTFPs through value addition of available resources.

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Influence of organic inputs on seed germination and seedling growth of *Oroxylum indicum* Vent.

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ABSTRACT: *Seed germination and seedling growth of Oroxylum indicum Vent. was studied under the lab and nursery conditions. In seed germination studies pre-treatment with panchagavyam showed maximum vigour index (556.2) when compared to other traditional and conventional methods. Organic inputs like panchagavyam and cow dung highly influenced seedling growth also under nursery conditions. Maximum shoot length (12.62 cm) and root length (2.72 cm) were observed in the case of panchagavyam treated seedlings. The use of cow dung also showed significant results. This study revealed that Oroxylum indicum can be effectively propagated using traditional methods and organic manuring. The use of organic inputs and traditional methods for germination and growth of Oroxylum indicum is recommended since it is a medicinal plant which is largely used in Indian systems of medicine.*

Key words: Seed germination, Panchagavyam,Vigour index.

Introduction

Oroxylum indicum Vent., a member of the family Bignoniaceae, is a small or medium–sized deciduous tree growing throughout India. All parts of the plant are used for medicinal purposes. Due to its high therapeutic value this plant is widely used in Ayurveda (Sivarajan and Balachandran 1994). The plant species is naturally propagated by seeds which have short viability period and poor seed set. Problems related to its natural propagation and indiscriminate exploitation for medicinal purpose has pushed *Oroxylum indicum* to the list of endangered plant species of India (Ravikumar and Ved 2000). This plant possesses antioxidant, antifungal, antimicrobial, anti-inflammatory, antibacterial, antiarthritic and anti-cancer properties (Warrier et al. 1995). *O. indicum* is extensively used in Ayurveda as an important ingredient of Dashmula which is a compound decoction of 10 roots (Anonymous 1987).

The species is generally propagated by seeds but its germination rate and viability is very low due to seed abortion (Dalal and Rai 2004; Talari et al. 2013). Destructive and non-sustainable collection methods coupled with low regeneration and habitat destruction have posed serious threats to the survival and availability of *O.indicum* (Yasodha et al. 2004). Raghu (2006) has carried out studies on seed germination of *O. indicum*. However, the role of organic nutrients in the augmentation of seed germination of this species is not yet studied. The main objective of this study was to study the influence of organic inputs like *panchagavyam* and cow dung on seed

germination and seedling growth of the plant, *O. indicum*. The seed biology of the plant is given in Table 1.

Table 1: Seed Biology of *Oroxylum indicum* Vent.

Collection place	Cherpulassery
Date of collection	06-12-2012
Flowering period	June-July
Fruiting period	November – January
Collection season	December- January
Quantity	3.821 g
Processing	Seeds are extracted from the pod and sun dried
Fruit weight	10- 11pod / kg
Shape of Fruit	Sword-shaped
Weight of single pod	0.111 – 124 kg
Fruit length (Avg)	59.58 - 61.83 cm
Fruit (Thickness)	8.86 - 9.85 mm
Breadth of middle of fruit	6.85 - 7.6 cm
Breadth of tip of fruit	5.68 - 6.93 cm
Seed weight	9038 - 9045/ kg
MC %	12.095 %
Seed emptiness	30.56 to 47.62 % among 13 pods
Average number of seeds per pod	355-400
Size of winged seed	7.08 cm X 4.22 cm
Size of seed without wing	1.62 cm X 1.14 cm
Size of Seed (Thickness)	0.78 - 0.85 mm
Insect Infestation	None observed
Fungal infection	Observed
Storage physiology	Orthodox
Viability period	up to one year
Storage Temperature	4^0 C
Germination type	Epigeal
Germination Percentage	80- 90%
Viability testing	Germination test

Materials and Methods

The present study was conducted over a period of seven months from 2012 February to 2013 August in the Kerala Forest Seed Centre, Kerala Forest Research Institute, Peechi, Thrissur (Latitude – 10^0 31′ 47′′ N; Longitude- 76^0 22′ 7.5′′E; Altitude – 45m above msl) as a part of the research project titled "Evaluation of indigenous methods of nursery techniques for medicinal plants" during 2012-15.

Well matured pods of *O. indicum* were collected from Cherpulassery, Palakkad District, Kerala State. After extracting seeds from the pods they were surface dried under shade for one week and used for study. The basic seed technology tests such

as fruit weight, seed weight, moisture content, etc. were carried out. The processed seeds were tested for moisture content on fresh weight basis over dry weight (ISTA 1999). The seeds with moisture content (6.97%) were soaked overnight with 3% *panchagavyam* (a mixure of 5:1 cowdung and ghee in a 5:3:3:5 cow's urine, curd, milk and water formulation) (Jayashree and George 2006) and cold water for germination studies. Unsoaked seeds served as control. After soaking seeds were washed with distilled water and then sown for germination using vermiculate medium (Chacko and Pillai 1997). The seeds were sown in trays and kept in the lab at an average temperature of 30°C for germination. Observations were made on germination starting from radicle emergence up to one week after sowing as detailed in Table 2. Germination was recorded daily from the date of sowing and continued till the germination ceased. Daily germination percentage was summed up to obtain cumulative germination percentage for each treatment on each assessment date. All the experiments were carried out with four replications of 30 seeds each. The germination trials were conducted in completely randomized design and tested for statistical significance using analysis of variance (ANOVA). The imbibition periods were recorded in all treatment combinations. Mean emergence time (MET) was calculated by modifying the following formula of Butola and Badola (2004), MET = $\sum \frac{(fx)}{\sum x}$. Where x is the number of newly emerged seedlings on each day, f the number of days after the seedlings were set to emerge and $\sum x$ the total number of seedlings emerged at the end of the experiment. The germination energy, defined as the germination percentage when the mean daily germination reached its peak, was also determined (Paul 1972). Germination Index expressed as speed of germination was also calculated (AOSA 1983).

The seedlings were allowed to grow under same environmental conditions for three months. The uniform sized seedlings were transplanted after one week to poly bags of 15 cm x 10 cm size containing potting mixture of Soil: Sand: Compost (2:1:0.5). Then after two weeks the plants were manured with cow dung powder (10g/plant) and 3% *panchagavyam* as foliar spray (Natarajan 2002) for growth performance studies. The experiment was started with three replications for each treatment with 24 seedlings in the 1stweek of March 2013. Measurements of shoot length, shoot girth, number of leaves, collar diameter, root length and root girth were taken from 6 plants each carefully uprooted at 21 days intervals and tabulated (Table 2).

Vigour Index of the seedlings was calculated according to Abdul- Baki and Anderson (1973) as germination percent X seedling total length (shoots and root length). Survival percentages of seedling were noted at 30 days after the germination. The data were subjected to one way analysis of variance and the treatment means were compared with LSD (Least Significant Difference) wherever necessary.

Results and discussion

'Days taken for the commencement of germination' was not influenced by different treatments when compared to control (Table 3). Germination of seeds commenced after 3 days of sowing in the control, and in the other treatments it happened after 7 days. Seeds treated with 3% *panchagavyam* showed the highest germination percentage (95.91%) among all the treatments but the variation was not statistically significant when compared with the control (95.83%). Pre-soaking of seeds in cold water overnight resulted in lower germination percentage (95%). In the case of vigour index, 3% *panchagavyam* treatment showed better results when compared with the other treatments.

In the growth performance experiment organic treatments like cow dung and *panchagavyam* showed maximum results in the case of all the parameters studied (Table 4 & Fig. 1-4). Mean shoot length of *O. indicum* treated with different fertilizers like cow dung, *panchagavyam* and control for the period of 21, 42 and 63 days is as presented in Table 3. Observation on 21st day showed that height of *O. indicum* was significantly higher when *panchagavyam* and cow dung were applied, when compared to the control plants. On the 42nd day, shoot length was higher in *panchagavyam* treated plants than in other treatments, followed by cow dung which also gave significantly taller plants. On the 63rd day also seedlings were taller in the case of cow dung and *panchagavyam* treated plants when compared to the control. Study of mean shoot girth on 21st day showed that *O. indicum* grew significantly thicker when fertilized with cow dung compared to those treated with *panchagavyam* or the control plants (Table 3). However subsequent results on the 42nd day and 63rd day were similar to those obtained in the first week. In total, shoot girth was thicker in *O. indicum* seedlings grown with cow dung when compared to *panchagavyam* which was in turn significantly thicker than those recorded in control seedlings. Mean numbers of leaves recorded from *O. indicum* fertilized with cow dung, *panchagavyam* and the control, observed on the 21st day intervals are shown in Table 3. The results on 21st, 42nd and 63rd days after sowing did not show significant variation in mean number of leaves in the treatments used. Mean collar diameter recorded in three intervals in this plant showed that the treatments did not induce any significant difference in the character. Mean root length of *O. indicum* recorded on the 21st day was significantly higher in the case of *panchagavyam* and cow dung treatments when compared to the control plants. On the 42nd day shoot length was higher in the case of cow dung when compared to other treatments. It was followed by *panchagavyam* which gave significantly taller plants. On the 63rd day also seedlings were taller with cow dung and *panchagavyam* when compared to those that did not receive any fertilizer. In the case of mean root girth also the application of cow dung and *panchagavyam* showed significant results than the control.

The biomass percentage per seedling was the highest in cow dung and *panchagavyam* that was significantly different from control. The lowest shoot biomass percentage and root biomass percentage were observed in control plants. There were studies which agreed with our results that organic manures like cow dung and *panchagavyam* improved the growth of plants at seedling level (Harisha et al. 2010; Jayashree and George 2006; Kumar et al. 2011; Wisdom et al. 2012). From the study it could be concluded that organic inputs like cow dung and *panchagavyam* play an important role in improving seedling vigour and seedling growth. Being a medicinal plant, use of organic inputs for its germination and growth is highly recommended.

Table 2: Effect of pre-germination treatments in *Oroxylum indicum*

No.	Treatment	Germination (%)	Shoot Biomass (%)	Root Biomass (%)	Seedling length (cm)	Vigour index
1	Control	*95.83*	10.308	27.975	5.43±.14	520.357
2	*Panchagavyam*	*95.91*	14.295	47.413	5.8±.14	556.278
3	Cold water	*95*	14.739	26.355	5.5±.16	522.53

Table 3: Germination characteristics of *Oroxylum indicum*

Treatment	Germination (%)	Imbibition period (Days)	Total germination period (Days)	MET	GE	GI
Control	95.83	3	20	8.66	72.5	1.92
Panchagavyam	95.91	7	12	8.29	42.5	2.78
Water soaking	95	7	17	8.4	65	2.31

Table 4: Growth performance of *Oroxylum indicum* seedlings with organic treatments

Treatment	Mean shoot length (cm)	Mean shoot girth (mm)	Mean number of leaves	Mean collar diameter (mm)	Mean root length (cm)	Mean root girth (mm)
After 21 days						
Control	7.92 ±2.18	1.87±.08	6±0.09	2.38±0.26	7.33±0.78	1.28±0.22
Cow dung	8.72±0.92	2.33±0.15	6±0.15	2.78±0.81	11±0.24	2.15±0.26
Panchagavyam	9.53±1.28	2.18±0.21	6±0	2.55±0.20	11.03±0.2	2.27±0.1
After 42 days						
Control	10.87±2.15	2.03±0.19	7.2±0.98	2.65±0.24	11.13±3.2	2.12±0.3
Cow dung	12.21±2.13	2.4±0.14	6.5±1.22	2.78±0.19	13.86±6.25	2.58±0.67
Panchagavyam	12.23±1.41	2.28±0.20	7±1.1	2.62±0.18	11.12±7.31	2.62±0.33
After 63 days						
Control	11.75±1.47	2.13±0.08	6.7±1.5	2.83±0.5	8.75±4.2	1.97±0.44
Cow dung	11.47±1.21	2.28±0.18	7±1.09	2.75±0.16	16.75±1.66	2.37±0.16
Panchagavyam	12.62±0.91	2.4±0.14	7±1.09	2.72±0.07	11.53±4.78	2.42±0.46

Figure 1: Evaluation of traditional and conventional fertilizers in *Oroxylum indicum* using *panjagavyam* and cowdung (Trial-I: After 21 days)

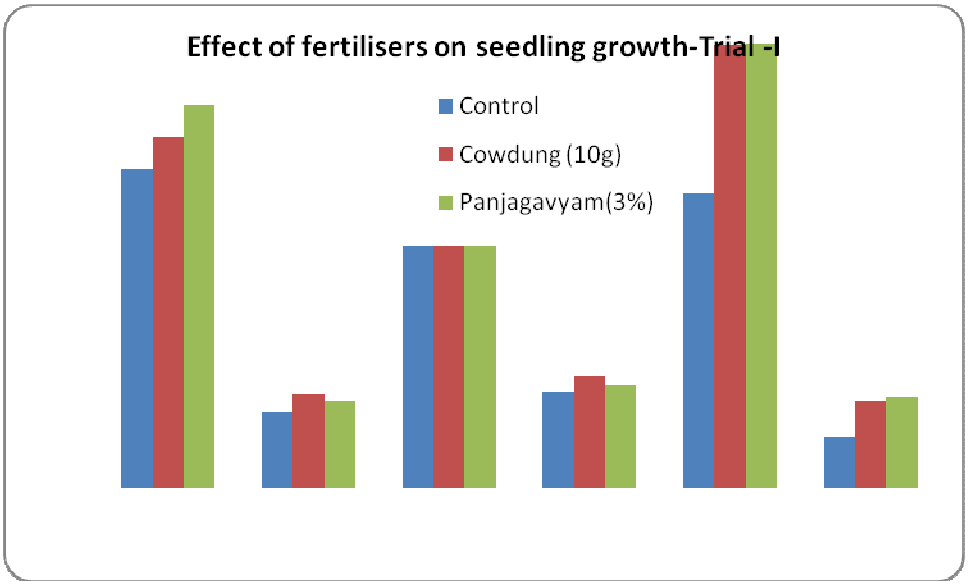


Figure 2: Evaluation of traditional andconventional fertilizers in *Oroxylum indicum* using *panjagavyam* and cowdung (Trial-2: After 42 days)

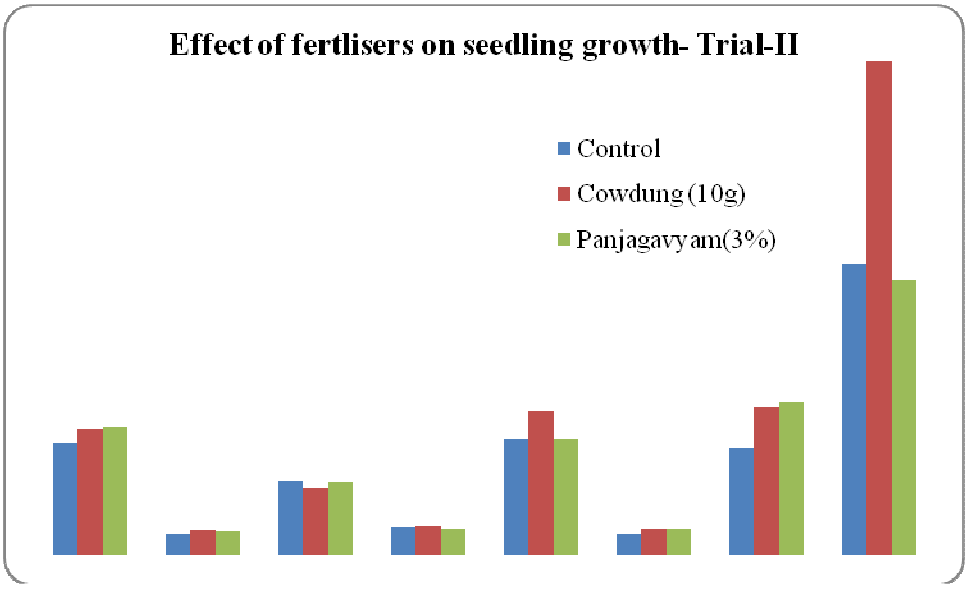


Figure 3: Evaluation of traditional and conventional fertilizers in *Oroxylum indicum* using *panjagavyam* and cowdung (Trial-3: After 63 days)

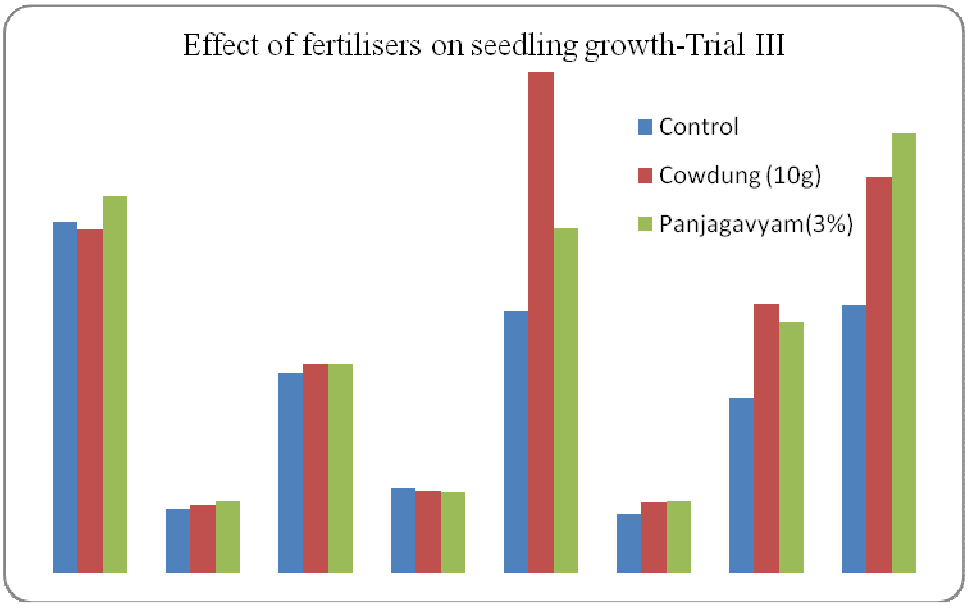
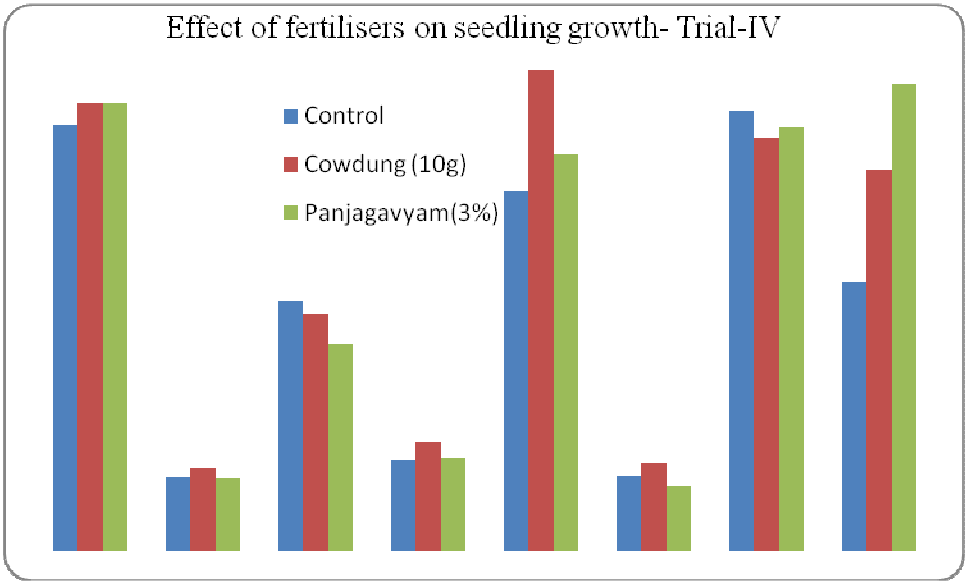


Figure 4: Evaluation of traditional and conventional fertilizers in *Oroxylum indicum* using *panjagavyam* and cowdung (Trial-4: After 84 days)



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Development of precision farming package for Bottle gourd under rainshelters of Kerala homesteads

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ABSTRACT: *Precision agriculture or Hi-tech agriculture makes use of the inputs most efficiently and judiciously to optimize the production and profit with least impact on soil and environment. Applications of plasticulture technologies are really a boon to Hi-tech agriculture by minimizing environmental pollution. Here we are recommending a precision farming package for bottle gourd under rainshelters of Kerala homesteads using plasticulture applications such as drip irrigation and fertigation. Results from the study indicated that modification of the KAU adhoc recommendation of irrigation and fertigation levels increased the crop yield and this can be adopted for year round production of bottle gourd under Kerala homesteads.*

Key words: *Bottle gourd, micro irrigation, plasticulture, precision farming*

Introducton

Cucurbits are vegetable crops belonging to family *Cucurbitaceae*, which primarily comprised species consumed as food worldwide. Bottle gourd [(*Lagenariasiceraria* (Mol.) Stand L.)] commonly known as Lauki or Ghiya in India is one of the most important member of the family *Cucurbitaceae* and believed to be originated in Africa (Whitaker, 1971). It is commercially grown in all the states of India in both rainy and summer seasons. Bottle gourd contributes significantly to dietary intake of vitamins (Vit A, B, and C), carbohydrates (2.9%), proteins (0.25%), fats (0.5%) and minerals (0.5%) such as calcium, iron, potassium, phosphorous. Tender fruits are used as vegetable and also for preparation of sweets and pickles especially in the hills. It has a cooling effect and prevents constipation and has diuretic and cardio-tonic properties. Fruit pulp has good medicinal values and is used as antidote against certain poisons.

In India it occupies an area of 9000 hectares with productivity of 28.9 tonnes per hectare (Anonymous 2005). To meet the stringent demands of both fresh market and processing industry, there is a need to boost the productivity and quality aspects of present day bottle gourd cultivars. Despite continuous efforts of various levels the bottle gourd productivity did not get momentum. A number of limiting factors could be attributed to the low productivity. Bottle gourd requires hot and moist climate for its cultivation.

It cannot withstand frost and can be grown in wide range of soils with a pH range from 6.5 to 7.5. This vegetable requires good drainage. Adding organic matter or Farm Yield Manure (FMY) will make soil rich so that better yield with quality vegetable can be expected.

Precision agriculture uses the inputs most efficiently and judiciously to maximize productivity and profitability with minimum impact on soil and environment. Precision in terms of both time and quantity of inputs and agronomic practices, envisages a prospect, which can help in decreasing the cost of production and not having any adverse effect on soil and environmental health. Thus the intent of precision farming is to match agricultural inputs and practices to localized conditions within a field to do the right thing in the right place, at the right time and in the right way. Micro-irrigation is a method of delivering slow, frequent applications of water to the soil near the plants through a low pressure distribution system and special flow control outlets. It can be considered as an efficient irrigation method, which is economically viable, technically feasible and socially acceptable. It is the slow and regular application of water directly to the root zone of the plants through a network of economically designed plastic pipes and low discharge emitter. Fertigation is the method of application of soluble fertilizer with irrigation water. It is a prerequisite for drip irrigation and offers precise control on fertilizer application and can be adjusted to the rate of plant nutrient uptake (Magen 1995). Maximization of crop yield, quality and minimization of leaching loss of nutrients below the rooting zone could be achieved by managing fertilizer concentrations in measured quantities of irrigation water using drip irrigation (Hagin and Lowengart 1995). When fertilizer is applied through drip irrigation, it was observed that the yield has been increased and about 30 per cent of the fertilizer could be saved (Sivanappan and Ranghaswami 2005). It is one of the most effective convenient methods of supplying nutrients of water according to the specific requirements of the crop to maintain optimum soil fertility and to increase the quality of the produce (Shingure et al. 1999). Fertigation is the most efficient method of fertilizer application, as it ensures application of the fertilizers directly to the plant roots and it allows nutrient placement directly into the plant root zone during critical periods of nutrient demand (Mikkelsen 1989; Rajput and Patel 2002).

This study is an attempt to develop a precision farming package for the production of vegetable crops like bottle gourd in rainshelters of Kerala homesteads.

Materials and Methods

The experiment was conducted in the experimental rainshelter of area 100/200 sq.m located at the instructional farm of Precision Farming Development Centre, KCAET, Tavanur, Malappuram District in Kerala during September- December 2015. The area is located at 10°51'8"N latitude and 75°59'11"E longitude 8.54 m above mean sea level. Rainshelter frame worked with stainless, cladding with U.V. stabilized polythene sheet (200microns) is used for the study. It is filled with sandy loamy soils rich in organic matter with good drainage and the pH ranges from 6.5 to 7.5. ArkaBahar- a pure line selection from a local collection (IIHR20) from Karnataka was used for the study.

The soil in the field is ploughed thoroughly using mini tiller and left idle for one week after lime application. The manures used were Neem cake: 1 sack of 25 kg, Trichoderma: pack of 250g in 50 L water, Cow dung: 400 kg. Seeds were treated with *Pseudomonas fluorescens* (10 grams/litre) seeds before sowing. Seeds were germinated in plastic seedling trays and transplanted into the field on 10th day. Beds are prepared with 20 m length, 1m base width and 80cm top width. The plants were grown at a spacing of 2 m X 2 m in Randomized Block Design (RBD) with three replications. Weeding and intercultural operations were done manually in periodic manner. Installed drip irrigation system with screen filter, main, sub-main and lateral tubes and online emitters at an interval of 2m and other accessories required. A 5 HP submersible pump was used to lift water from the bore well and supply to the drip irrigated plot. The main and sub main pipelines used for drip irrigation were made of PVC pipes of 63 mm and 50 mm diameter respectively. Linear Low Density Poly Ethylene (LLDPE) pipes of 16mm diameter were used for laterals in the drip irrigation treatments. Drippers of 2 litres per hour (2lph) capacity were fitted on the laterals at a spacing of 2 m. Fertigation pump is used as the fertigation device. The application of fertilizer to various treatments was controlled by using control valves provided in the sub main and lateral flow control valves provided at the off take of laterals. Duration of the crop is 120 days, so the fertigation was scheduled as 40 splits with the frequency of once in three days from planting till the end of crop. Weekly foliar application of micronutrients was also provided.

Four experimental treatments were applied including control as follows; T1=70 % of KAU adhoc recommended fertigation schedule, T2=90 % of KAU adhoc recommended fertigation schedule, T3=110% of KAU adhoc

recomended fertigation schedule, Control= 100% of KAU adhoc recommended fertigation schedule. The irrigation schedule is constant (2 l/day) for all the treatments. Plants yield characters such as fruit weight (kg), fruit length (cm), number of fruits/plant and yield per plant (kg) were calculated based on treatment and replication wise.The data was statistically analyzed using *Microsoft Excel*. The level of the significant difference (LSD at $P < 0.05$) was used in the ANOVA to test the effect of different fertigation treatments on different response variables. The data were presented as mean ± SE.

Result

Crop yield is always an important effective and economic index consideration in the crop development. The number of fruits per plant, fruit weight and fruit size are important determinant of yield in bottle gourd. The treatment T2 (90% of the KAU adhoc recommendations) with 100% irrigation showed comparatively better performance in yield characters as compared to the other treatments including control. The highest mean fruit weight (1.48 kg), mean fruit number per plant (15.67), mean fruit length (41.20) and yield per plant (54.38) in the fertigation level at 90% KAU adhoc recommendations (Table 2). Fertigation treatments had significant effects ($P < 0.05$) on all the yield parameters.

Conclusion

Irrigation and fertilizer management is important to crops yield. High fertilizer application and inefficient irrigation is the norm in current farming practice, we should take the measure of reducing nutrient application level. Indian agriculture today faces the challenge of meeting demand for safe and quality food. Care has to be taken in protecting the natural resources and the environment in the race for food security. Water is a major input in agriculture. The water use efficiency of the crops has to be increased in order to reduce the water loss from the field. Drip irrigation system is considered as the most effective micro irrigation method, as water is applied directly into soil at the crop root zone. The application of fertilizer through irrigation increases the uptake by plants and reduces their loss in soil.

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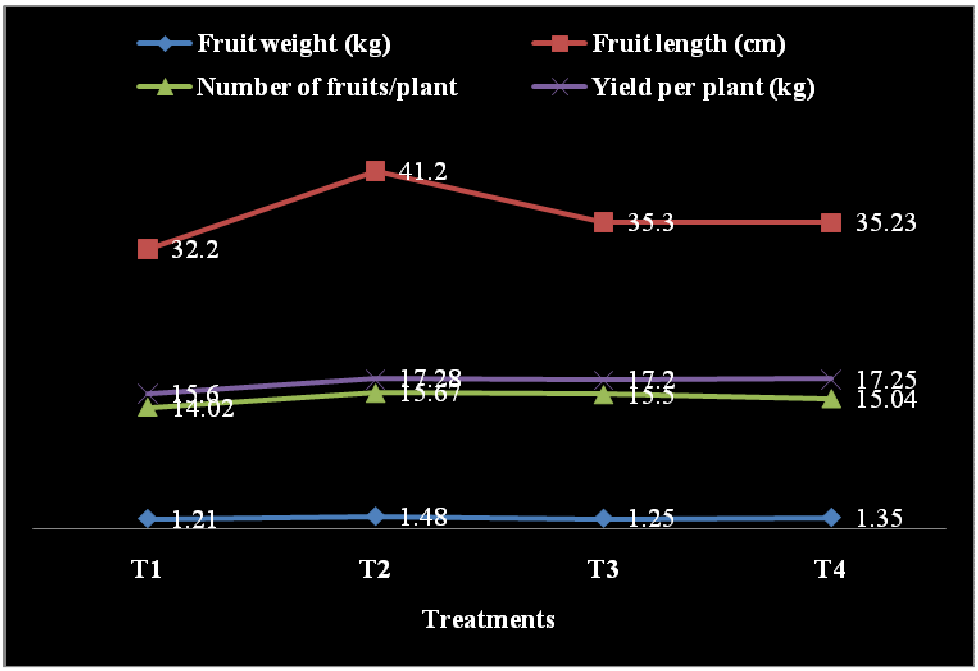
Table 1: Fertigation schedule details

Crop	Total NPK (Kg/ha)	Basal P	Establishment stage (6 doses)	Vegetative stage (12 doses)	Fruiting stage (22 stages)
Bottle gourd	175, 125, 300	62.50	35, 12, 60	70, 24, 120	70, 24, 120

Table 2: Effect of various fertigation treatments on yield parameters of bottle gourd

Yield parameters	Treatments			
	T1	T2	T3	T4
Fruit weight (kg)	1.21 ± 0.02	1.48 ± 0.04*	1.25 ± 0.01	1.35 ± 0.01
Fruit length (cm)	32.20 ±0.08	41.20 ± 0.1*	35.30 ± 0.02	35.23 ± 0.01
Number of fruits/plant	14.02 ± 0.03	15.67 ± 0.01*	15.50 ± 0.03	15.04 ± 0.02
Yield per plant (kg)	15.6 ± 0.02	17.28 ± 0.05*	17.20 ± 0.01	17.25 ± 0.02

Figure 1: Effect of different fertigation treatments on major yield parameters of bottle gourd



Genetic Diversity of *Maranta arundinacea* L. accessions of Kerala

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Abstract: *Root and tuber crops occupy a prominent position in the food security of the developing world due to their high caloric value and carbohydrate content. In this context the cultivation of root crops needs more attention to improve the agricultural and rural economy. Being a medicinal plant as well as a good source of quality starch, Maranta arundinacea L. is found to be a suitable crop for cultivation in Kerala. Selection of promising genotypes having high yield and superior quality starch has immense scope in the crop improvement of Maranta arundinacea. With this objective, a field experiment for the analysis of sixty accessions of Maranta arundinacea based on genetic variability, heritability and genetic advance was undertaken at the Department of Botany of University of Calicut, Kerala during 2012-2015. Sixty diverse accessions were collected and these accessions were observed in the experimental plot in randomized block design for three consecutive crop seasons by adopting standard cultural practices. Data on fourteen growth, yield and quality characters were recorded by destructive sampling and analyzed statistically to study the extent of genetic variability existing in the crop based on these characters. Among the characters studied, highest GCV and PCV were shown by number of primary fingers and the highest genetic advance by starch content. Heritability of characters varied from 16.69% to 99.22% and highest heritability was shown by starch content, which was followed by plant height (79.08) and yield per plant (72.13%). Highest heritability indicated the limited influence of environment on these characters. Genetic advance under selection was maximum for starch content (70.75%) followed by number of primary fingers (50%) and number of tillers (49.65%), thus showing the usefulness of these characters in selection programmes.*

Key words: *Variability, Maranta arundinacea, heritability, genetic advance*

Introduction

Root and tuber crops are important to agriculture food security and income for 2.2 billion people in developing countries. They are major source of energy in developing countries with fast population growth and high urbanization rates. These crops are expected to contribute significantly to the increased income generation and nutritional well- being of the people in the tropics in the next decades. Among the root tuber crops, *Marantana arundinacea* (West Indian

arrowroot) place a superior position because of its high quality medicinal starch. West Indian arrowroot is a perennial herb widely cultivated for its starchy rhizomes throughout the tropical countries of the world. The plant is an erect, perennial herb, shallow rooted with rhizomes penetrating in to the soil. The plant thrives best in light, well drained loamy or sandy soil with partial shade. It is mainly propagated by the tips of the rhizomes known as "bits" which carries 2- 4 nodes. The small rhizome containing eyes were also used for planting (Anonymous 1962). Once revered by the ancient Mayans and other inhabitants of Central America as an antidote for poison-tipped arrows, the herb is mainly used today to soothe the stomach and alleviate diarrhea. It has also been popular for centuries in the culinary arts and is still used in many American kitchens as a thickening agent. While arrowroot is native to Central America and widely cultivated in the West Indies, it can also be found growing in many tropical regions of the world, including Southeast Asia, South Africa, Australia, and in Florida in the United States (Edison et al. 2006).

The primary objective of a conservation program is to ensure that the existing genetic variation is maintained among a population. This genetic variation is the result of some billion years of evolution which represents the evolutionary legacy of a species. More importantly, loss of genetic variation has a variety of harmful effects on characteristics of individuals that are important o the continued existence of a species (Zouros and Foltz 1987). Furthermore, the loss of variation is expected to reduce the ability of populations to adapt to changing environmental conditions and to increase their susceptibility to extinction. Genetic variability studies are found to be more important for the selection of superior genotypes and for the crop improvement program. Being a medicinal plant as well as a good source of quality starch, *Maranta arundinacea* is a potential crop for cultivation in Kerala and hence the present study needs importance.

Materials and methods

The experiments were carried out in the experimental field of the Genetics and Plant breeding division, Department of Botany, University of Calicut, Kerala, India. The University of Calicut is located 23 km south of the historical city of Calicut located 75°46'E longitude and 11° 15'N latitude at an elevation of 40-60m from MSL. The experiments were laid out in randomized block design (RBD) with 3 replications (Fig. 1). Sixty accessions of *Maranta arundinacea* L. collected from different locations in the northern districts of Kerala were used for the study (Table 1).

Table 1: Accesions of *Maranta arundinacea* studied

SI No.	Accession No.	Source	District
1	CUW 1	Chavakkad	Thrissur
2	CUW 2	Panambra	Malappuram
3	CUW 3	Varadoor	Wayand
4	CUW 4	Areekode	Malappuram
5	CUW 5	Pathappiriyam	Malappuram
6	CUW 6	Kalikavu	Malappuram
7	CUW 7	Kuniyil	Malappuram
8	CUW 8	Puthur Vayal	Wayanad
9	CUW 9	Villunnial	Malappuram
10	CUW 10	Mundakkulam	Malappuram
11	CUW 11	Alinchuvad	Malappuram
12	CUW 12	Vadakkummuri	Malappuram
13	CUW 13	Athinjal	Kasaragod
14	CUW 14	Kanjangad	Kasaragod
15	CUW 15	Trikkarippur	Kasaragod
16	CUW 16	Puthiyiruthi	Thrissur
17	CUW 17	Eramangalam	Malappuram
18	CUW 18	Punnayurkulam	Thrissur
19	CUW 19	Kanjiramukku	Malappuram
20	CUW 20	Pananthara	Thrissur
21	CUW 21	Nayarangadi	Thrissur
22	CUW 22	Kizhoor	Thrissur
23	CUW 23	Nakkola	Malappuram
24	CUW 24	Edakazhiyoor	Thrissur
25	CUW 25	Kanippayyur	Trissur
26	CUW 26	Anjoor	Thrissur
27	CUW 27	Mayanad	Calicut
28	CUW 28	Mangalassery	Malappuram
29	CUW 29	Parambilpeedika	Malappuram
30	CUW 30	Puthiyatheru	Kannur
31	CUW 31	Pavaratty	Thrissur

32	CUW 32	Monnamkallu	Thrissur
33	CUW 33	Edakkara	Thrissur
34	CUW 34	Kaplengad	Thrissur
35	CUW 35	Erinjoli	Kannur
36	CUW 36	Mayyazhi	Kannur
37	CUW 37	Ponnyam West	Kannur
38	CUW 38	Ponnyam East	Kannur
39	CUW 39	Kathiroor	Kannur
40	CUW 40	Kollanpadi	Malappuram
41	CUW 41	Kalpetta	Wayanad
42	CUW 42	Vazhavatta	Wayanad
43	CUW 43	Munderi	Wayanad
44	CUW 44	Karaparamba	Calicut
45	CUW 45	Kannadikkal	Calicut
46	CUW 46	Thamburanpadi	Thrissur
47	CUW 47	Mangalam	Palakkad
48	CUW 48	Iritty	Kannur
49	CUW 49	Vavannoor	Palakkad
50	CUW 50	Mulayankavu	Palakkad
51	CUW 51	Kulukallur	Palakkad
52	CUW 52	Nhangattiri	Palakkad
53	CUW 53	Chalissery	Palakkad
54	CUW 54	Kodakara	Thrissur
55	CUW 55	Vetharkandam	Wayanad
56	CUW 56	Athichal	Wayanad
57	CUW 57	Kalluvayil	Wayanad
58	CUW 58	Thamarassery	Calicut
59	CUW 59	Cheruvathani	Thrissur
60	CUW 60	Meenangadi	Wayanad

Figure 1: Overview of the experimental field

Fourteen characters including six growth characters and eight yield characters (Table 2) were subjected to analysis of variance (ANOVA) to test the significance of variations between the accessions with reference to standard F table (Fisher and Yates 1963). Phenotypic variance, genotypic variance, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (broad sense) and genetic advance were analyzed to study the extent of variation in the case of each character. Phenotypic and genotypic coefficients of variations were estimated following Burton and Devane (1953). Heritability (Broad sense), the fraction of the total variance that is heritable was estimated as the percentage of genotypic variance over phenotypic variance (Jain 1982). Genetic advance under selection was calculated the formula proposed by Abraham (2000).

Table 2: Characters observed for the study of genetic variability

SI. NO.	CHARACTERS
Growth characters	
1	Plant height (cm)
2	Number of tillers
3	Number of leaves per tiller
4	Leaf length (cm)
5	Leaf breadth (cm)
6	Leaf area (cm^2)
Yield characters	
1	Yield per plant (g)
2	Number of rhizomes
3	Length of rhizome (cm)
4	Diameter of rhizome (cm)
5	Number of primary finger
6	Length of primary finger (cm)
7	Diameter of primary finger (cm)
8	Starch content (mg/g)

Results and discussion

Study of variability of genetic resources of a crop is the first step towards the understanding of the genetic diversity of the genetic stock so as to use them in crop improvement programmes. Such variability studies have been reported in cardamom (Radhakrishnan et al. 2006), rice (Mini 2006), coffee (Raghu et al. 2003). Most of the agronomic characters of the crop plants are polygenic and *Maranta arundinacea* is not an exemption. Polygenic characters show different levels of heritability based on their response to environmental factors. Among the growth characters, highest GCV and PCV were shown by number of tillers; highest heritability was shown by plant height and highest genetic advance by number of tillers. This shows that number of tiller is the most important growth character with the highest GCV, PCV and genetic advance. More over this character shows 49.65% heritability also (Table 3). Among the growth characters, heritability (broad sense) was found to be the highest in the case of plant height.

Table 3: Genotypic variance, phenotypic variance, GCV, PCV, heritability (broad sense) and genetic advance of the characters studied in *Maranta arundinacea*

SI.No	Characters	Genotypic variance	Phenotypic variance	GCV (%)	PCV (%)	Heritability (%)	Genetic advance (%)
Growth characters							
1	Plant height**	143.80	181.85	9.87	11.10	79.08	18.09
2	No. of tillers**	2.29	3.70	30.62	38.95	61.89	49.65
3	Number of leaves per tiller**	9.66	18.01	17.65	24.06	53.64	26.59
4	Leaf length**	1.57	2.49	4.00	5.05	63.05	6.56
5	Leaf breadth**	0.21	0.30	4.26	5.09	70.00	7.34
6	Leaf area	259.35	379.10	6.88	8.32	68.41	11.73
Yield characters							
1	Yield per plant**	6291.17	8721.55	24.42	28.75	72.13	42.72
2	No. of rhizome per plant**	3.12	6.10	20.68	28.86	51.15	30.40
3	Length of rhizome**	4.25	22.97	8.38	19.50	18.50	7.43
4	Diameter of rhizome**	0.06	0.01	9.76	14.63	46.15	13.19
5	No. of primary finger**	1.15	3.06	39.48	64.58	37.58	50.00
6	Length of primary finger*	2.34	14.02	15.61	38.16	16.69	13.12
7	Diameter of primary finger**	0.10	0.54	18.39	41.96	18.52	16.01
8	Starch content**	3885.74	3916.14	34.48	34.61	99.22	70.75

Among the yield character studied, highest GCV and PCV were shown by number of primary fingers. Highest heritability was shown by starch content, which indicates the limited influence of environment on this character. Characters such as length of rhizome, diameter of rhizome, number of primary fingers, length of primary finger and diameter of primary finger showed heritability below 50%, where as all other statistically significant characters showed above 50% heritability. The low heritability in the case of some characters may be due to the influence of environmental factors as suggested by earlier workers (Tripathy et al. 2000). Genetic advance of characters in percentage of mean is a very effective indicator of the characters that could be utilized in selection program. It is a measure that derived from heritability. Genetic advance under selection was maximum for starch content (70.75%) followed by number of primary fingers (50.00%) and number of tillers (49.65%), thus showing the usefulness of these characters in selection. Characters with highest genetic advance could be utilized for selection programmes as reported by earlier works (Jayasree et al. 2006; Radhakrishnan et al. 2006).

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Purified caulophyllumine-a from *Solanum mauritinum* scop.-its antihemolytic and antioxidant potentiality

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ABSTRACT: *Currently, traditional plant-based medicines for primary health care needs are a challenge. However, the majority of plants have not yet undergone comprehensive chemical, pharmacological and toxicological studies to investigate their bioactive compounds. Bug weed (Solanum mauritianum Scop.) is an evergreen woody species of Solanaceae, native of South America. Ripe fruits are used by the local people as vegetable during famine periods and the leaves are used to cure various ailments. The present investigation was designed to isolate the lead alkaloid molecule from S. mauritianum and to evaluate its antihemolytic and antioxidant potentialities. Crude alkaloid was isolated, purified yielded a bluish coloured fraction and its identity was confirmed by NMR as caulophyllumine-A. Further, the antioxidant activity was assayed using the DPPH radical scavenging and FRAP assay. The* IC_{50} *values ranged from 34.2 to 45.89 µg/ml. Protective effects of caulophyllumine-A, against* H_2O_2 *induced oxidative damage in plasmid pBR322 DNA was noticed at the tested doses (µg/ml). Finally the cytotoxicity of caulophyllumine-A was analyzed by examining haemolytic activity against human blood erythrocytes, whereby the percentage lysis of RBCs was found to be in the minimal range of 1.65 to 4.01% comparable with the control.*

Key words: *Solanum mauritianum*, Antioxidant, Cytotoxicity, NMR analysis

Introduction

Solanaceae is a large plant family containing more than two thousand species and nearly half of them belong to a genus, *Solanum*. This family includes a large number of species known for the presence of a variety of natural products of medicinal importance. *Solanum* species are rich in steroidal glycoalkaloids and flavonoids, an unique group of phytochemicals. These compounds are initiators for the synthesis of steroidal drugs. Plants have been used for the treatment of various human diseases since time immemorial. They maintain the health and vitality of individuals, and also cure a variety of diseases, including cancers with minimal side effects. More than 50% of all modern drugs in clinical use were derived from natural products and many of them were used to control cancer cell growth. It was also reported that more than 60% of cancer patients use vitamins or herbal products as therapy.

Materials and methods

Solanum mauritianum Scop. is a medium sized tree found growing in the higher altitudes of Kerala served as the material for the present study. The plant can be easily identified by the presence of prominent cataphylls, stellate velvelty trichomes at both the adaxial and abaxial foliar surfaces. Berries are produced in clusters through out the year. Greenish fruits turns yellowish brown at maturity producing pungent smell.

40g of shade dried powdered sample was subjected to soxhlet extraction with ethyl acetate. The lyophilized residue was adsorbed to 0.25g silica gel and loaded at the top of the freshly packed column with silica of mesh size 60-120. 100% chloroform was used for primary elution of bluish coloured fraction (caulophyllumine A). Further purification was done with a combination of petroleum ether and chloroform (4:1). Identification of the compound was done with the help of H^1 NMR.

DPPH free Radical Scavenging Assay

0.1 mM solution of DPPH in ethanol was prepared. 1 ml of solution was added to 3 ml of different concentrations of caulophyllumine-A (5, 10, 15, 20, 25, 30 µg/ml). The mixture was shaken vigorously and allowed to stand at room temp for 30 min. and absorbance was measured at 517 nm by using spectrophotometer. Reference standard compound being used was ascorbic acid, quercetin and experiment was done in triplicate. For the determination of IC_{50} values by the DPPH free radical scavenging assay method described by Iqbal et al.(2005) with some modification was used. The IC_{50} values were calculated from the plot of the regression equation against percentage scavenging and concentrations of samples used. Three replicates were recorded for each sample. The percentage scavenging by DPPH was calculated from the following equation, $[(A_{blank} - A_{sample})/A_{blank}] \times 100$, where A is the absorbance.

Determination of Reducing Power

The determination of the total antioxidant activity (FRAP assay) is a modified method of Yen et al. (2000). The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$. The temperature of the solution was raised to 37 °C before use. Caulophyllumine-A (150 µL) were allowed to react with 2850 µl of the FRAP solution for 30 min in the dark condition. Readings of the coloured product (ferrous

tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $FeSO_4$. Results are expressed in µM Fe (II)/g dry mass and compared with that of ascorbic acid and quercetin. The reducing power of the caulophyllumine-A was determined according to the procedure described by Yen et al. (2000). Caulophyllumine-A samples containing 5–100 µg/mL was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%); the mixture was incubated at 50 °C for 20 min, then 10% trichloroacetic acid (5 mL) was added, and the mixture was centrifuged at 980 × *g* for 10 min at 5 °C in a refrigerated centrifuge. The upper layer of the solution (5 ml) was diluted with distilled water (5ml), 0.1% ferric chloride (1ml) was added and the absorbance at 700 nm noted.

Antioxidant Activity by DNA Protection Assay

To evaluate the antioxidant activity of caulophyllumine-A by the DNA protection assay the method described by Kalpana et al. (2009) was used. pBR 322 DNA (0.5 µg/µL) was diluted up to two-fold (0.5 µg/3 µL) using 50 mM pH 7.4 sodium phosphate buffer. The diluted pBR 322 DNA (3 µL) was treated with test sample (5 µL). After this 30% H_2O_2 (4 µL) was added in the presence and absence of different concentrations of caulophyllumine-A. The volume was made up to 15 µL with sodium phosphate buffer (pH 7.4). The relative difference in migration between the native and oxidized DNA was then examined on 1% agarose by horizontal DNA gel electrophoresis using a Bio-Rad wide mini system. The gels were documented by a Syngene model Gene Genius unit.

Cytotoxicity Studies

The cytotoxicity was determined by testing the haemolytic activity of caulophyllumine-A using the method with some modification described by Powell et al. (2000). Caulophyllumine-A at a concentration of 1 mg/mL in 10% DMSO were prepared. 3 mL of freshly obtained human blood was placed in heparinized tubes to avoid coagulation, gently mixed and poured into a sterile 15 mL Falcon tube and centrifuged for 5 min at 850*g*. The supernatant was poured off and RBCs were washed three times with chilled (4° C) sterile isotonic phosphate buffer saline (PBS) solution (5 mL), adjusted to pH 7.4. The washed RBCs were suspended in chilled PBS (20 mL). Erythrocytes were counted on a heamacytometer. The RBCs count was maintained to 7.068×10^8 cell/mL for each assay. The caulophyllumine-A (20 µL) were taken in 2 mL Eppendorf tubes and then diluted blood cell suspension (180 µL) was added. The samples were incubated for 35 min at 37 °C. After incubation and agitation for 10 min, the tubes were placed on ice for 5 min. and centrifuged for 5 min. at 1500*g*. After centrifugation supernatant (100 µL) was taken from the tubes, and diluted with chilled PBS (900 µL). All Eppendorfs were maintained on ice after dilution. After this mixture from each Eppendorf (200 µL) was added into 96 well

plates. For each assay, 0.1% Triton X-100 was taken as a positive control and phosphate buffer saline (PBS) as a negative control. The absorbance was noted at 576 nm.

Statistical Analysis

All the experiments were conducted in triplicate unless stated otherwise stated and statistical analysis of the data was performed by analysis of variance, using the STATISTICA 5.5 software. A probability value of difference $p \leq 0.05$ was considered to denote a statistically significant.

Results and discussion

Chromatographic elution of powdered fruit sample yielded purified bluish coloured fraction with petroleum ether and chloroform in the ration 4:1. The fraction upon solvent vapourisation was subjected to H^1 NMR for identification (Fig. 1). Absorption peaks revealed the identity of the compound as caulophyllumine-A (Fig. 2).

Figure 1: H^1 NMR absorption peaks of caulophyllumine-A

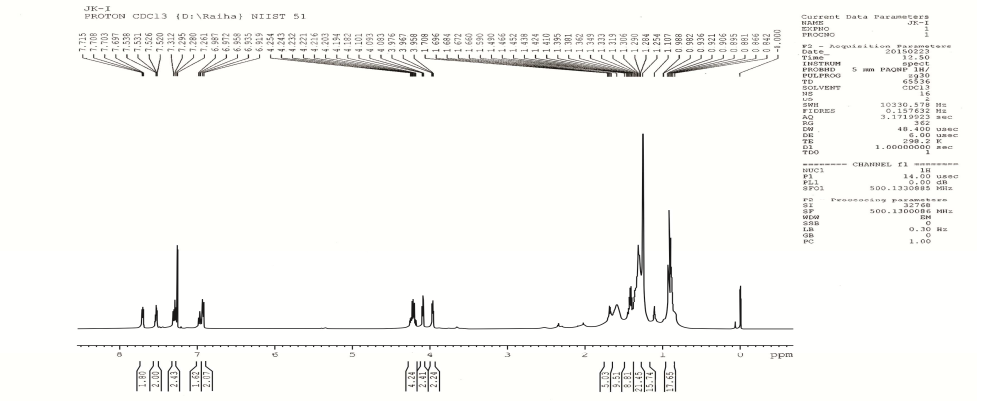
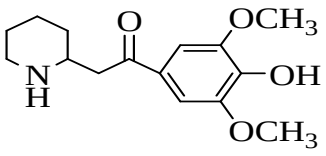


Figure 2: Structure of caulophyllumine-A



Antioxidant Activity

The DPPH radical, which has a deep violet color, reacts with hydrogen donor species such as alkaloids and upon receiving a proton loses its color and becomes yellow. The IC_{50} value for caulophyllumine-A was 45.89 ± 0.06 µg/mL. The smaller value of IC_{50} represents an optimal antioxidant activity. Derwich et al. (2011) reported that 1,8-cineole, germacrene, limonene, pulegone *β*-pinene and *a*-pinene are good antioxidants. Thymol also behaves as an antioxidant (Undeger et al. 2009). Polyphenols are proven in antioxidant and antimicrobial properties. The reducing power of caulophyllumine-A was showed in Table 1. The reducing power of the phytoconstituents is associated with their antioxidant potential. The reducing power of the caulophyllumine-A increased in a concentration-dependent manner. Therefore reducing power evaluation might be taken as important parameter for the assessment of antioxidant activity.

Table 1: Reducing power of caulophyllumine-A

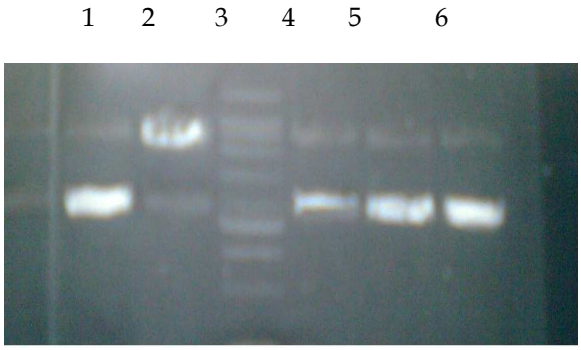
Conc. (µg/ml)	DPPH % inhibition ± SD	FRAP µm/g ± SD
10	9.5 ± 0.4	132.4 ± 0.24
20	31.2 ± 0.14	196 ± 0.03
40	44.5 ± 0.02	246 ± 0.31
60	62 ± 0.04	359 ± 0.04
80	74 ± 0.24	409 ± 0.01
100	80 ± 0.29	472 ± 0.14
Ascorbate (100 µg)	96%	501±0.01
Quercetin (100 µg)	94%	512±0.02

Antioxidant Activity by DNA Protection Assay

The antioxidant activity of different concentrations of caulophyllumine-A in the protection of plasmid pBR322 DNA from H_2O_2 induced damage is shown in Fig. 3. From the figure it was clear that in the first lane the plasmid pBR322 DNA present without any treatment might be in super coiled form. When comparing the results of the second lane with the other lanes, which contain pBR322 DNA that was exposed to H_2O_2, that caused damage in plasmid pBR322 DNA, in the second lane the damage to the DNA strand, which occurred due to conversion of the super coiled form of pBR322 DNA into an open linear form, leaving behind the untreated DNA (first lane) can be seen. In the fourth to sixth lane 10 to 1,00 µg/mL of caulophyllumine-A was added in pBR322 DNA to observe their protective effects.

The results in Fig. 3, when compared with each other, show the protective effects of caulophyllumine-A at a concentration of 100 µg/mL (sixth lane) which displays a band almost equal to the pure pBR322DNA (first lane). The caulophyllumine-A at a concentration of 10 µg/mL (fourth lane) showed less protective effect on DNA and the band in this lane was similar to the damaged DNA (second lane treated with H_2O_2). Caulophyllumine-A at 1,00 µg/mL protected the DNA, perhaps by scavenging the oxidation products that damage the DNA and this did not allow the H_2O_2 to open the coiled DNA so it remained in protected form. The protective effect of DNA could be due to its alkaloidal nature and that scavenges the free radicals and oxidation products.

Figure 3: Antioxidant Activity of caulophyllumine-A by DNA Protection Assay. Lane 1 = Plasmid pBR322 DNA without treatment (super coiled); Lane 2 = Plasmid pBR322 DNA treated with H_2O_2 (open circular or damaged); Lane 3 = 1 kbp DNA ladder; Lane 4 = Plasmid pBR322 DNA treated with 10 µg/mL caulophyllumine-A + H_2O_2; Lane 5 = Plasmid pBR322 DNA treated with 100 µg/mL caulophyllumine-A + H_2O_2; Lane 6 = Plasmid pBR322 DNA treated with 1,00 µg/mL caulophyllumine-A + H_2O_2.



Cytotoxicity Studies by Haemolytic Activity

The cytotoxicity was studied by examining haemolytic activity against human red blood cells (RBCs) using Triton X-100 as positive control. The percentage of lysis was evaluated by comparing with the absorbance of sample and the Triton X-100. The positive control showed about 100% lysis, where as the phosphate buffer saline (PBS) showed no lysis of RBCs. When the effects of caulophyllumine-A was compared with the controls, different percentage lysis of RBCs caused by different

concentrations such as 6.25, 12.5, 25, 50 and 100 µg/ml of caulophyllumine-A 6.969 ± 0.03, 22.71 ± 0.02, 56.72± 0.02, 64.45 ± 0.01, 68.39 ± 0.01, respectively (Table 2). The LC 50 - 49.55ug/ml was determined using ED50 plus V 1.0 software. The mechanical stability of the membrane of red blood cells (RBCs) is a good indicator to evaluate in vitro the effects of various compounds when screening for cytotoxicity. Treating cells with a cytotoxic compound can pose different problems to human beings. The cells may undergo loss of membrane integrity and burst rapidly as a result of cell lysis.

Table 2: Cytotoxicity Studies by haemolytic Activity

Sample Concentration (µg/ml)	Percentage lysis
Control (Triton X-100)	100
6.25	6.969 ± 0.03
12.5	22.71 ± 0.02
25	56.72± 0.02
50	64.45 ± 0.01
100	68.39 ± 0.01

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Effects of water stress on morphological, physiological and biochemical characters in two varieties of *Momordica charantia* (L).

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ABSTRACT: *Drought is one of the most adverse factors for plant growth and productivity, and is considered generally as a severe threat for sustainable crop production. Momordica charantia (L.), belonging to the family Cucurbitaceae, is an annual, monoecious vegetable crop, with two varieties viz., M. charantia var. charantia and var. muricata based on fruit size, shape, colour and surface texture. The present investigation examined the effect of water deficiency on morphological, physiological and biochemical responses of cultivated and wild varieties of M. charantia under controlled conditions. The experiments were conducted in control and treated plants that grew in polythene bags filled with garden soil, sand and cow dung in ratio 2:1:1. The duration that affected the severity of water stress was determined as 9th and 15th days for the cultivated and wild varieties respectively. The experimental groups were subjected to water stress for a period of 15 days during vegetative (15-28 days of maturity) and flowering stage (32-40 days of maturity) by withholding irrigation, to find out the changes in morphological, physiological and biochemical characters. The study noticed a reduction in the yield related morphological traits, relative water content and pigment concentration in cultivated variety at vegetative and flowering stages than in wild. Biochemical analysis revealed an increase in level of enzymatic and non enzymatic antioxidants, primary metabolite and rate of lipid peroxidation in wild variety compared to that of the cultivar. The study emphasized the superiority of the wild variety in response to tolerance to drought stress.*

Key words: *Drought stress, Momordica charantia, wild, cultivar*

Introduction

Drought is an extended abnormal dry period, which occurs in a region when it consistently receives below the average rainfall. It can be defined as the absence of adequate soil moisture, necessary for a plant to grow normally, and to complete its life cycle (Manivannan et al. 2008). It is considered as one of the most important environmental stresses limiting plant growth and crop productivity (Terzi et al. 2006). Drought is a multidimensional stress affecting plants at various levels of their organization (Hsiao 1973). Plant water deficit depends both on the supply of water to soil and the evaporative demand of atmosphere. According to World Bank, drought is the world's most expensive disaster, destroying the economic livelihood and food source for those dependent on Agricultural sector.

Momordica charantia L., one of the most important vegetable crops, cultivated in tropical regions belongs to family Cucurbitaceae. It is commonly known as bitter

gourd, karela, balsam pear or bitter melon. The species is classified into two botanical varieties based on fruit size, shape, colour and surface texture viz., *M. charantia* var. *charantia* with large fusiform fruit having numerous triangular tubercles giving the appearance of a "crocodile back" and *M. charantia* var. *muricata* (Wild) with small and round fruits with tubercles and more or less tapering ends (Chakravarty 1990). Both the varieties are widely cultivated throughout the tropical and subtropical regions of India. It was noticed that the cultivars were less tolerant to water deficits compared to wild variety (Beevy and Bai 2012 unpublished). The present investigation was carried out to assess drought tolerance in wild and cultivated varieties of *M. charantia* by giving special attention to identify the duration that resists severity of water stress in wild and cultivated varieties, to analyse morphological, physiological and bio-chemical characters in wild and cultivated varieties of *M. charantia* during stress period and to identify the superior tolerant variety.

Materials and Methods

M.charantia var. *charantia* (seeds) procured from the College of Agriculture, Vellayani, Thiruvananthapuram, and the variety *muricata* obtained from the Department of Botany, University of Kerala, Kariavattom served as the material for present study. The duration that affected the severity of water stress in wild and cultivated varieties of *M. charantia* was determined by subjecting them to maximum water deficit during this investigation.

The effects of water stress on the two varieties of *Momordica* were studied under controlled conditions. 10 seeds each of uniform size of two varieties were sown in polythene bags of size 120×40 cm. The bags were filled with 5.5kg soil mixture containing garden soil, sand and cow dung in ratio 2:1:1. Chemical properties of soil were analysed to find out the impact of stress on pH, micro and macro elements in it. Plants with same height and number of leaves were selected after 12 days and planted in different bags and maintained under controlled conditions. The plants were then divided into test and control groups and were subjected to water stress for a period of 15 days during vegetative (15-28 days of maturity) and flowering stage (32-40 days of maturity) of development by withholding irrigation. Morphological, physiological and biochemical characterization of test and control plants of two varieties were carried out during the study.

Morphological characters of test and control plants were analysed during the vegetative and reproductive stages of development. The yield related quantitative characters selected for the present investigation are: Plant height, Number of branches/plant, Specific leaf area, Number of fruits/plant, Number of seeds/fruit, Length of fruit, Weight of 100 seeds, Foliar trichomes/unit area, Number of

tendrils and Weight of ten fruits. The dimensions were taken using a measuring scale, and the arithmetic mean was calculated using the formula X=∑X/N. Here ∑X iss sum of all observations and N is number of observations. The data collected were subjected to Analysis of Variance (ANOVA, SPSS, Inc, Chicago IL, USA).

Estimation of relative water content (RWC)(Castillo 1996)

Topmost full expanded leaves of test and control plants were used for estimation of RWC. 5-10 leaf discs of around 1.5 cm diameters were hydrated to full turgidity by floating on de-ionized water in a closed petri dish for 4 hours under normal temperature. After 4 hours the samples were taken out and removed the surface moisture quickly with filter paper and immediately weighed to obtain fully turgid weight. Then the samples were dried in an oven at 80°C for 24 hours, and weighed to determine the dry weight of the sample.

Estimation of pigments

Total chlorophyll and carotenoids were estimated following the procedure of Moran and Porath (1980). 0.1g of leaf material was ground in 8ml of 80% acetone and extract was centrifuged at 18000 rpm for 10 minutes. Then supernatant was collected and its absorbance was recorded at wave lengths of 480nm, 645nm and 663nm using spectrophotometer. Estimation of chlorophyll and carotenoid content was calculated following Inskeep and Bloom (1985).

Biochemical Characterization

The total proline content was calculated accoriding to Bates et al. 1973. The red colour intensity measured at 520 nm. The same method was adopted for conducting experiments with D-proline as standard. The level of lipid peroxidation was measured by determining Malondialdehyde (MDA) content using standard procedure of Heath and Packer (1968). The absorbance of the sample was determined at 535nm against blank.

Estimation of Primary Metabolites

Total carbohydrate content was calculated followed the method by Hodge and Hofreiter 1962. Total protein estimation was done according to the method of Lowry et al. (1951). Total protein was calculated by calibrating the measured absorbance with that of Bovine Serum Albumin (BSA) as standard.

Estimation of Stress Enzymes

Activity of peroxidase was determined following the procedure of Rodriguez and Sanchez (1982). Activity of Catalase was determined following the procedure of Sadasivam and Manickam 2005. Activity of Ascorbc acid Oxidase was determined following the procedure of Malik and Sing 1994. The total ascorbate was calculated according to Sadasivam & Manickam, 1996. The concentration of phenol in the test sample was calculated from the standard curve and expressed as mg phenol /100 g material and followed by Sadasivam and Manickam 2005.

Results and Discussion

Morphological characters

The observations on morphological characters of the varieties of *M. charantia* under water stress along with the control plant is given in Table 1 & Fig. 1a-d and 2 a-d. It was found that morphological characters differed significantly in both varieties of *M. charantia* during vegetative and flowering stages of stress. During the period of flowering stage of stress, treated and control of wild and cultivar showed significant variations. Treated plants of cultivated variety showed decrease in size of fruit length, fruit diameter and in number of seeds per pod compared to control. Reduction in number of fruit/plant, number of seed/pod, length of fruit, plant height, leaf area and number of tendrils were observed in cultivar compared with that of wild. Reduction in plant height, fruit size and number of fruits per plant was observed in cultivar when stress was given at vegetative stage. The reduction in plant height due to an increase in water stress in *Sesame* has been reported by Mensah et al. (2006). Timpa et al. (1986) reported the same in cotton cultivar. According to Abdalla and El-Khoshiban (2007) the shoot length of the treated wheat was declined when duration of drought period increases. Reduction in the number of branches in species of *Sophora* was reported at different water stress levels in field conditions (Li et al. 2009). Ruiz Sanchez et al. (2000) considered the reduction in leaf area under water stress as an avoidance mechanism which minimizes water loss.

Figure 1: Fruit morphological variations in the varieties of *M. charantia* under water stress during vegetative stage. a. CVC-Cultivar vegetative stage control, b. CVS-Cultivar vegetative stage stress, c. WVC - Wild vegetative stage control and d. WVS-Wild vegetative stage stress.

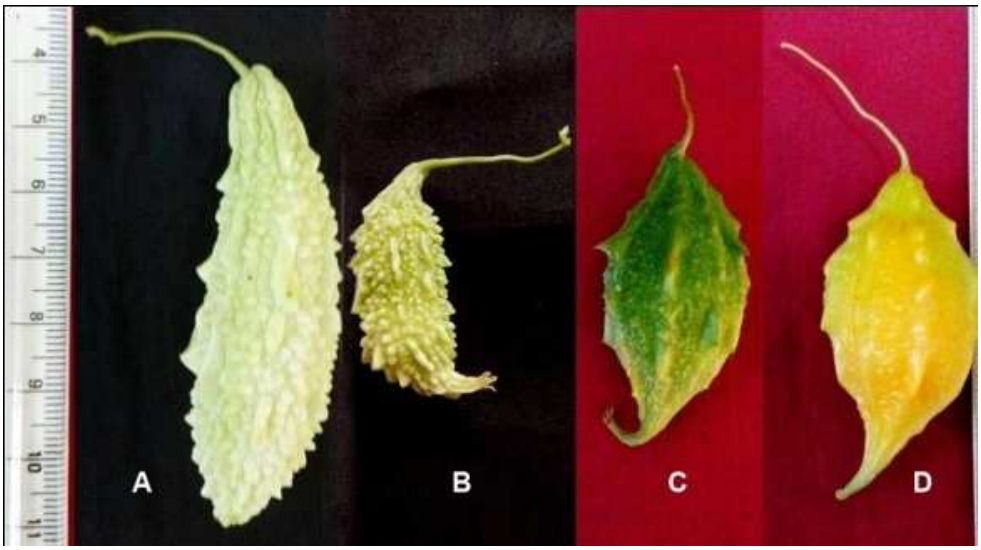
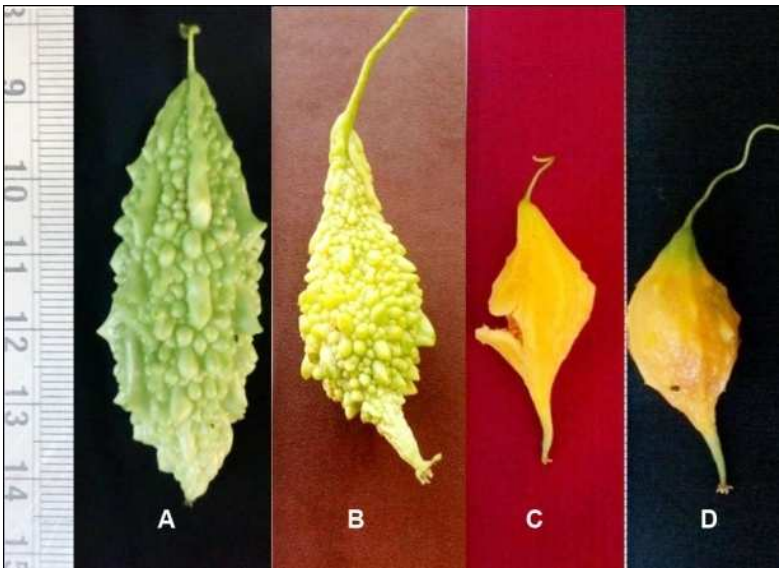


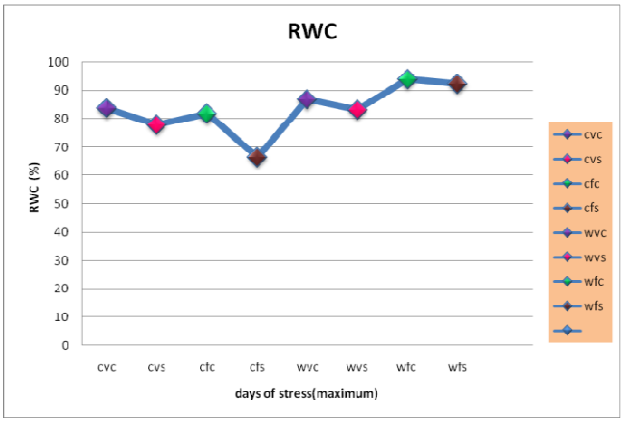
Figure 2: Fruit morphological variations in the varieties of *M. charantia* under water stress during flowering stage a. CVC-Cultivar vegetative stage control, b. CVS-Cultivar vegetative stage stress, c. WVC - Wild vegetative stage control and d. WVS-Wild vegetative stage stress.



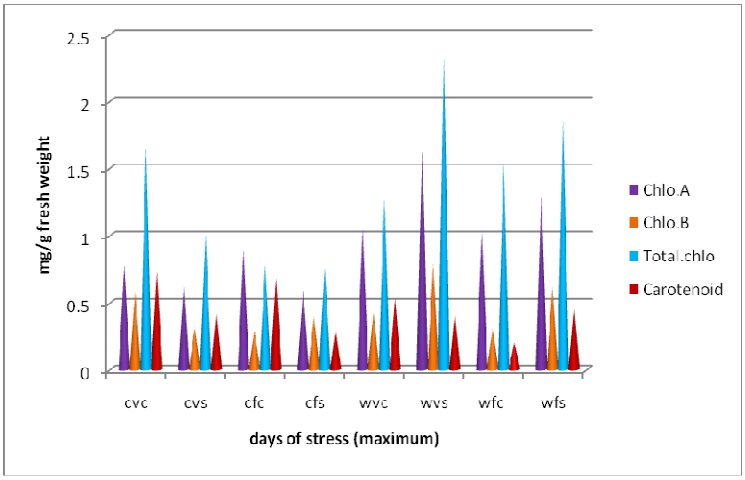
Physiological characters

Relative water content (RWC) can be used as a better indicator of water stress than other growth/biochemichal parameters of plants (Sinclair and Lundlow 1985; Alizadeh 2002). Changes in relative water content in two varieties of *M. charantia* during vegetative and flowering stages of stress are shown in Graph 1. Relatively higher amount of water content was maintained by wild varieties than cultivar in both stages of stress. However, the level of water content was decreased in flowering stage of stress in both the varieties. The quantity of plant pigments such as chlorophyll a, b, total chlorophyll and carotenoid analyzed at two stages of stress (vegetative and flowering stage) are shown in Graph 2. It was found that cultivar had decreased amount of pigment in the flowering stage of stress compared to vegetative stage. However, the pigment concentration was high in wild varieties. According to Pastori et al. (1992); Sairam (1998) and Kraus et al. (2004) high chlorophyll and carotenoid content is associated with stress tolerance of plants. Chandrasekharan et al. (2000) described the reduction in carotenoid content under water stress in Wheat. Low level pigment content found in cultivar in the present study may be due to decrease in the relative water content.

Graph 1: Changes in relative water content during different stages of stress



Graph 2: Changes in pigment concentration during the vegetative and flowering stage of stress



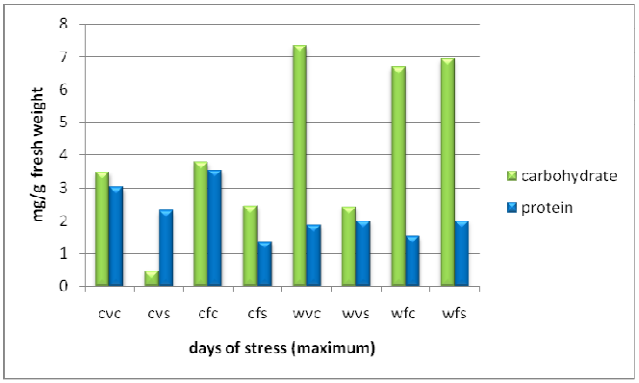
Biochemical characterization

The proline concentration during different stages of stress in cultivar and wild variety is given in Table 2. The amount of proline concentration increases as mg/100g tissue. The proline concentration increased in vegetative and flowering stages of stress both in wild and cultivar. But concentration was not much higher in cultivar during flowering stage of stress. However, wild variety showed a higher concentration of proline. Increased production of proline during stress at all stages of development was reported in rice (Roy et al. 2009). Water stress induced proline accumulation has been reported by Singh et al. 1973. The lipid peroxidation level in leaf of two varieties of *M. charantia* was determined as content of Malondialdehyde (MDA) present in it. Malondialdehyde formed from the break down of poly unsaturated fatty acids serve as a convenient index for determining the extent of peroxidation reaction which increases with increase in water stress (Smirnoff, 1995). In the present investigation, the level of MDA showed variations with stress treatments (Table 2). The vegetative stage stress showed an increased level of MDA than flowering stage stress, particularly in cultivar compared with wild variety. It indicates that cultivars are more throne to water stress during the vegetative stage. Moller (2007) has been considered the content of MDA as an indicator of oxidative damage.

Protein estimation of two varieties of *M. charantia* subjected to water deficit is given in Graph 3. It was found that quantity of protein was higher in wild variety, during vegetative and flowering stages of stress. High protein in wild reveals that variety is

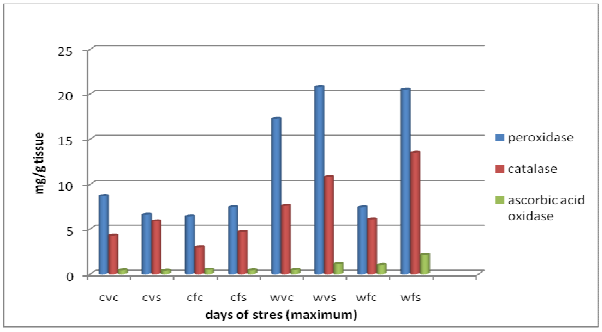
tolerant to drought. Increased protein content during stress was reported in Cowpea varieties (Akhila et al. 2007). Bray (1993) and Palva (1994) reported that certain protein, probably associated with stress tolerance is synthesized in response to water stress. Significant difference in concentration of carbohydrate was observed in two varieties of *M. charantia* under different stages of stress (vegetative and flowering) (Graph 3). The cultivar had less amount of carbohydrate during the periods as compared with control. The variety *muricata* (wild) was showed significant variations in both stages of stress than in cultivar. Garg et al. (2004) and Akhila et al. (2007) suggested that water stress significantly decreases starch content in bean genotypes and cow pea varieties respectively. Decrease in carbohydrate content due to drought was also reported in rice and wheat (Hassan et al. 2004; Pline et al. 2003).

Graph 3: Changes in the concentration of primary metabolite during vegetative and flowering stages of stress



The activity of AAO showed a gradual increase as the stress duration increases (Graph 4), in both the varieties. But the control and treated plants of wild variety were with higher ascorbic acid oxidase activity compared to the cultivar during the period of vegetative and flowering stages of stress. Increased production of AAO helps to protect wild variety from oxidative damage. Same phenomenon was reported in *Vigna unguiculata* (Akhila et al. 2007) and in wheat (Sairam et al. 1998). According to Smirnoff and Colombe (1998) the oxidative enzymes like AAO are known to deplete the pool of free radicals from accumulating to toxic levels during stress situations.

Graph 4: Changes in the activities of enzymatic antioxidant during vegetative and flowering stages of stress in the wild and cultivated variety

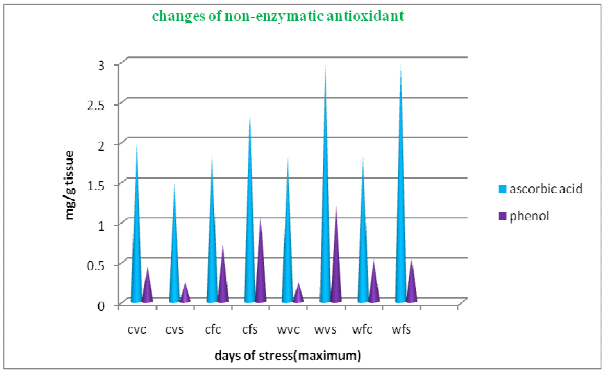


The activity of peroxidase showed significant differences between treated and control plants of wild and cultivated varieties both in the vegetative and flowering stages (Graph 4). Higher peroxidase activity observed in wild variety during flowering stage of stress may helps the plant to thrive well against the harmfull effects of oxidative stress. According to Fazeli et al. (2007) and Vaseva et al. (2012) peroxidase activity was found to be sufficiently high to enable the plants to protect themselves against oxidative stress. Drought stress induced the activity of catalase in both the cultivated and wild varieties of *M. charantia.* The treatment resulted in a significant increase in catalase activity in wild compared with cultivar as shown in Graph 4. Increased activity of catalase during drought has been reported in rice by Roy et al. (2009).

The amount of phenol showed significant differences between test and control of the two varieties of *M. charantia* during the vegetative and flowering stages of stress (Graph 5). However, wild variety *M. charantia* var. *muricata* showed an increased phenol concentration in flowering stage as compared to vegetative stage. Higher phenol content in wild variety indicates its high antioxidant property. Increased antioxidant activity during drought correlates with total phenolic content in blue berry was reported by Ehlenfeldt and Prior 2001. Plant phenolics have been considered to be the main lines of cell acclimation against stress in plants (Dixon et al. 1992; Rivero et al. 2001). Ascorbic acid plays a protective role in plants against ROS that are formed from photosynthetic and respiratory processes (Guo et al. 2005). The present study noticed a slight difference between control and treated plants of *M. charantia* in amount of ascorbic acid (Graph 5). However, wild showed higher amount of ascorbic acid concentration than cultivar during the vegetative and flowering stages of stress. Jaleel (2009) reported an increase in ascorbic acid corresponding to the age in water stressed plants of *Withania somnifera*. Ascorbic

acid increases with increase in water stress in *Vigna seedling* (Mukherjee and Choudhuri 1983).

Graph 5: Changes in the concentration of non-enzymatic antioxidants during vegetative and flowering stages of stress



Conclusions

The present investigation analysed the morphological, physiological and biochemical characterizations of cultivated and wild varieties of *Momordica charantia* L. subjected to water stress during the vegetative and flowering stages. The study found out a reduction in the morphological characters of the cultivated variety *M. charantia* var. *charantia* particularly in the number, length and weight of fruits and other yield parameters, both in the vegetative and flowering period of stress. High relative water content and enhanced production of pigments in wild varieties during the vegetative and flowering stages of stress indicate that they are tolerant to water deficit. The amount of proline accumulation, rate of lipid peroxidation and concentration of primary metabolite showed significant variations both in the control and treated varieties of *M. charantia.* Enhanced enzymatic and non-enzymatic antioxidants in the wild variety suggest that their efficiency to overcome water stress. The morphological, physiological and biochemical analysis of the varieties of *M. charantia* subjected to water stress at the vegetative and flowering stages revealed that the wild variety, *M. charantia* var. *muricata* is more tolerant to water deficit than the cultivar *M. charantia* var. *charantia*.

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Table 1: Changes in morphological characters during water deficit in two varieties of *M. charantia* Cultivar **- CVC-** vegetative stage control **CFC**- flowering stage control, **CVS**- vegetative stage stress **CFS-** flowering stage stress, Wild **- WVC**- vegetative stage control, **WFC**- flowering stage control **WVS**- vegetative stage stress **WFS**- flowering stage stress

Plant	Height cm	No.of branch	No.of leaf /plant	No.of tendril	Leaf area	N.of fruit/plant	No.of seed/pod	Length of fruit	Ten fruit wt.	Wt.of 100 seed
					Vegetative stage stage of stress					
CVC	237.0±5.90	8.50±0.57	77.00±4.14	13.75±1.25	107.9±3.78	15.25±1.79	21.00±1.68	17.52±0.75	121.63±6.31	20.66±0.89
CVS	218.0±9.77	9.25± 1.70	74.00±3.53	12.25±1.50	106.32±3.4	4.75±0.85	8.00±0.91	8.13±0.76	101.02±2.87	15.98±0.60
WVC	244.10±4.43	9.75±1.70	111.5±5.01	24.25±1.93	35.35±3.35	41.75±1.93	9.50±0.64	4.00±0.24	16.78±0.73	3.84±1.14
WVS	243.5±14.73	9.25±0.95	104.75±6.6	26.50±1.84	37.20±3.85	43.25±2.52	6.50±1.04	4.25±0.18	15.80±0.11	3.86±043
					Flowering stage of stress					
CFC	232.92±5.98	9.75±1.25	83.75±1.79	23.00±2.21	138.88±2.0	13.00±0.91	18.00±3.11	17.25±6.98	125.12±6.98	21.27±1.11
CFS	211.5±18.32	7.75±0.95	70.75±2.95	14.00±1.08	110.65±3.5	3.00±0.85	5.25±1.54	106.19±3.7	106.19±3.78	10.19±1.14
WFC	249.12±7.14	9.75±0.10	108.75±4.0	23.75±0.85	35.81±3.09	42.50±2.38	7.50±1.32	17.12±0.57	17.12±0.57	4.96±0.28
WFS	247.01±4.09	9.50±1.29	108.5±3.45	21.75±1.11	34.94±1.87	41.25±1.70	8.00±1.08	16.17±0.82	16.17±0.82	4.19±0.16

Table 2: Changes in the rate of lipid peroxidation and proline

Plant type		**Lipid peroxidation**		**Proline**	
		Water stress		Water stress	
		control	treated	Control	treated
Cultivar	Vegetative stage	0.509± 0.322	0.790 ± 0.172	3.207 ± 0.440	3.095 ± 0.150
	Flowering stage	0.846± 0.530	0.698 ±0.4526	3.295 ± 0.161	3.314 ± 0.858
Wild	Vegetative stage	0.643± 0.338	1.399 ± 0 .208	5.640 ±0 .236	5.896± 0.931
	Flowering stage	0.558± 0.363	1.841 ± 0.0752	5.3722 ± 0.243	6.285 ± 0.552

Changes in peroxidase and protein in relation to sex differentiation in *Coccinia grandis* (l.) Voigt. (Cucurbitaceae)

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ABSTRACT: *Sex determination is an important developmental event in the life cycle of all sexually reproducing organisms. Proteins and isoenzymes have a vital role in determining the sex of plants during the reproductive phase. Peroxidase plays a role in the regulation of growth substances in plants. The candidate species, Coccinia grandis, is a eudicot belonging to the family Cucurbitaceae which shows strong dioecy and heteromorphic mode of sex determination. Present investigation analysed the activities of peroxidase enzyme and proteins in the vegetative and floral meristems of male and female plants of C. grandis using spectrophotometric and electrophoretic methods. Level of peroxidase activity was found to be higher in the vegetative apical meristem compared to the flower primordial stage of both the sexes, and that was higher in female than in male flower primordia. The polyacrylamide gel electrophoresis revealed that the expression of protein in the species varied in relation to sex. The study discussed the significance of protein and peroxidase in determining the sexual phenotypes in C. grandis.*

Key words*: Coccinia grandis, Peroxidase, Protein, Dioecious, Sexual phenotype.*

Introduction

Coccinia grandis (L.) Voigt, a dioecious perennial herb, commonly known as Ivy gourd, Scarlet gourd, Tindori and Kovakkai (in Malayalam), is a tropical plant species belonging to the family Cucurbitaceae. Dioecy is a rare condition in which male and female flowers are produced on different plants. About 38% of angiosperms have this feature, of these only 40 representatives have sex chromosomes (Charlesworth and Guttman 1999; Givinish 1980; Ming et al. 2007). Dioecy appears to be rather more common among dicot genera than in monocots and particularly prevalent in some families including Cucurbitaceae (Renner and Ricklefs 1995).

Flowering is a complex biological process that is regulated by both environmental and developmental factors. Many physiological and biochemical changes were taking place during the vegetative to reproductive development. Researchers have revealed that genetic and molecular mechanisms of transition occur from vegetative growth to flowering in the dioecious species, *Arabidopsis thaliana* (Mouradov et al. 2002). According to Lloyd 1980; Lovett and Harper (1980) male and female plants perform different reproductive functions that impose very different resource demands on the plant. Meagher (1984) stated that certain physiological differences between the sexes are thought to be involved for plant sexuality. Among these, influence of plant growth

substances and differential enzyme activity are the major aspects of the physiology of sex expression that have been studied in species like *Phoenix dactylifera* (Suganuma and Wasaki 1983), *Ricinus communis* (Jaiswal and Kumar 1983) *Spinacea oleracea* (Onyekwelu and Harper 1979) and in *Carica papaya* (Minoz et al. 1982). Studies on variations in the enzyme activities in the male and female plants of *Coccinia grandis* was reported by Gulati (1989). However, the role of enzyme and protein in the transition of vegetative bud to floral stage was not yet reported. The present investigation thus aimed to determine the changes in protein and peroxidase content in the dioecious species *Coccinia grandis,* in relation to the vegetative to floral development and senescence.

Materials and Methods

Male and female plants of *C.grandis* with similar environmental background from Kariavattom Campus were used for the present investigation (Fig. 1 A&B). Vegetative (Fig. 2A), floral buds (10-20 days after initiation of floral bud) (Fig. 2B&C) and mature flowers (28-29 days after floral bud formation) were selected for the analysis.

100mg of the sample was extracted in 3ml of ice cold 0.1M phosphate buffer (pH 7) by grinding in a pre cooled mortar and pestle and centrifuged at 18000g for 15 minutes at 5^0C. Then 3ml of buffer solution, 0.05 ml of guaiacol (240mg/100ml) and 0.03 ml hydrogen peroxide (0.14ml of 30% H_2O_2/100ml) were added to 0.1ml of the supernatant. Then the time required to increase the absorbance from 0.05 to 0.1 at 436 nm absorbance was noted. The protein estimation was carried out according to Lowry et al. (1951). 100 mg of the sample was extracted in phosphate buffer (pH 7), centrifuged and the supernatant was collected for the analysis.

Crude extract containing protein was subjected to electrophoresis using 7.5% of polyacrylamide gel for 2.5 hours. The gels were incubated in a mixture containing 0.4 ml of guaiacol and 0.3 ml hydrogen peroxide in 0.1 M sodium phosphate buffer (pH 6.1) for visualizing the isoenzymes of peroxidase.

Figure 1: *Coccinia grandis* (A). male and (B). female plant

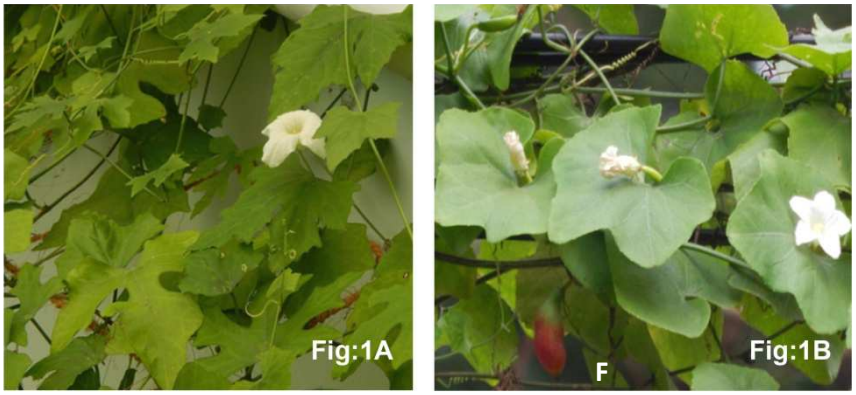


Figure 2: (**A).** Vegetative buds, (B). Female floral bud and (C). Male floral buds

Results and Discussion

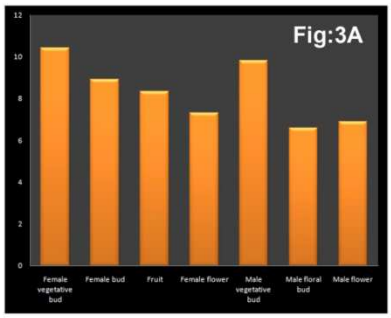
Isoperoxidases are important in plant development and differentiation. Significant differences in peroxidase activity were observed in the present investigation at the vegetative and reproductive stages in *C. grandis* (Fig. 3A & B). During vegetative stage an increase in enzyme activity was noted, and it suggests the role of the enzyme in the regulation of plant growth substances at the early period of development. Levels of peroxidase activity had also shown to differ between the male and female flowers of *C. grandis* indicating the significance of peroxidase in the sex expression of the dioecious species. Differential expression of peroxidase isozyme had been reported in species such as *Actinidia chinensis* (Hirsch and Fortune 1984) and *Mercurialis annua* (Kahlem 1976).

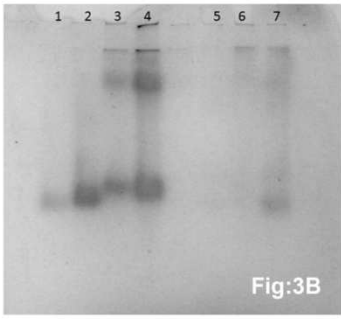
In reproductive development, the study noticed higher peroxidase activity in the female bud than in male (Fig. 3A). More or less similar observations were reported in *C. sativus* by Sriram and Mohan (1984). It was found that the mature flowers showed more isozyme activity than floral buds, and this may be a consequence of senescence. According to Costa et al. (2005) isozyme activity was increased in the senescence stage

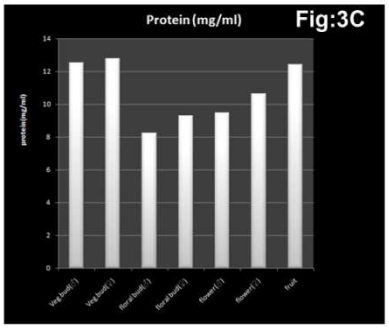
of floral organs due to the disintegration of the cell membranes and organelles and the formation of radicals and reactive species (ROS), such as O_2 and H_2O_2. Hossain et al. (2006) reported an increase in the level of radicals and reactive species in petals of *gladiolus* L. and suggested that it is due to the regulation of programmed peroxidase activity, which seems to be a prerequisite for the onset of senescence. The results confirmed previously expressed considerations, indicating that peroxidase is involved in the senescence process and in the control of cell wall stability, primarily by the elimination of ROS, and also by cross linking components of the secondary cell wall (Passardi et al. 2004). Bartoli et al. (1995) also observed an increase in peroxidase activity in petals of *Chrysanthemum morifolium* during senescence. According to Lima et al. (1999), peroxidase appears at a low concentration in green tissues and at high concentrations in mature tissues.

In the present study, significant changes were observed in relation to total protein content in the vegetative and floral bud and in mature flowers of *C. grandis*. Protein content was tended to increase in vegetative buds in both the sexes, but decreased in reproductive stage (Fig. 3C). The increase in protein content during vegetative stage can be considered as an adaptation for survival under stress. Sood et al. (2006), noted higher protein content in the young plants of rose. The present investigation noticed a decrease in the nature of the protein content in mature flowers than floral buds (Fig. 3C). Changes in the protein levels during flower development were reported in *Chamelaucium uncinatum* (Olley et al. 1996) and *Agapanthus africanus* (Nihal et al. 2009). Higher protein concentrations in the floral buds of *C. grandis* obtained in the present investigation may be explained by the view of Woodson and Handa (1987) that the synthetic processes and post-translational modifications regulating protein content have an important implication for the successful control of flower development. The decrease in the level of protein content from bud to flowering stage observed in the study may be due to the protein loss as a result of increased protease activity that leads to accelerated senescence of flower as suggested by (Gietl and Schmid 2001). Decrease in protein content during senescence was also observed in petals of *Sandersonia aurantiaca* (Eason et al. 2002) and *Dendrobium* (Lerslerwong et al. 2009). Al-Khatib and Paulsen (1984); Zavaleta-Mancera et al. (1999) and Ueda et al. (2000) had reported a decrease in total protein concentration due to the accelerated leaf senescence. Reid and Wu (1992) reported that senescence is associated with derepression of specific genes, increasing polyribosome activity and changes in the pattern of protein synthesis.

Figure 3: (A). Estimation of peroxidase in male and females of *C. grandis*, **(B).** Isozyme profiling for peroxidase (1- Male bud, 2-Female bud, 3-Male vegetative bud, 4-Female vegetative bud, 5-Male flower, 6-Female flower, 7- Fruit) and (C). Estimation of total protein content in vegetative and reproductive stages of development.







Conclusion

The present study revealed that peroxidase and proteins had an important role in the transition of vegetative to reproductive development and senescence of the flowers in the dioecious species, *C. grandis.*

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Antioxidation and mitochondrial functions of *Oryza sativa* L. exposed to high light stress

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ABSTRACT: *Although light is crucial for the survival of plants, exposure of a plant to light exceeding what is utilized in photochemistry leads to the production of reactive oxygen species and the inactivation of photosynthesis. The effects of high intensity light stress (2,000 µmolm−2s−1) on various physiological and biochemical parameters of rice seedlings (*Oryza sativa *'Annapoornna') at different time intervals (0, 2, 4, 6 and 8h) were investigated. The results revealed that high light stress adversely affected various physiological and biochemical features in rice seedlings with increase in high light exposing period. Malondialdehyde content and the mitochondrial activity was found to be increased upon increasing the duration of high light exposure and the maximum increase was recorded at 8h of high light exposure. In order to assess the role of the enzymatic and non enzymatic antioxidative defence system in rice seedlings subjected to varying periods of high light exposure, total ascorbate, proline content, guaiacol peroxidase (EC 1.11.1.7) and catalase (EC 1.11.1.6) activity were monitored and it was found that content of non enzymatic and acivity of enzymatic antioxidants increased in* O. sativa, *with increase in period of high light exposure, which aid in countering the reactive oxygen species generated. Antioxidant defence machinery in rice seedlings was found to enhance its functioning with increase in high light exposing period and reached maximum at 8h of high light stress application. Ascorbate and guaiacol peroxidase was found to have a prominent role in antioxidation capacity of* O. sativa *exposed to high light stress.*

Key words*: Catalase, Guaiacol peroxidase, Malondialdehyde, Reactive oxygen species.*

Introduction

Cereals are the most significant resource of calories to humans. Rice, a common cereal, is staple food for more than half of the population. It is expected that we will have to produce 25% more rice by the year 2030 (Khush 2012). As the 21st century unfolds, global rice production has showed signs that it may no longer be secure in the future. There are many constraints and challenges to reduce rice food shortage, as climatic change highly affects the agricultural productivity. According to Mahajan and Tuteja (2005), the abiotic stresses- salinity, low temperature, excess light and drought in particular- are responsible for most of the reduction in agricultural productivity.

Light is essential for plant's life, but it is also a potential cause of photooxidative damage to plants. When the absorbed light energy exceeds the rate of the photochemistry, photoinhibition occurs. After a prolonged period of photoinhibition, the generated reactive oxygen species oxidatively damage the plant cells, resulting in photodestruction by structural damage to chloroplasts and other organelles (Zhang et

al. 2011). In plants, the damage due to excess light is caused in part by reactive oxygen species (ROS) generated by electrons leaking from the photosynthetic electron transport system. Photoprotection of plants against excess light includes direct removal of excess excitation energy within the light-capturing system i.e. thermal dissipation and/or removal of active oxygen species formed in the photochemical apparatus by various components of antioxidant defence system (Jiao et al. 2004).

In the above contests, studies concerning effects of high light stress on various physiological and biochemical parameters of rice seedlings seems to be high significance and are indispensable. In this study, we investigated the effects of high light stress on malondialdehyde (MDA) content, mitochondrial activity and enzymatic and non enzymatic antioxidants in rice seedlings after high light exposure.

Materials and methods

Rice seeds (*Oryza sativa* 'Annapoornna') were collected from RARS, Pattambi, Kerala. Rice seedlings were raised in half strength Hoagland medium (Hoagland and Arnon 1940) after surface sterilizaion with 0.1% $HgC1_2$ for 4 min. and they were grown in a plant growth chamber under controlled environmental conditions (light/dark cycles 14/10 h at 300 $\mu molm^{-2}s^{-1}$, 24+2°C and RH 55+5%).

Rice seedlings were exposed to high intensity light stress (2,000 $\mu molm^{-2}s^{-1}$), provided by 1000 W PAR64 (Philips) metal halide lamps, after 9 day of plant growth. To protect the seedlings from the heat generated by the lamp, a trough of transparent glass of 20 cm depth with circulating water was placed under the lamp. Light intensity at the surface of the leaves was measured by a solar radiation monitor (EMCON, Cochin, India) and various physiological and biochemical analyses were measured at different time intervals (0, 2, 4, 6 and 8 h) in the seedlings exposed to high intensity light.

MDA was extracted and estimated according to the method of Heath and Packer (1968). MDA concentration was calculated using its molar extinction coefficient of 155 mM L^{-1} cm^{-1}. Mitochondrial isolation was carried out according to Kolloffel (1967) after the plant materials were homogenized in ice cold isolation buffer (0.05 M phosphate buffer with 0.4 M sucrose and 5 mM EDTA, pH 7.2). Oxygen consumption by mitochondria was measured at 25°C using a Clark-type O_2 electrode (DW1/AD, Hansatech, Norflok, UK) as per the protocol of Schmitt and Dizengremel (1989) and the oxidation rate of NADH was calculated in terms of µmol O_2 consumed min^{-1} mg^{-1} protein. The protein content in the mitochondrial preparations was determined by the method of Bradford (1976).

Ascorbate content was measured according to the method of Gillespie and Ainsworth (2007). A standard curve was prepared using commercial L-ascorbic acid. Free proline

content was extracted from leaf using 3% sulphosalicylic acid and estimated following the method of Bates et al. (1973) using L-proline as standard.

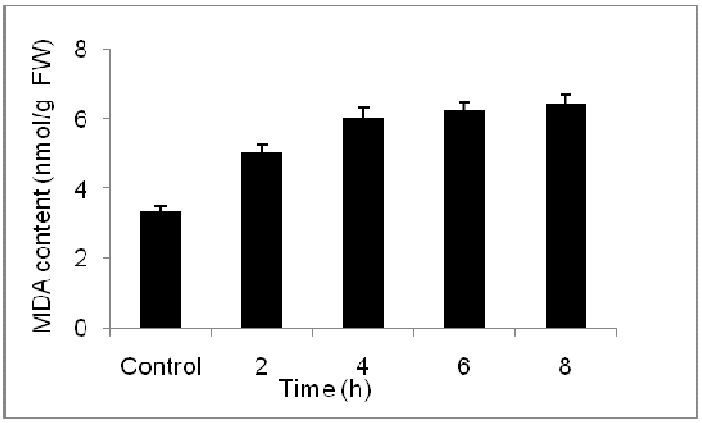
For assessing antioxidant enzymatic activity in rice seedlings, fresh leaf tissues were homogenized in 50 mM potassium phosphate buffer (pH 7.0) using a prechilled mortar and pestle. The homogenized extract was filtered through 2 layers of muslin cloth and the filtrate was centrifuged at 10,000 g for 15 min at 4°C. The supernatants were collected and used for the enzyme assay (Yin et al. 2009).

The activity of catalase (CAT EC 1.11.1.6) was determined by analyzing the decrease in absorbance at 240 nm for 1 min following the decomposition of H_2O_2 (Kar and Mishra 1976). One unit of enzyme activity was defined as µmoles H_2O_2 decomposed per minute per mg protein. Guaiacol peroxidase (GPX, EC 1.11.1.7) activity was measured by following the change of absorption at 420 nm due to guaiacol oxidation (Polle et al. 1994). One unit of enzyme activity was defined as µmoles of guaiacol oxidized per minute per mg protein.

Results

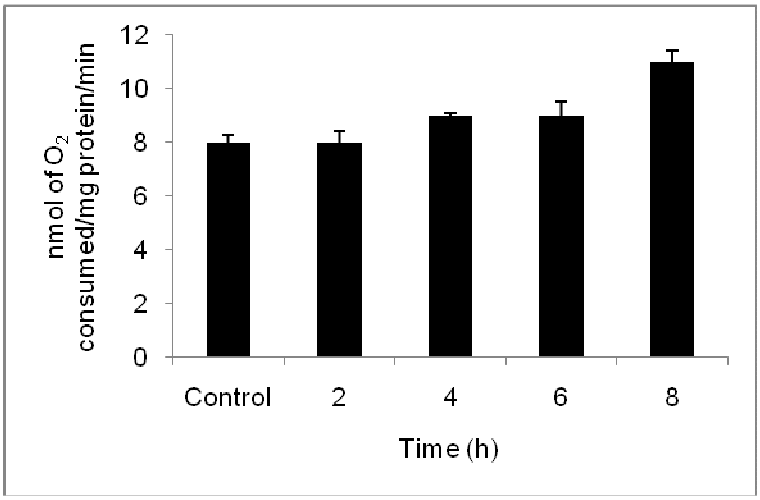
In order to study the effect of high light stress on primary metabolic functions and the tolerance mechanisms developed to cope with this adverse environmental condition in rice seedlings, various physiological and biochemical parameters were analyzed in detail in control and high light treated plants. MDA content was found to be increased upon increasing the duration of high light exposure in rice seedlings and reached maximum at 8h of high light exposure (92% as compared to the control), whereas it was only 50% increase after 2h of high light stress application. The percentage increase in MDA content at 4 and 6h of high light exposure was 79 and 87% respectively as compared to the control plants (Fig. 1).

Figure 1: MDA content of rice seedlings exposed to high light (0-8h). Values are the mean ± SE of three independent experiments.



Under high light stress conditions, mitochondrial activity got enhanced in rice seedlings. The increase in mitochondrial activity under varying high light exposing period was highest during 8h (38% as compared to the control) and rate of increase after 4 and 6h of high light stress exposure was 13% as compared to control. There was no any change in mitochondrial activity of rice seedlings upon treatment with 2h of high light exposure (Fig. 2).

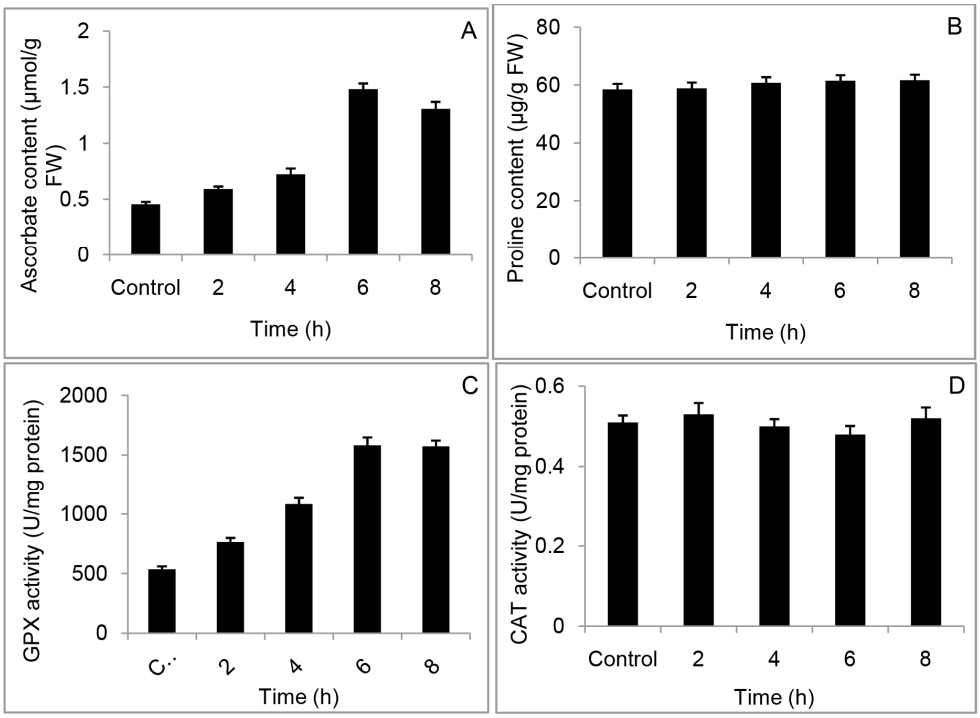
Figure 2: Mitochondrial activity (nmol of O2 consumed/mg protein/min) of rice seedlings exposed to high light (0-8h). Values are the mean ± SE of three independent experiments.



In order to study the role of non enzymatic antioxidant system in countering high light stress in rice seedlings, ascorbate and proline contents were monitored. Ascorbate content was found to increase significantly upon increasing the high light exposure period (0-8h) and rate of increase was more prominent at 6h of high light exposure (224% as compared to control plants). High light exposure resulted in minimum increase of total proline content of rice seedlings as compared to control plants (5%) after 6 and 8h of high light stress (Fig. 3).

The antioxidant enzymes activities were measured in the seedlings of *O. sativa* subjected to four different time intervals (0, 2, 4, 6 and 8h). GPX activity of rice seedlings increased upon increasing the high light exposure period and reached maximum at 6 and 8h of high light exposure (195%) as compared to control. The percentage increase of GPX activity at 2 and 4h of high light exposure was 42 and 102% respectively as compared to control seedlings. From the results obtained, it was evident that there was no significant change in CAT activity of rice seedlings upon treatment with varying periods of high light stress (Fig. 3).

Figure 3: Antioxidant components [ascorbate (A), proline (B), GPX (C) and CAT (D)] in rice seedlings exposed to high light (0-8h). Values are the mean ± SE of three independent experiments.



Discussion

The increased level of MDA is counted as an index of membrane damage due to lipid peroxidation by the effect of ROS generated under different environmental stress conditions. This is in correlation with our results of increasing MDA content in rice seedlings after high light treatment for different time intervals. It was earlier found that MDA content was elevated in *Arabidopsis thaliana* (Distelbarth et al. 2013) and *Prorocentrum minimum* (Park et al. 2009) under high light stress.

Although mitochondria is not known to have any direct role in high light stress alteration, the less increase in mitochondrial activity of rice seedlings may be due to the metabolic interactions occurring between chloroplasts and mitochondria. Under high light conditions, the respiratory chain is thought to play role in dissipating excess reductants produced in the chloroplasts (Raghavendra and Padmasree 2003). The non-phosphorylating pathways like alternative oxidase pathway in the respiratory chain, a mechanism for plant photo-protection, are considered to be efficient dissipation systems for these reductants. This pathway, which is generally considered as energy-wasteful, is assumed to consume excess reductants and thus avoid ROS production,

especially under stressful conditions (Umbach et al. 2005). Yoshida et al. (2008) demonstrated that leaf alternative oxidase is up-regulated in *Arabidopsis* under highlight stress.

To assess the role of antioxidative defence system to detoxify ROS produced during high light stress, the activities of major antioxidants were monitored in *O. sativa* subjected to varying periods of high light stress. Ascorbate is the most abundant and water soluble antioxidant which aid in minimizing the damage caused by ROS in plants (Toth et al. 2011). In this investigation too, ascorbate was found to accumulate greatly in high light exposed rice seedlings. It has been reported that ascorbate plays a crucial role in protection against high light stress in plants (Talla et al. 2011). A significant increase of ascorbate was recorded in *Arabidopsis thaliana* after high light exposure (Zechmann et al. 2011). The accumulation of proline under various stressed conditions is certainly considered an adaptive response of seedlings subjected to high light stress also (Hayat et al. 2012). In this study, it was noticed that even at severe high light treatment caused only slight increase of proline in rice seedlings. In *O. sativa*, high light treatment preferentially enhanced the activity of GPX, to counter the increase of ROS generated in seedlings as a result of high light stress. A positive correlation between the antioxidant enzyme activities and stress tolerance were already reported in plants (Jisha and Puthur 2014). Coinciding with our results, Gechev et al. (2003) found that the increase in GPX activity and no significant change in CAT activity of tobacco plants when treated with high intensity light.

The results conclusively proved that high intensity light stress adversely affected various physiological and biochemical features in rice seedlings with increase in high light exposing period. Antioxidant defence machinery in rice seedlings was found to enhance its functioning with increase in high light exposing period and reached maximum at 8h of high light stress application. Ascorbate and GPX could be having a prominent role in *O. sativa* for ameliorating the negative effects of high light stress.

Acknowledgements

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Spirulina platensis: an ideal organism for metabolic labelling using mini bioreactor

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ABSTRACT*: Microalgae like Spirulina platensis contain very high amount of provitamin A precursor β-carotene and have the ability to synthesise β-carotene photosynthetically, making this organism ideal for metabolic labelling of carotenoids. Stable isotope labelling for studies involving pro vitamin A of algae appears to be a safe and cheapest alternative in predicting Vitamin A status and bioavailability. Stable isotopes could be traceable by using mass spectrometry. Production of provitamin A precursors and its labelling intensity could be easily modulated by manipulating the culture conditions. We have developed a mini bioreactor system for metabolic labelling of algae using heavy water. Three to four fold increase in β-carotene production could be achieved by modulation of light intensity. Light intensity of 75 to 100 μ mol m-2 s-1 was found to be ideal for optimum biomass and β-carotene production under laboratory conditions. Increased biomass production was found possible by bubbling CO_2 air mixture. Maximum effective utilization of costly label and biomass output using the spent medium was achieved with the use of mini bioreactor. Mass Spectrometric analysis of extracts of algal biomass revealed shift in mass to charge ratio (m/z) of H in β-carotene ($C_{40}H_{56}$) molecule. The results illustrate that over 60% of 56 hydrogen atoms, replaced with deuterium when compared to unlabelled control. High level of the label incorporation is essential for human intervention trials where β-carotene recovery is very little from samples like plasma or tissue after a feeding experiment. Spirulina grown in D_2O enriched water, apart from being a source of deuterated compounds other labelled macromolecules could also be useful for bioavailability studies involving human beings.*

Key words*: Algae, Spirulina, Labelling, β-carotene, Stable isotope, Vitamin A deficiency*

Introduction

Photosynthetic algae have been increasingly being utilized as food, bio fuels, nutraceuticals and useful biomass. Intrinsically labelled precursors could be produced by growing suitable algae in controlled conditions. Tagging of metabolites using radiolabelled precursors has been widely used to trace unknown metabolic reactions in model organisms including human beings. For instance, vitamin A deficiency is a serious problem in many parts of the world especially in many developing countries (Beaton et al. 1993). In South East Asia, it is estimated that quarter of a million children go blind each year because of this nutritional deficiency (Sommer 1982). Developments in the methodologies particularly stable-isotope dilution method, provides an accurate determination of vitamin A/provitamin A status in humans where other invasive methods are not possible. Stable isotope labelling for studies (Perker et al. 1992; Tang et al. 1999; Tang 2000; Tang 2014) involving pro vitamin A appears to be a safe alternative but intrinsic labelling in natural resources seems complicated (van Vliet et al. 1996).

However, many stable isotope tracer production approaches depends largely on chemically synthesized, complex and expensive reagents.

Spirulina is a fresh water micro alga with very high nutritive value (Key 1991; Vonshak 1997) and has attracted much attention because of its use as human and animal protein source and has very high content of β-carotene than any other assorted vegetables. It contains valuable micronutrients such as biotin, vitamin B_{12}, folic acid and other vitamin B complexes (Venkataraman 1993) and certain strains accumulate large amounts of γ-linolenic acid (Mahajan and Kamat 1995). This micro algae has the ability to synthesise β-carotene photosynthetically, making it an ideal organism for metabolic labelling of carotenoids. Efficiency of production of metabolically labelled provitamin A depends mainly on the type of culture system, growth conditions and nature of precursor molecules. Therefore, present study aimed at to produce intrinsically labelled deuterated carotenoids using carotenoid rich blue green algae, *S. platensis* in culture in a bioreactor.

Materials and methods

Zarrouk's medium (Zarrouk 1966) was used for the liquid culture of *S. platensis*. Sterilised media was prepared by autoclaving and used for developing asceptic cultures in flasks. Two percent agar slants were also made with same constituents of Zarrouk's medium for the maintenance of pure cultures obtained from CFTRI, Mysore. Initial cultures were kept at ambient temperature (25.0°C ± 2.0°C) in subdued light of 30-50 μ $molm^{-2}s^{-1}$ and incubated the cultures at 25°C with light phase of 10-12 hours and dark phase of 8-10 hours. Liquid stock cultures were put on rocking shakers with speed of 30-40 rpm. Subsequent subcultures were made as and when required. *S. platensis* was grown initially in H_2O and subsequently D_2O enriched water (D_2O content 99.4% v/v) purchased from BARC, Mumbai, India.

A laboratory scale micro bioreactor was fabricated using Borosil glass. Transparent vertical column mini bioreactor with illumination and agitating system having a working volume of 1.5 litter capacity was developed the design is shown schematically (Fig. 1). Main part of the bioreactor consists of an illuminated region with column of 100 cm length and diameter of 5 cm so as to hold 1.5 litre of the culture. Top of the column opened to desiccators filled with silica gel and the bottom of bioreactor column fitted with stoppered outlet for withdrawal of culture. Magnetic stirrer was used to agitate and controlled mixing of the culture at desired speed (150 rpm). Reactor column was illuminated with a set of cool white florescent lamps (4 x 20 W) fitted on a movable stand, which in turn provided appropriate photosynthetic flux density of 75μto 100μmol m^{-2} s^{-1}. Increased biomass production was found possible by bubbling CO_2-air mixture at laboratory conditions.

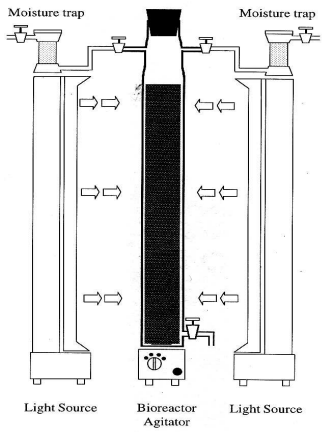
In order to compensate lag in the biomass production of deuterated cultures and to ensure maximum utilisation of costly deuterium in the medium the cultures were allowed to grow further until the next stationary phase (second cycle), thus the same

medium was used up to 6th cycle. This was done initially using the normal water and then extended to ^{2}[H] cultures.

Freshly inoculated medium was allowed to grow and the biomass increase was assessed by withdrawing aliquots of culture from lag phase to log phase to stationary phase on every 5th day. The growth kinetics in terms of biomass (dry weight) of the algal cells was determined by measuring the absorbance (Becker 1994). Biomass in suspension cultures up to 30 to 40 days was harvested at stationary phase by withdrawing the cultures in 50ml polypropylene tubes and centrifuged at 900 x g for 10 minutes and biomass pellets were freeze-dried, weighed and stored under nitrogen at -20°C.

Harvested biomass was suspended in acetone: petroleum ether (1:1) v/v and kept overnight under 4°C for better and easy recovery of carotenoids. Then the mixture was centrifuged at 100x g for 20 minutes. After repeated extractions (4 times) the supernatants were pooled and washed twice with distilled water using a separating funnel. Washed extracts were dried over anhydrous sodium sulphate and reduced to minimum volume by evaporating the solvents using N_2 stream. The crude extracts were reconstituted in hexane and kept for further separation and fractionation of carotenoids in amber coloured containers under nitrogen stored in -20°C. All operations were done at subdued light under N_2 atmosphere. Carotenoid extracts were subjected to liquid chromatographic (LC) analysis (Waters LC). Injection volume was 10µl and total run time of 40 minutes was given and individual carotenoids were separated. Then the beta carotene fraction was passed on to the Mass Spectrometer (Waters) attached to the LC. Relative abundance and mass to charge ratio (m/z) of each fraction were computed and mass spectrum was constructed. Statistical analysis of the data was done using Student's t-test (Bennett and Franklin 1967).

Figure 1: Schematic outline of the bioreactor showing cylindrical body of volume 1.5 litters and movable light source (arrows indicate direction) with cool white fluorescent lamps on the either side. Magnetic stirrer was used to agitate the culture inside the bioreactor and moisture trap for preventing atmospheric exchange of deuterium is attached.



Results

Mini bioreactor system was designed and tested for scaling up the production of biomass and labelled compounds. Productivity of the bioreactor was compared with flask cultures (Fig. 2) the results show that in the growth cycle, a lag phase of 15 to 20 days was observed in bioreactor and in flask cultures. On the 35$^{t\ h}$days after inoculation, significantly higher dry biomass (300 mg/L) was produced when *Spirulina* was grown in the bioreactor compared to that in the flasks in the stationary phase (180 mg/L) in the deuterated medium. In order to maximise the utilisation of the deuterium label, reuse of spent heavy water medium was tried. The spent D_2O medium was used in repeated cycles and effectiveness in biomass and β-carotene production was studied. Fig. 3 shows the kinetics of biomass production. The quantity of biomass produced was not affected up to 4th cycle. Mean dry biomass production was about 360 mg/L in the first cycle. However, the amount of biomass produced was less in the 6th passage when compared to 1st and 5th cycle.

Biomass obtained from different cycles of growth was extracted and carotenoids were separated using liquid chromatography. Components of total carotenoids were analysed using LC. Lutein and *trans*-β-carotene were the prominent carotenoids. Table 1 shows the relative amounts of different carotenoids present in the *Spirulina* grown in D_2O medium in bioreactor. Separated carotenoid fractions were passed on to Mass Spectrometer for determining the labelling intensity of β-carotene molecule by replacement of H by deuterium. Mass spectrum of β-carotene isolated from the *Spirulina* under optimum conditions of growth and replacement of [H] atom in the β-carotene in terms of charge-to-mass ratio is about 40-60% as evidenced from the shift in the m/z ratio from 534 to arange of 555 to 570, mass spectrum of β-carotene extracted from *Spirulina* grown in D_2O in bioreactor when compared to unlabelled β-carotene with m/z ratio of 534.

Figure 2: Kinetics of growth of *Spirulina* cultures in flasks and bioreactor using deuterated water as medium. Suspension cultures of *S. platensis* were allowed to grow under controlled light and temperature in culture flasks and in bioreactor. Dry biomass production was assessed every five days. Cells grown in flasks were used for the comparison. Values given are mean ± SD (n=3).

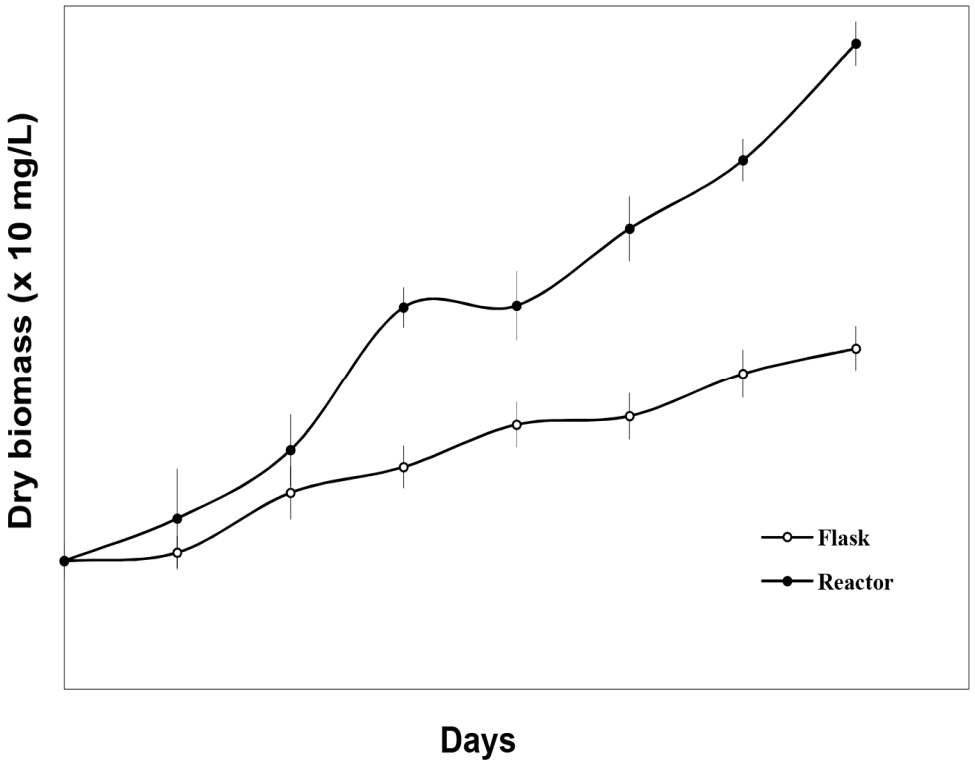


Figure 3: Kinetics of growth of *Spirulina* in repeated cycles in bioreactor. *Spirulina* culture in bioreactor was harvested by centrifugation and 1/5th of the cells were reinoculated into the spent medium and allowed to grow further under same conditions. This was repeated for up to 6th cycle. Biomass content of each passage was determined. Values are the average of three different cultures analyzed in duplicate ($p<0.05$).

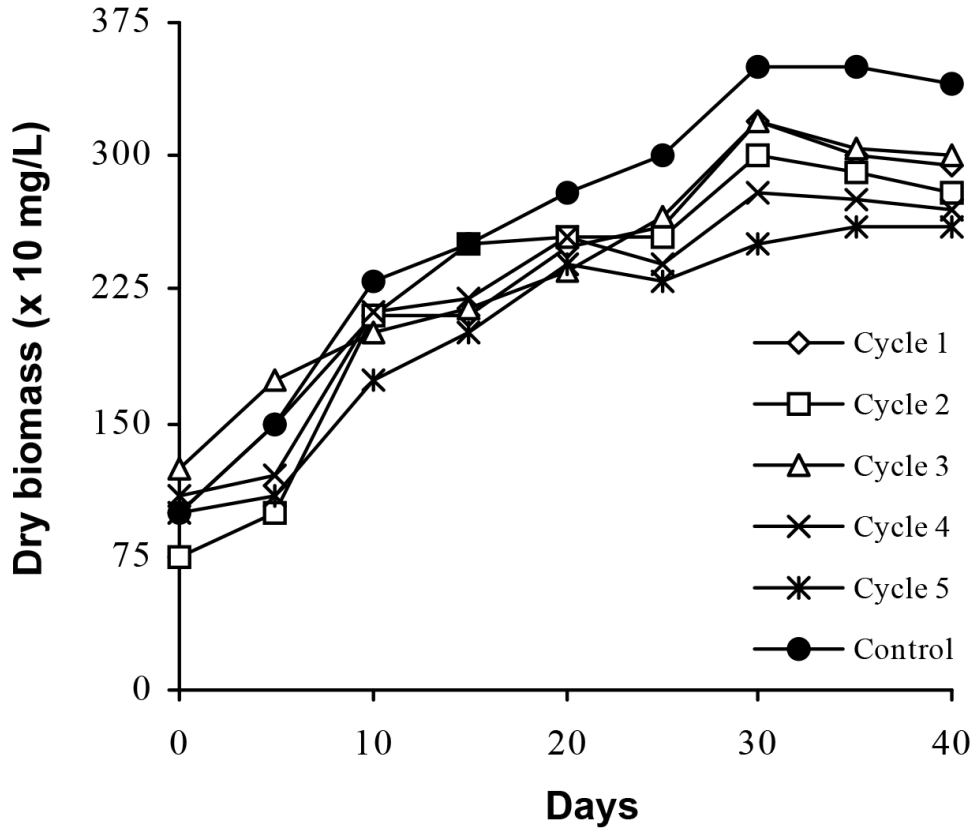


Table 1: Relative quantities of different carotenoids in *Spirulina platensis* grown in D_2O enriched medium in different cycles in bioreactor. *S. platensis* cultures were grown in deuterium enriched medium in 6 cycles in bioreactor for 40 days each. The biomass was harvested extracted and analysed for various carotenoids using LC. Analysis was done in triplicate and the mean values were expressed as percentages of the total amount of carotenoids

Cycles	1	2	3	4	5	6
Total carotenoids (μg g^{-1} dry mass)	**740**	**670**	**560**	**590**	**525**	**512**
Individual carotenoids						
Lutein	27	31	29	36	22	20
Zeaxanthin	10	8	11	9	8	7
Cryptoxanthin	2	1	2	1	0.5	0.75
13-*cis*-β-carotene	1	1	1	1	1	0.5
α-carotene	0.5	1	1	0.8	1	1.0
trans-β-carotene	18	17	21	21	17	13.0
9-*cis*-β-carotene	4	4	5	4	3.5	3.0

Discussion

S. platensis is blue-green algae which usually grows in warm temperature, especially in tropical regions and is reported to grow abundantly in alkaline waters in salty lakes (Farrar 1966; Ciferri 1983). It has very high nutritive value owing to the presence of high content of proteins with the essential amino acids and several other micronutrients. Apart from important pigments like chlorophyll, xanthophyll and phycocyanin, *Spirulina* has a carotene content of ten times that of carrots and iron content 12 times that of any other food and is the richest vegetarian source of vitamin B_{12}. *Spirulina* has been easily incorporated in medical formulations owing to its excellent nutrient composition (Becker 1994). *Spirulina* containing intrinsically labelled provitamin A carotenoids can be used to study the influence of various factors on the absorption, bioavailability and bioconversion of provitamin A carotenoids.

After standardisation of small-scale production of deuterated carotenoids in flasks, a mini laboratory scale bioreactor was designed and tested, for scaling up the production of biomass and labelled compounds. Productivity of the reactor was tested by growing the algae up to 40 days and compared it with growth in flasks. Comparatively high biomass was obtained from the bioreactor culture.The difference in biomass production may be due to the high rate of photosynthesis, uniform distribution of algal

cells and temperature stability in the bioreactor. This indicates that the proposed bioreactor is more productive than the conventional cultures in flasks. As the reactor was designed for the production of intrinsically labelled β-carotene, *Spirulina* growth was monitored and the biomass production was found to be considerably increased when compared with heavy water cultures in conical flasks (Gireesh et al. 2001) under controlled conditions, is one of the advantages of this bioreactor where high biomass production may lead to increased label incorporation.

The results of MS analysis demonstrate over 60% incorporation of deuterium into the β-carotene produced by algal cells as evidenced from the shift of m/z ratio to 566 compared to unlabelled β-carotene. High level of label in corporation is essential for tracer analysis studies in human intervention trials where only very little amount of the β-carotene may be recovered from the blood samples following a feeding experiment (Wilson et al. 1997). While using heavy water in deuterium incorporation studies, one of the problems is the dilution of heavy water with moisture. This may lead to lower deuterium incorporation. The present bioreactor was fitted with moisture trap to limit moisture exchange to circumvent this problem. Repeated use of spent medium in reactor reduces the cost of production. *Spirulina* cultured in deuterated medium is rich sources of carotenoids other than β-carotene. In addition to this, algal cultures grown in heavy water may be unique in their physical properties due to deuterium substitution. It is also important to note that this reactor system has the option to control the degree of isotopic labelling by moisture exclusion, this could be possible and each isotopomer with specific m/z ratio would be useful in tracer analysis studies. The results show that the *Spirulina* grown in D_2O enriched water, apart from being a source of deuterated compound also will be useful to study the bioavailability of provitamin A.

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Rootstock induced epigenetic variation in *Hevea brasiliensis*

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ABSTRACT: *The heritable variation within a species is the consequence of the difference in the primary DNA sequence of different individuals. The term epigenetics is generally used to refer the study of heritable change in gene expression that is independent of DNA sequence. Epigenetic changes can result in altered gene transcription and serves as an important mechanism in regulating gene expression during development and in response to stimulus. It is reported that heritable epigenetic marks persists through meiosis and are stably transferred to the next generation resulting in trans-generational epigenetic inheritance. Rubber tree is commercially propagated by bud grafting and the effect of rootstock on the performance of scion is still debated. In the present study we made an attempt to identify the impact of stock scion interaction on the epigenome of rubber by comparing the methylation pattern of uniform, own rooted seedlings and their bud grafted counter parts. Multiple uniform seedlings of Hevea brasiliensis were developed through half ovulo embryo culture method. Genetic as well as epigenetic uniformity of these seedlings was confirmed by RAPD as well as MSAP (Methylation Sensitive Amplified Length Polymorphism). One among the uniform seedlings was multiplied by bud grafting to divergent rootstocks and were again tested for their genetic and epigenetic uniformity. The study revealed that there is no genetic variation among the multiple seedlings and among the bud grafted counterparts. However, epigenetic variation was observed among the bud grafted plants which were maintained under uniform environmental conditions. Since rootstock is the only source of variation here, it is presumed that the variation was induced by rootstock. The study assumes importance in Hevea because accumulation and maintenance of epigenetic changes during various cycles of vegetative propagation may eventually lead to an altered phenotype and can result in intraclonal variability.*

Key words*: Hevea brasiliensis, RAPD, MSAP, Variability*

Introduction

Hevea brasiliensis (Willd. ex A, Juss.) Muell. Arg. (Para rubber tree), a tropical tree species belonging to the family Euphorbiaceae, is preferred over alternative sources of natural rubber worldwide. Commercial planting materials are raised through bud grafting technique where the desired scion is grafted on to assorted rootstocks grown from cross pollinated seeds. Despite this vegetative mode of propagation, large tree to tree variation in growth and yield among bud grafted *Hevea* trees was reported (Combe 1975; Omokhafe 2004). Strong rootstock effect on scion yield was demonstrated by many workers by comparing different types of rootstocks on the same scion (Combe & Gerner 1977; Negi et al. 1981; Sobhana et al. 2001; Gonçalves & Martins 2002; Cardinal et al. 2007). Though factors like soil heterogeneity and G x E interactions are partly responsible for these variations, it is mainly attributed to the genetically divergent nature of the rootstocks used for propagation (Nayanakantha and

Seneviratne 2007). The key mode by which the rootstock controls growth and properties of scion is yet to be unravelled. The possibility of epigenetic modifications like RNA-directed DNA methylation in the scion which may result in divergent phenotypic characters was suggested by different researchers (Kanehira et al. 2010; Zhang et al. 2012). The signals emanating from the rootstock by the above modes have their impact on target genes resulting in the reprogramming of their expression profiles based on the site of action (Koepke and Dhingra 2013). In the present study we made an attempt to identify whether rootstock influences the epigenome of the scion, by comparing the methylation pattern of uniform, own rooted seedlings and their bud grafted counter parts.

Materials and methods

Induction of multiple, uniform, own rooted seedlings

Uniform own rooted multiple seedlings were developed through the induction of zygotic polyembryony, by *in vitro* culture of immature fruits following half *ovulo* embryo culture technique (Rekha et al. 2015). The well-developed plantlets were transferred to small polythene bags filled with sand, soil, and soil rite mixture and kept in environmentally controlled growth chamber for 2–3 weeks, for hardening. Acclimatized plantlets were field planted. Among the multiple seedlings, eye buds from one of the plantlet was bud grafted to assorted seedlings for comparison.

Molecular analyses

DNA extraction

Genomic DNA was isolated from own rooted seedlings and their bud grafted counter parts following a modified CTAB method of Doyle and Doyle (1990). The DNA concentration and purity were determined spectrophotometrically. Integrity of DNA samples was also checked on 0.7 % agarose gel (Sigma). Working solutions of DNA stock for PCR were adjusted to 10ng/µl and stored at 4 °C.

RAPD analysis

Four arbitrary decamer primers, OPA10 OPA7, OPA1 and OPA15 primers (Operon Technologies, Inc., USA), were used for this study. Amplifications were performed in a DNA Thermal Cycler (Gene Amp 9600 PCR system, Perklin Elmer, USA) in a reaction mix containing 50 ng of DNA template, 20 pmol of primer, 2 mM MgCl2, 200 µM dNTPs, 0.7 units of *Taq* polymerase with 1X PCR buffer (Promega), in a final volume of 25 µl for each reaction. The PCR program consisted of an initial denaturation at 94 °C for 5 min, followed by 40 cycles of 30 s at 94 °C, 1 min at 36 °C and 2 min at 72 °C with a final extension for 10 min at 72 °C. The amplified products were analyzed by electrophoresis in 1.4 % agarose (Sigma-Aldrich, India) gels, stained with ethidium

bromide (0.5 µg/ml of TAE buffer), and photographed using a Gel Documentation system.

MSAP analysis

To detect MSAP, two reactions were set up at the same time. In the first reaction, 1 µg of genomic DNA of the three polyembryony derived plants was digested with 10 U of *Eco*RI plus 10Uof *Msp*I in a final volume of 50 µl containing 50 mMTris–HCl (pH 8.0), 10 mM $MgCl_2$, and 50 mM NaCl by incubating overnight at 37 °C. The second digestion reaction was carried out as above with the exception that *Hpa*II was used in place of *Msp*I. Following ligation with *Eco*RI adaptor [(5′-CTCGTAGACTGCGTACC-3′/3′-CATCTGACGCATGGTTAA-5′)] and *Msp*I-*Hpa*II adaptor [(5′-ACGATGAGTCTAGAA-3′/3′-CTACTCAGATCTTGC-5′)] pre-amplification reactions were performed with *Eco*RI primer (E+A primer: 5′-GACTGCGTACCATTCA-3′) and *Msp*I-*Hpa*II primer (Met+T primer: 5′-ACGATGAGTCTAGAACGGT-3′) with one selective base each. Pre-amplified mixtures were diluted 1:50 from their original volume with sterile Milli Q water. Selective amplifications were conducted with *Eco*RI primer with three selective bases (E+AAG/E+AGT/E+AGC/E+AGA/E+ AGG) and *Msp*I-*Hpa*II primer with three selective bases (Met+TAC/Met+TAG) respectively. Adaptor ligation, pre amplification and selective amplifications were performed as per standard AFLP procedure. The selectively amplified products were mixed with an equal volume of formamide gel loading buffer and denatured and electrophoresed on 6 % (w/v) denaturing polyacrylamide gel containing 7 M urea and 1×TBE. Gels were run at 1,200 V for 4 h and stained by the silver staining method. The same procedure was followed for analysing the bud grafted plants also.

Results

Induction of multiple embryos and plant regeneration

Multiple uniform seedlings of single zygotic origin were successfully developed by *in vitro* culture of immature fruits (Fig. 1a). All the plants were phenotypically similar (Fig. 1b). The regenerated plants were acclimatised and field planted. Growth of the plants was found to be on par with seedlings raised from assorted seeds. Among the uniform seedlings three field established plants of single zygotic origin was utilized for further studies.

RAPD analysis

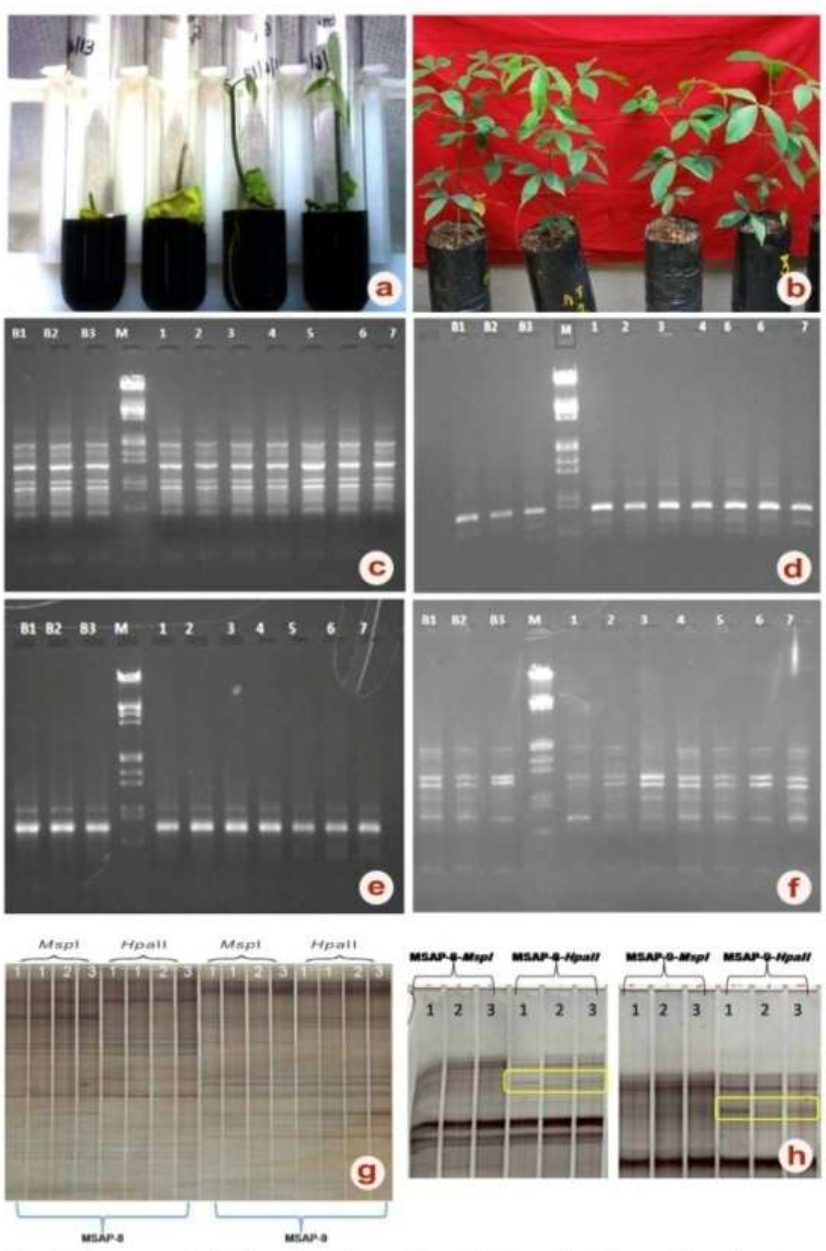
RAPD experiments revealed the own rooted plants as well as the bud grafted counterparts showed similar banding pattern with the tested primers (Fig. 1.c-f). Lane B_1, B2 and B_3 in the figure show the RAPD profile of own rooted seedlings of single

zygotic origin. Lane 1-7 shows the RAPD profile of bud grafted plants. The same banding pattern obtained with the same primers ensures the genetic fidelity.

MSAP analysis

Similarity/dissimilarity in the epigenome of the three own rooted plants and their bud grafts was determined by assessing their global genomic DNA methylation pattern using the MSAP technique. Notable variations were not observed among the three plants in the *Msp*I as well as *Hpa*II digest sets for all the primer combinations analysed (Fig. 1g). In the figure, the amplification of DNA samples with 2 primers after digestion with methylation insensitive (*Msp*I) methylation sensitive (*Hpa*II) are given. This indicates that there is no epigenetic variation among the own rooted seedlings. All the primer combinations analysed showed clear variation in the banding pattern between the *Msp*I and *Hpa*II digests. However the three plants showed same banding pattern with both the digests. When bud grafted plants were subjected to MSAP analysis, variations were observed with two primer combinations in the methylation sensitive *Hpa*II digest set indicating epigenetic variations among these plants (Fig. 1h). In the MSAP digest set, the banding pattern was uniform.

Figure 1: Root stock induced epigenetic variations in *Hevea brasiliensis*. (a & b). development of multiple uniform seedlings, (c-f). RAPD analysis of uniform seedlings and their bud grafted counter parts with different primers, (g). MSAP analysis of uniform seedlings and (h). MSAP analysis of bud grafted seedlings.



Discussion

Genetically similar uniform seedlings generated *via* induction of zygotic polyembryony, and their budgrafted counterparts were used in the present study in order to minimise the error. The uniformity and single zygotic origin of polyembryony derived plants of *H. brasiliensis* has been already established (Rekha et al. 2015). Molecular analysis proved that the plants developed were genetically similar. Conventional molecular markers based on sequence polymorphisms like RAPD as well as methylation sensitive AFLP which could detect sequence structure independent DNA modification like methylation changes, were utilized for the purpose. The budgrafted plants derived from these seedlings also showed same RAPD profile with tested primers, indicating their genetic uniformity (Fig. 1.c-f). When MSAP was performed, notable variations could not be detected among the initially developed three polyemryony derived plants for all the primer combinations tested which implies that there exists no methylation variation among them i.e. they are epigenetically uniform (Fig. 1g). On the contrary, in the bud grafted plants MSAP results showed polymorphic bands among with methylation sensitive *HpaII* digest indicating the presence of epigenetic changes (Fig. 1h). Though *Hevea* genome is reported to be highly methylated (Uthup et al. 2011) the absence of the variation in the MSAP profile in polyembryony derived plants and the presence of the same among the bud grafts appears to be exciting since the only source of variation could be the divergent rootstock. Other factors like growing environment, soil properties and nutrient availability were kept uniform. Therefore the possibilities of these factors influencing the epigenome are minimal when compared to the genetically different rootstock. Epigenetic traits are heritable changes associated with chemical modification of DNA without altering the primary DNA sequence. Depending on the site as well as type of tissue in which it occurs, DNA methylation can mediate the transmission of an active or silent gene either for short-term during mitosis or for long-term across generations during meiosis (Saze 2008). Therefore, phenotypic changes induced by variations in DNA methylation patterns may either be transient or heritable in nature depending on the type of tissue in which they occur. This is important in a species like *Hevea*, where vegetative mode of reproduction is commercially accepted. The change in the DNA methylation pattern is an indication of influence of rootstock on the scion. If it happens in a coding region of DNA, that will be reflected in gene expression also. Epigenetic changes are reversible or irreversible and some irreversible changes are likely to get accumulated over generations and can alter the phenotype of the plant. There is every chance that similar changes that are observed in the present study may be perpetuated among the vegetatively propagated generations of the composite plant. During subsequent cycles of budding, changes may happen again and they may get accumulated finally altering several properties of the original rubber seedling. A series of responses due to the influence of rootstock on scion physiology, gene expression, and protein function parameters in plants are already reported in other plants (Koepke

and Dhingra 2013). Stock induced variation in DNA methylation pattern as observed in the present study can be attributed to stock scion interaction leading to intraclonal variability which is a major reason for destabilising the productivity in rubber plantations. However further extensive studies are needed in this direction for identifying the extent of variation, its stability and frequency. Thus the present study could facilitate a better understanding of the stock-scion interaction process in rubber. This attempt towards unravelling the intricacies of stock-scion interaction in *Hevea* through methylation studies is a first step in this direction.

Acknowledgements

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Comparative morpho-physiological and biochemical responses in *Oryza sativa* 'Aathira' and 'Mattatriveni' subjected to Zn stress

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ABSTRACT: *The effects of Zn stress in seedlings of two rice cultivars (Aathira and Mattatriveni) were analysed and the morpho-physiological and biochemical responses were compared between the two cultivars. Seedlings of both the cultivars showed a differential response towards two different concentrations of ZnSO4 treatments (2 and 6 mM ZnSO4). The growth of seedlings reduced with increasing concentration of ZnSO4 (as analysed by dry weight, fresh weight and shoot length) in both the cultivars and the reduction was higher in Aathira. Zn stress had a negative effect on photosynthetic pigment composition and the reduction in total chlorophyll content and carotenoid content was more in Aathira. Changes in metabolite content in response to Zn stress was studied by anlysing total sugar, total amino acids. Total sugar content increased with increasing concentrations of Zn in Mattatriveni whereas in Aathira it decreased. Total amino acid got enhanced under Zn stress and the maximum increase was observed in Aathira subjected to 2 mM ZnSO4. Chlorophyll a fluorescence parameters studied were area (area above the fluorescence curve), activity of the water-splitting complex on the donor side of PSII (Fo/Fo), the maximum quantum yield of non-photochemical de-excitation (ΦDo) and performance index (PI). Treatment with ZnSO4 reduced area, Fv/Fo and PI whereas it increased ΦDo. These changes in chlorophyll a fluorescence parameters were more in Aathira as compared to Mattatriveni. From the above results it can be concluded that Mattatriveni was more tolerant to Zn than Aathira as evidenced by morphological, physiological, biochemical and chlorophyll a fluorescence parameters.*

Key words: *Rice cultivars, Aathira, Mattatriveni, Biochemical*

Introduction

Rice (*Oryza sativa* L.) is the second most widely grown cereal crop and the staple food for majority of the population (Nguyen and Ferrero 2006). Moreover, is the staple food for majority of the human population. Rice productivity is affected by many biotic and abiotic stress factors which includes heavy metal stress. Sources of heavy metals in paddy fields include application of fertilizers and use of waste materials for fertility amendment in soil (Shuman and Wang 1997).

The essential element Zn is required in low quantities and this micronutrient have an important role in growth and development of plants particularly paddy (Alloway 2009). Although Zn is an essential element for growth and development of plants elevated concentrations are found to be toxic and it will retard crop yield (Alloway et al. 1990). Higher concentration of Zn causes inhibition of plant growth, nutrient imbalance, chlorosis of leaves, and reduction in photosynthetic rate (Cambrolle et al.

2012). In addition Zn can damage cell membranes, change enzyme specificity and interrupt cellular functions (Aery and Sarkar 1991). The objective of this work was to study the effects of Zn stress in two rice cultivars in Aathira and Mattatriveni by analysing morphological, physiological and biochemical parameters.

Materials and methods

Rice seeds of the cultivars Aathira and Mattatriveni were obtained from Regional Agricultural Research Sstation, Pattambi. Seeds were surface sterilized with 0.1% $HgC1_2$ solution for 20 minutes. The seeds were grown in plastic bottles (22×12cm) containing absorbent cotton soaked with distilled water (control). All the analysis was done on 10 day old seedlings exposed to two different concentrations of $ZnSO_4$ (2 and 6 mM $ZnSO_4$). Seedlings were grown in a plant growth chamber set at 14/10 h light-dark cycles at 300 $\mu molm^{-2}s^{-1}$, 24+2^oC and RH 55+5%.

Total chlorophyll and carotenoid content were estimated by the method of Arnon (1949). Estimation of total sugar was done by the method of Dubois et al. (1956). Total amino acid estimation was done by the method of Moore and Stein (1948). Chlorophyll a fluorescence transient were measured with the Plant Efficiency Analyzer (Handy PEA, Hansatech Ltd., Norfolk, UK). All measurements were performed on the upper surfaces of the youngest fully expanded leaves following a dark adaptation period of 20 minutes using the leaf clips. Maximal fluorescence was induced by a 1 s pulse of white light (3000 $\mu molm^{-2}s^{-1}$) with the gain adjusted automatically to 0.7 to avoid over scaling errors. Thereafter, Chlorophyll *a* fluorescence signals were analyzed with the Biolyzer HP3 software. The average values from 30 measurements recorded on second leaves of the plant for each treatment are shown.

Results

The effects of Zn ($ZnSO_4$) stress in seedlings of two rice cultivars (Aathira and Mattatriveni) were analysed with respect to changes in growth parameters, photosynthetic pigments, metabolites and chlorophyll *a* fluorescence parameters. In both cultivars, growth parameters such as fresh weight, dry weight and shoot length per plant were found to be reduced under Zn stress. Maximum reduction in fresh weight and dry weight was observed in 6 mM $ZnSO_4$ in both the cultivars. The reduction in fresh and dry weight of Aathira was 60 and 46% and in Mattatriveni the reduction was 46 and 31% respectively at 6 mM $ZnSO_4$. Treatment with $ZnSO_4$ reduced the shoot length of the seedlings almost to half. Aathira showed maximum reduction in shoot length (64% at 6 mM $ZnSO_4$) (Table 1).

Table 1: Effect of $ZnSO_4$ (0, 2 and 6 mM) on fresh weight, dry weight and shoot length per plant in Aathira and Mattatriveni. The data is an average of recordings from three independent experiments each with three replicates (ie. n=9). The data represent mean ±standard error.

Cultivars	Treatments	Fresh weight (mg per plant)	Dry weight (mg per plant)	Shoot length (cm per plant)
Aathira	Control (0 mM)	54.81+1.24	9.5+0.22	12.45+0.25
	2 mM $ZnSO_4$	23.36+0.88	6.84+0.2	5.77+0.25
	6 mM $ZnSO_4$	22.16+1.14	5.13+0.28	4.33+0.2
Mattatriveni	Control (0 mM)	63.03+0.5	10.45+0.076	14.41+0.53
	2 mM $ZnSO_4$	40.38+1.25	8.9+0.19	7.75+0.38
	6 mM $ZnSO_4$	33.78+1.19	7.19+0.22	6.51+0.4

Treatment with $ZnSO_4$ negatively affected photosynthetic pigments. Maximum reduction in both total chlorophyll and carotenoid content (76 and 60%), respectively was noticed in Aathira subjected to 6 mM $ZnSO_4$ (Fig. 1 A & B). Under Zn stress total sugar content decreases irrespective of the Zn concentration in Aathira whereas it increases in Mattatriveni with increase in concentration of Zn (Fig. 1C). Zn treatment brought an enhancement in the content of total amino acids in both the cultivars and Aathira showed maximum increase in amino acids (30%) in 2 mM $ZnSO_4$ as compared to control (Fig. D).

When rice seedlings were grown in two different concentrations of $ZnSO_4$, the relative values (relative to the control) of chlorophyll a fluorescence parameters were altered. Area above the fluorescence curve reduced under Zn stress and the reduction was more in Aathira as compared to Mattatriveni. Fv/Fo, which indicates the activity of the water-splitting complex on the donor side of PSII also reduced under Zn stress in both the cultivars. The reduction recorded in Aathira was almost to the same extent in both concentrations of $ZnSO_4$. But in Mattatriveni subjected to 2 and 6 mM $ZnSO_4$ 39 and 30% reduction in Fv/Fo was observed. The maximum quantum yield of non-photochemical de-excitation (Φ_{Do}) increased under Zn stress. The increase in Φ_{Do} was more in Mattatriveni at 2mM $ZnSO_4$ (35%). Performance index (PI) represents the sample vitality (Strasser et al. 2000) and it reduced to a range of 62 - 68% in Aathira under Zn stress (Table 2).

Figure 1: Effect of Zn stress on (A). total chlorophyll content, (B). carotenoid content, (C). total sugar and (D) total amino acids in Aathira (AR) and Mattatriveni (MT) exposed to $ZnSO_4$ (0, 2 and 6 mM).

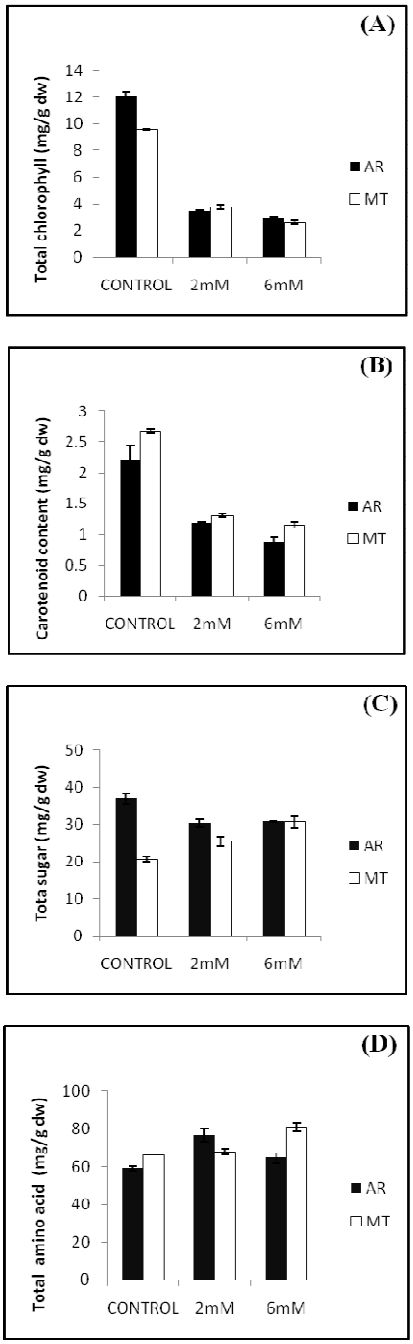

Table 2: Effect of $ZnSO_4$ (0, 2 and 6 mM) on chlorophyll *a* fluorescence related parameters in Aathira and Mattatriveni. The data is an average of recordings from three independent experiments each with three replicates (ie. n=9). The data represent mean ±standard error.

Cultivars	Treatments	Area	Fv/Fo	Φ_{Do}	PI(abs)
Aathira	Control (0 mM)	16247+348	2.6224 +0.06	0.2761+0.0088	4.301+0.086
	2 mM $ZnSO_4$	9684+242	1.8852+.039	0.3466+0.01	1.3957+0.032
	6 mM $ZnSO_4$	9170+229	1.8459+0.35	0.3514+0.008	1.6309+0.046
Mattatriveni	Control (0 mM)	12036+261	2.0662+0.049	0.3261+0.0078	2.3307+0.066
	2 mM $ZnSO_4$	8419+186	1.2552+0.033	0.4434+0.009	1.2513+0.038
	6 mM $ZnSO_4$	8860+213	1.4562+0.03	0.4071+0.011	2.132+0.059

Discussion

In our study it was found that fresh weight, dry weight and shoot length of both the rice cultivars were retarded under Zn stress. This was due to the negative effect of Zn on the growth of the plant and as a result of reduced enzyme activities as well as low level of growth hormones (Mazher et al. 2007). Similar observations (52% reduction in plant biomass) were obtained in *Phyllostachys pubescens* treated with higher concentration of $ZnSO_4$ (800 mg per kg soil) (Peng et al. 2015). Like growth parameters photosynthetic pigments (total chlorophyll and carotenoid content) were also negatively affected by Zn stress. This reduction can be correlated with reduced synthesis of photosynthetic pigments and enzymes involved in their biosynthesis (Gonzalez et al. 2012) and/or by the breakdown of chlorophyll by the substitution of Mg^{2+} in chlorophyll molecules by Zn^{2+} (Kupper et al. 1998). Similar reductions in total chlorophyll content was observed in barley cultivars (Gonzalez et al. 2012) and reduction of total carotenoid content was observed in *Morus alba* (Tewari et al. 2008) under Zn stress.

Under Zn stress, total sugar content decreases in Aathira whereas it increases in Mattatriveni. Accumulation of sugars in plants subjected to Zn stress can be correlated with maintenance the osmotic potential and as a part of keeping carbohydrate metabolism at optimum level even under stress (Gibson 2005). The decrease in total sugar may be due to the retardation in carbohydrate metabolism. Zn stress brought an enhancement in total amino acid content in Aathira and Mattatriveni. The increase in amino acids under Zn stress may either due to the enhanced synthesis of amino acids which have a role in stress tolerance or due to the breakdown of proteins (Widodo et al. 2009). Similar results were obtained in *Cyamopsis tetragonoloba* were total sugar

increased at lower concentrations (50 and 100 mg per kg soil) and decreased at higher concentrations of $ZnSO_4$ (150, 200 and 250 mg kg^{-1}) (Vijayarengan 2012).

The area above the fluorescence curve is proportional to the pool size of the electron acceptors Q_A on the reducing side of PS II. It was found to be reduced in both Aathira and Mattatriveni under $ZnSO_4$ treatment and Aathira showed maximum reduction to a range of 40-44%. Reduced area under Zn stress indicates the reduction in the number of electron acceptors Q_A. Similar results were obtained in barley cultivars under high light stress (Kalaji et al. 2012). Fv/Fo is a good indicator of photosynthetic quantum conversion rate and it was found to be sensitive to $ZnSO_4$. Reduction in Fv/Fo activity was due to the reduction in the activity of water-splitting complex on the donor side of PSII under Zn stress, which decreased (10%) under metal treatment in barley cultivars (Gonzalez et al. 2012).

The maximum quantum yield of non-photochemical de-excitation (Φ_{Do}) increased in Zn treated plants. Increase in Φ_{Do} under Zn stress may be due to the down-regulation of PS II activity during stress and thereby avoiding over-reduction of Q_A (Xu et al. 2010). It indicates the presence of a mechanism for dissipation of excess excitation energy to protect the photosynthetic apparatus in seedlings from possible photodynamic damages that is induced by Zn. When *Solanum melongena* L. seedlings were treated with Cd there was an enhancement in Φ_{Do} (Singh and Prasad 2014). Performance index depends on the concentration of reaction centre per chlorophyll, primary photochemistry and electron transport and it was found to be reduced under Zn stress in both the cultivars. PI is sensitive to changes in either antenna properties, trapping efficiency or electron transport beyond Q_A. The reduction in PI was more in Aathira. The decrease of PI in Zn treated plants was due to the decrease in the photochemical efficiency. Similar results were observed in *Hordeum vulgare* L. under drought stress (Oukarroum 2007).

Effects of $ZnSO_4$ in two rice cultivars Aathira and Mattatriveni revealed that fresh weight, dry weight, shoot length and photosynthetic pigments were reduced in metal treated seedlings than control. Among the two cultivars treated with $ZnSO_4$ minimum changes with regard to control in the above parameters were found in Mattatriveni which indicates that this cultivar is more tolerant to Zn. Two cultivars showed differential response to Zn stress in case of metabolites. Total amino acids increased in both the cultivars but total sugar decreased in Aathira and increased in Mattatriveni which indicate that Aathira is highly sensitive to Zn drastically affecting the sugar metabolism. While a lower concentration of $ZnSO_4$ (2mM) drastically affected most of the parameters studied, still a higher concentration of $ZnSO_4$ (6mM) was found to impart stress in Mattatriveni, which indicate the higher tolerance potential of the latter towards Zn as compared to the former cultivar.

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Nacl induced changes in osmotic status and primary metabolic activities of *Acanthus ilicifolius* L. grown under Hydroponic conditions

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***ABSTRACT**: The effects of short-term (15d) exposure of NaCl (0-600 mM) on the physiological responses of a mangrove plant, Acanthus ilicifolius L., cultured in half strength Hoagland nutrient medium were analyzed in this study. Stem cuttings of A. ilicifolius were grown in Hoagland nutrient solution for a period of 35 days and were then treated with different concentrations of NaCl (0-600 mM). Parameters like relative water content (RWC), osmotic potential (OP), photosystem I (PSI), photosystem II (PSII) and mitochondrial activities were studied. The leaf RWC of control plants were 94% and that of 200, 400 and 600 mM NaCl treated plants were 86, 78 and 43%, respectively. Similarly, the leaf OP in the control as well as the NaCl stressed (600 mM NaCl) plants ranged from -2.59 to -8.41 MPa. Both photochemical and mitochondrial activities showed significant increase upto 400 mM NaCl and upon further increase of NaCl, the activities rapidly declined. In 200 and 400 mM NaCl treated plants, PSI activity showed an increase of 30, 39% and PSII showed an increase of 32, 66% respectively over the control plants. But in the case of 600 mM NaCl, a rapid decrease in the activity of PSI and PSII was recorded, i.e., 74 and 64%, respectively. Consequently, the maximal mitochondrial activity was recorded in 400 mM NaCl treated plants, i.e., 6.53. The above results indicates that A. ilicifolius possess potential adaptive physiological responses against NaCl stress which helps in lowering the toxic effects of NaCl upto a concentration of 400 mM.*

***Key words:** 3, 4 dichlorophenyl-1, 1-dimethyl urea, 2, 6 Dichlorophenolindophenol, Indole-3-butyric acid, Methyl viologen.*

Introduction

Halophytes are physiologically interesting group of plants as they form potential models for studying the mechanism of stress tolerance, especially salinity stress. Salinity can inhibit plant growth due to various factors, including ion toxicity, impairment of mineral nutrition and changes in the water relations. The extent to which each of these factors can affect growth depends on plant genotype and on environmental conditions (Munns 2002). The increase in salt content due to intrusion of sea water into aquifers leads to an increase of salinity of the agricultural soils, which reduces plant growth and crop productivity in many arid and semi-arid regions of the word (McKersie and Leshem 1994). Utilization of salt-tolerant plants is expected to be an effective method to improve saline soil. Hence, better understanding of the mechanisms that enable plants to adapt to salt stress is necessary for exploiting saline soil. Even though the use of halophytes in bioremediation of saline soils has long been

recognized, only a few reports have been published concerning the plant species suggested for exploitation in bioreclamation of salt affected lands (Qadir and Oster 2002).

It is well documented that salinity stress can bring about negative impacts in plant–water relations and photosynthesis, including diminished productivity as stress intensifies (Salpeter et al. 2012). High NaCl concentration seems to impair electron transport in chloroplast and mitochondrial membranes, and lead to formation of reactive oxygen species (ROS) (Foyer and Noctor 2002). For most halophytes, the lethal salt concentration is well above 300 mM (generally between 700-1000 mM), but growth inhibition will occur before this lethal level is reached. Physiological adaptations allow higher accumulation of salt in most halophytes, with tissue concentration often exceeding 500 mM (Atwell et al. 1999). *Acanthus ilicifolius* L. is a salt excreting mangrove that can tolerate saline conditions by evoking a number of salt tolerance mechanisms. In this context, it is interesting to study the effect of salinity, to know how the osmoticum is maintained and metabolics function operates under saline situation. Therefore, in the present study, salt tolerance of *A. ilicifolius* was examined in terms of analysis of the osmotic adjustment and primary metabolic functions of the plant.

Materials and methods

Plant materials and growth conditions

Stem cuttings of *A. ilicifolius* (15-20 cm) were collected from mangrove forests of Kadalundi Vallikkunnu Community Reserve (KVCR), Kerala, India. Cuttings were then transferred to half strength Hoagland medium (Hoagland and Arnon 1940) after treating with 15µM IBA (2 hrs.) for hydroponic culture. Plants were maintained in green house under controlled condition of temperature (28±2^{0}C), light intensity (100 µmolm^{-2}s^{-1}) and humidity (60±5%). Plants in Hoagland's medium were designated as control and those in the medium containing 0-600 mM NaCl were the treated plants.

Relative water content

Relative water content of the fully expanded leaves was calculated according to Barrs (1968).

Leaf osmotic potential

Osmotic potential of the cell sap from leaves of treated and control plants were determined using a vapor pressure osmometer (Wescor 5520, USA). Calibration of the osmometer chamber was done using 290 and 1000 mMkg^{-1} standard solutions supplied by the manufacturer.

PSI and PSII activities

Thylakoids from leaves were isolated according to Puthur (2000) and the photochemical activities of the isolated thylakoids were assayed polarographically with a Clark-type oxygen electrode (DW1/AD, Hansatech, Norflok, UK) which was connected to a digital control box (OXYG1, Hansatech) at 4°C. The light dependent O_2 uptake/evolution was measured by irradiating the sample with saturating intensity of white light (-1800 µmol photons $m^{-2}s^{-1}$), provided by a 100W halogen lamp (LS2, Hansatech). Chlorophyll content of the thylakoid samples was estimated according to the method of Arnon (1949).

PSI activity was measured in terms of oxygen consumption by using artificial electron donor DCPIP and methyl viologen (MV) as exogenous electron acceptor. The reaction mixture contained reaction buffer, ascorbate (600 µM), MV (500 µM), sodium azide (1 mM), DCMU (5 µM), $DCPIPH_2$ (0.1 mM). Chloroplast suspension equivalent to 10 µg chlorophyll was added and the volume was made up to 2 mL with the buffer. PS II activity was measured in terms of oxygen evolution by using pBQ as artificial electron acceptor. The reaction mixture consisted of reaction buffer, pBQ (500 µM), chloroplast suspension equivalent to 20 µg chlorophyll, which was finally made up to 2 mL with the buffer.

Mitochondrial electron transport activities

Mitochondrial isolation was carried out according to Kolloffel (1967). Oxygen consumption by mitochondria was measured at 25°C using a Clark-type O_2 electrode (DW1/AD, Hansatech, Norflok, UK) as per the protocol of Schmitt and Dizengremel (1989). Reaction medium contained 935 µl of assaying buffer (0.3 M sucrose, 10 mM potassium phosphate, 10 mM Tris, 5 mM $MgCl_2$ and 10 mM KCl; pH 7.2), 40 µl mitochondrial preparations (equivalent to 0.3 mg protein) and 50 µl of 200 mM sodium succinate. The oxygen uptake rate measurements were started, once the substrate was added. The oxidation rate of succinate was calculated in terms of µmol O_2 consumed $min^{-1}mg^{-1}$protein. The protein content in the mitochondrial preparations was determined by the method of Bradford (1976).

Results

For the preliminary screening of NaCl tolerance, plants were subjected to different concentrations of NaCl, (viz. 100, 200, 300, 400, 500 and 600 mM) added to half strength Hoagland medium. The photosynthetic pigment content and the Chl *a* fluorescence parameters of all the treatments along with the control plants were analysed (data not shown). From the results of these parameters it was clear that NaCl 200 mM imparted mild stress, NaCl 400 mM imparted moderate stress and NaCl 600 mM imparted

severe stress to *A. ilicifolius*. Therefore these concentrations were selected for further studies.

Relative water content

The leaf RWC of control plants was ~ 94% and it did not vary much during the treatment period of 15d. Under mild stress of NaCl (200 mM), RWC showed a slight reduction of 6%. But in the case of plants treated with 400 mM, RWC recorded was 78%. However, exposing the plants to extreme NaCl conditions (600 mM), leaf RWC tend to decrease upto 43% and the leaves were mostly wilted (Table 1).

Leaf osmotic potential

The leaf osmotic potential in the control as well as the NaCl stressed plants (0-600 mM) ranged from -2.59 to -8.41 MPa (600 mM NaCl) respectively during the period of treatment. In the 200 and 400 mM NaCl treated plants; the OP recorded was -3.01 and -4.64 MPa respectively on 15d (Table 1).

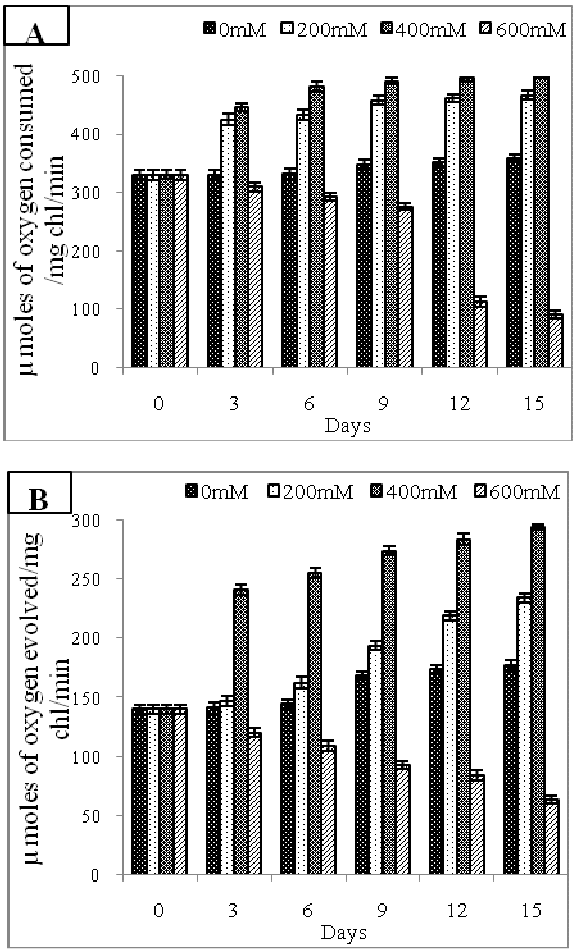
Table 1: Effect of NaCl in the RWC% and OP (MPa) of *A. ilicifolius L.* cultured in Hoagland solution (15d) containing 0-600 mM NaCl. Values are the mean ± SE of three independent experiments.

NaCl (mM)	RWC%	OP (MPa)
0	94.05 ± 3.76	-2.59 ± 0.12
200	87.84 ± 4.13	-3.01 ± 0.15
400	77.54 ± 3.13	-4.64 ± 0.23
600	42.67 ± 2.05	-8.41 ± 0.39

PSI and PSII activities

NaCl treatment promoted the PSI and PSII activities in *A. ilicifolius* upto 400 mM and at higher concentration (600 mM) there was a sharp decline in activity. In 200 and 400 mM NaCl treated plants, PSI activity showed an increase of 30, 39% and PSII showed an increase of 32, 66% respectively over the control plants. But in the case of severe stress of 600 mM NaCl, a rapid decrease in the activity of PSI and PSII was recorded, i.e., 74 and 64% respectively as compared to control (Fig. 1A & B).

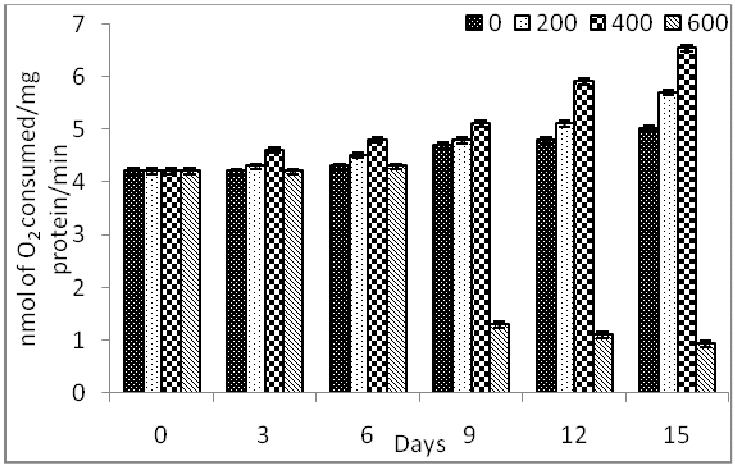
Figure 1 A and B: Effect of NaCl on PSI (a) and PSII (b) activities (nmol of O_2 consumed/evolved $mg^{1}chl^{-1}min^{-1}$) of *A. ilicifolius* L. Values are the mean ± SE of three independent experiments.



Mitochondrial electron transport activities

Mitochondrial activity of *A. ilicifolius* as assessed by the oxidation of exogenous sodium succinate was found to increase up to NaCl 400 mM beyond which it decreased rapidly, i.e., on 15d of treatment, the 600 mM NaCl treated plants exhibited a low value (0.92 nmol), when compared to the control (5.01 nmol). The maximal activity was recorded in the 400 mM NaCl treated plants, i.e., 6.53 nmol (Fig. 2).

Figure 2: Effect of NaCl on the mitochondrial activity (nmol of O_2 consumed mg^{-1} $protein^{-1}min^{-1}$) in the leaves of *A. ilicifolius* L. Values are the mean ±SE of three independent experiments.



Discussion

Halophytes constitute a diverse group of plants with varying degrees of NaCl tolerance, and they sequester Na^+ in cell vacuoles as the major means of adjusting the osmoticum. Water potential, solute potential and turgor potential are inter-related in plant cells and are markedly affected when plants are exposed to NaCl stress. RWC determines the leaf survival and indicates active metabolic processes that occur during saline stress (Johns et al. 2014). The RWC% in leaf of plants treated with 400 mM NaCl shows that, the plants will be in a position to carry on the metabolic process actively. But the very low RWC% in leaves of plants treated with 600 mM NaCl can hamper the metabolic activities of the cell and will result finally in dehydration at cellular level. As a general principle, when plants experience high osmotic stress because of low external water potential, a lowering of the solute potential is initiated, a process referred to as osmotic adjustment. The rate of water uptake is generally lower in mangroves due to high external salinity. To cope with the situation, mangroves have to maintain a high negative osmotic potential in their leaf cells. Decreasing external water potential produces a net accumulation of solutes in cells, which lowers the cell osmotic potential necessary for maintaining the turgor pressure (Navarro et al. 2003). Thus, the decreased OP recorded in NaCl treated *A. ilicifolius* leaves is a stress tolerance mechanism for maintaining the water balance of the cells developed against the high NaCl content present in the external medium. However, excessive NaCl accumulation (600 mM) is deleterious for living cell metabolism, as it dehydrates the cytosol and denatures several essential metabolic enzymes.

Subjecting *A. ilicifolius* to 200 and 400 mM NaCl resulted in the activation of a pool of PSI centres involved in cyclic electron flow there by increasing the PSI activity. Likewise the increase in PSII activity under 200 and 400 mM NaCl indicates that no damage occurred in PSII acceptor/donor sides and the resultant electron transport in plants subjected to these concentrations of NaCl. The enhanced photochemistry of NaCl treated (200 and 400 mM) *A. ilicifilius* was similar to that recorded in certain other halophytes, *Avicennia officinalis* (Saravanavel et al. 2011), *Kandelia candel* (Tattini 1997), etc. The decreased photosynthetic activity of 600 mM NaCl treated plants could be mainly due to partial stomatal closure, decreasing carboxylation efficiency and CO_2 saturated photosynthesis and inhibition of the light reaction mechanism (Mudrik et al. 2003). Moreover, the high accumulation of Na^+ in the cytoplasm or chloroplast when the vacuole can no longer sequester toxic ions can affect the integrity and function of photosynthetic membranes (Bastías et al. 2004).

Similarly, the results of mitochondrial activity studies indicates that functions of mitochondria in plants treated with 400 mM NaCl were better maintained than that in plants treated with 600 mM NaCl. The immediate targets of NaCl stress are the chloroplasts and mitochondria. Superoxide radicals and other ROS are formed in the chloroplasts during photosynthetic light reactions at the acceptor side of PS I, reducing site of PS II (Asada 2000), and at the oxygen evolving complex. Generation of superoxide radical due to reduction of oxygen could also take place at different points of the respiratory chain such as the flavoprotein region of NADH dehydrogenase and the ubiquinone–cytochrome region in the mitochondria (Arora et al. 2002). NaCl stress has been shown to cause damage to mitochondrial electron transport in *Zea mays* (Hamilton and Heckathorn 2001). However, the increase in activity upto 400 mM NaCl is due to the efficiency of mitochondria in generating ATP through the oxidation of exogenous sodium succinate. However, the oxidation rates were significantly lower in the case of 600 mM NaCl treated plants.

Thus it can be concluded that upto NaCl 400 mM, *A. ilicifolius* is having an efficient osmotic adjustment mechanism which help the plant to function normally and beyond which extreme lowering of OP causes damage to cell constituents as reflected in impaired cell functioning. However, *A. ilicifolius* showed high tolerance to NaCl up to 400 mM, which indicates that either Na^+ or Cl^- had no toxic effects on the photochemistry and mitochondrial activity of the plant even at this concentration of NaCl. But beyond 400 mM, NaCl was found to be toxic to the plant as it rapidly declines the photochemical and mitochondrial activities.

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Identification of new sources of resistance to Fusarium root rot, for breeding studies in *Vanilla*

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ABSTRACT: *Root and stem rot (RSR) disease of vanilla caused by Fusarium oxysporum f. sp. radicis-vanillae (Forv), is the most destructive disease in major vanilla growing countries. Chemical and biological agents are least effective in controlling the disease. Hence, the use of resistant varieties is the method of choice for controlling Fusarium diseases. The present study describes the aetiology of RSR and phenotypic evaluation of vanilla germplasm available at Centre Biological Resources (CRB) which maintains different species, interspecific hybrids and progenies of vanilla in Reunion Island. Three hundred and seventy seven single- spored Fusarium isolates were recovered from vanilla plantations in the South West Indian ocean (SWIO) region. The isolates were characterized by molecular markers (192) and pathogenicity (117). The taxonomic identity of the Fusarium isolates was resolved by sequencing the EF1a and IGS gene regions. The pathogenicity of the isolates was assessed using plantlet root inoculation method (PRIM) and AUDPC analysis on susceptible cultivated vanilla accessions. These experiments demonstrate that F. oxysporum was the principle species responsible for RSR. The phylogenetic analysis shows that the majority of the isolates (79%) recovered were F. oxysporum. It also shows that the isolates were highly polyphyletic regardless of geographic origin or pathogenicity. In order to assess the resistance of vanilla to Forv, the PRIM assay was again conducted using a well characterized highly pathogenic isolate, Fo072. The evaluations for resistance in controlled conditions were performed on 103 accessions (79 Vanilla planifolia, 15 accessions from 11 Vanilla species and 9 interspecific hybrids) of in-vitro grown vanilla plants. The vanilla accessions exhibited a wide range of survival rates against the pathogenic isolate with the AUDPC values ranging from 0 to 30. The hierarchical clustering analysis split the accessions into different classes, highly susceptible (hs), susceptible (s), moderately susceptible (ms) slightly resistant (sr) and resistant (r) based on the AUDPC values. The resistant class is occupied with 19 accessions, comprising of eight V. planifolia accessions, four V. bahiana, four other species such as V. pompona, V. crenulata, V. costaricensis and V. phalaenopsis and three interspecific hybrids including V. pompona and V. phaeantha as a parent. Further the field evaluation of vanilla plants grown in naturally infected soil showed a strong correlation between field and in-vitro assay. This molecular and pathogenicity data confirms that F. oxysporum is the sole responsible for the RSR. The high level of diversity within the isolates resulted in no clusters that correspond to a geographic origin, or pathogenicity levels. The new sources of resistance identified could be incorporated in the studies of breeding and genes responsible for resistance to Forv in vanilla.*

Key words: *Fusarium oxysporum, Phylogenetic analysis, In-vitro assay, AUDPC, Pathogenicity tests, Vanilla planifolia.*

Introduction

Root and stem rot disease (RSR) caused by *Fusarium oxysporum* f. sp. *radicis vanillae (Forv)*, is one of the most deadliest fungal disease in major vanilla growing countries around the globe (Koyyappurath et al. 2015; Tucker 1927). The disease has been reported in every country where vanilla is grown (Tombey and Liew 2010). The disease is a major limiting factor in the intensification of production in leading vanilla producing countries such as Madagascar, Reunion Island and Mexico (Benezet et al. 2000; Delassus 1963; Hernandez 2011).

The RSR of vanilla is very poorly controlled by chemical, biological and crop management methods currently available (Pinaria et al. 2010). On the other hand, the low genetic diversity of the cultivated vanilla species (*V. planifolia* and *V. tahitensis*) increases the vulnerability of the culture with respect to the pathogen (Bory 2008). So identifying and using resistant genotypes is the best control measure. No resistance to *Forv* has been reported in cultivated vanilla. However, a few sources of resistance to RSR are reported in some wild species such as *V. pompona*, *V. phaeantha*, *V. barbellata*, *V. aphylla* and *V. andamanica* (Divakaran et al. 2006; Knudson 1950; Theis and Jimenez 1957). Therefore identifying sources of resistance and using them is a good strategy for the management of RSR in vanilla.

The aim of the present study was to i) study the aetiology of RSR in Reunion Island and Madagascar, which are included in the major vanilla producing countries. The experimental approaches include the collection and molecular characterization of fungal isolates collected from different vanilla growing plots of the countries, ii) finding the sources of resistance to RSR in the available germplasm, which can contribute for the resistance breeding studies in vanilla. The experimental approach consists of a robust *in-vitro* screening and rating methodology.

Materials and methods

Survey and sampling

Surveys and samplings were conducted in vanilla growing areas of Madagascar (2009) and Reunion Island (2011). Root and stem samples were collected from vanilla plants showing RSR symptoms. The samples collected were stored in polythene bags and stored in cool conditions until further processing at the laboratory. The sampling covered vanilla plants growing in different conditions such as shade house, field and under-forest cultivation systems. A non-pathogenic *Fusarium oxysporum* isolate (Alabouvette 1986) was used as a reference isolate in the diversity and pathogenicity analysis studies.

Isolation and morphological identification

The explants collected were washed in distilled water. They were then rinsed in 1% sodium hypochlorite solution and washed thoroughly in distilled water and dried properly. The explants were then trimmed to 3-5 mm using a surgical blade. The explants from root (whole or cylinder only) and stems (whole or cylinder only) were plated on potato dextrose agar (PDA), supplemented with Streptomycin (100 Mg/L). The PDA plates were incubated in dark for 7 days at 25°C.

Genomic DNA extraction

Total DNA was isolated from about 100 mg of the mycelium scrapped from the plates containing PDA using 2 ml of lysis buffer (50 mM tris-HCl (Ph 7.5), 50 mM EDTA and 3% SDS). DNA was extracted using the method described by Edel (2001). Quantity and quality of the DNA was estimated using a Nanodrop spectrophotometer.

PCR and sequencing

The isolates were identified at the species level using sequencing the *EF1a* and IGS gene regions. Polymerase chain reaction (PCR) amplification was done using the primer pairs *EF1/EF2* (O'Donnell et al. 1998) and CNL12 (Anderson and Stasovsky 1992)/ CNS1 (White et al. 1990). PCR reactions were performed using a 96-well thermocycler machine (Applied Biosystems, USA). The 25 µl reaction mixture consists of PCR reaction buffer, 50 nmol of $MgCl_2$, 1 U of *Taq* polymerase (GoTaq, Promega, USA), 5 nmol dNTPs, 10 nmol of each forward and reverse primer and 40 ng of genomic DNA. For the *EF1a* locus, PCR conditions consisted of an initial denaturation at 96°C for 5 min, 35 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 1 min, and extension at 72°C for 1 min, and a final extension at 72°C for 7 min. For IGS, PCR conditions were 94°C for 3 min, followed by 35 cycles at 94°C for 30s, 60°C for 30s, 72°C for 2 min, and a final extension of 72°C for 10 min. The amplified products were visualised on a 1.5% agarose gel. The products were sequenced in both directions by Beckman Coulter Genomics (United Kingdom).

Vanilla genotypes and disease assessment

The *in-vitro* plants for identifying the sources of resistance to *Forv* were provided by the VATEL Biological resources centre (CRB-VATEL) which maintains different species, hybrids and progenies of vanilla in Reunion Island. The *in-vitro* plants were grown in basal MS media without any growth hormones.

The *in-vitro* plants were infected with a highly pathogenic isolate Fo072 using the root-dip inoculation method (Koyyappurath et al. 2015). In this method, the roots of *in-vitro* plants were dipped in conidial suspension (10^6 conidia/mL) for 5 minutes. The *in-vitro* plants were then transferred to plastic pots containing sterile coco fibre and baggase as substrate. The plants were incubated in a growth chamber with controlled conditions. The control plants used in the study were dipped in sterile distilled water. Disease

symptoms were recorded in every alternate days and accession susceptibility was calculated using AUDPC values (Koyyappurath et al. 2015).

Data analysis

The nucleotide sequences obtained after Sanger sequencing were edited manually using Bioedit 7.1.3.0 (Hall 1999). Similarity searches were performed in the GenBank database using the BLAST search algorithm. Phylogenetic trees were constructed using the MEGA 6 (Tamura et al. 2013) and Clustal W for alignment and Maximum Likelihood (ML) reconstruction methods.

Results

Survey and symptomatology

The explants were collected from 52 vanilla plots surveyed in Reunion Island and 19 in Madagascar. The survey covered majority of the vanilla growing areas. The sampled plots were diverse in terms of age of plants/plantation, plant nutrition, looping and pollination intensity.

RSR symptoms were observed in all the plots (Fig. 1). The early symptoms include the browning and death of the underground roots. It was observed that the aerial roots also get infected when it reaches the infected soil. This results in the proliferation of new aerial roots to re-establish soil contact, which actually weakens the plant.

Figure 1: Figure showing the typical root rot symptoms of a cultivated *Vanilla planifolia*

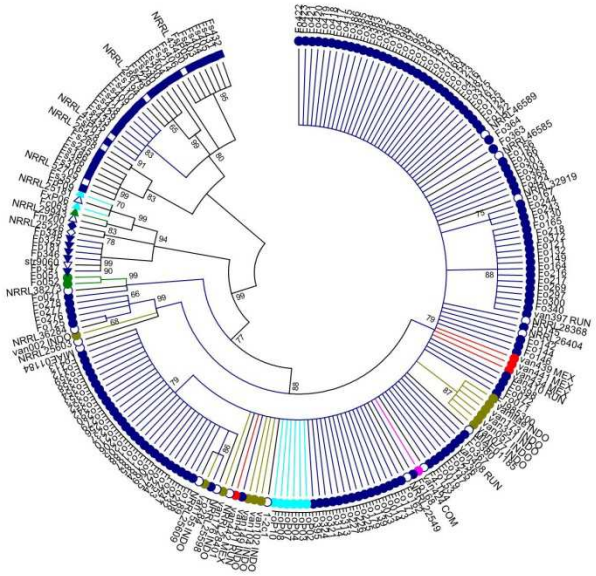
Fungal isolation and DNA extraction

A total of 316 single-spored isolates were recovered from Reunion Island and 49 from Madagascar. Majority of the isolates were recovered from *V. planifolia.* The recovered isolates were selected based on the different explants part and area surveyed for the DNA extraction. High quality DNA was extracted from a total of 192 isolates.

Molecular characterization

EF1/EF2 primers successfully amplified in selected 155 isolates (149 sequences from Reunion Island and 6 from Madagascar). While the CNL12/CNS1 primers amplified a total of 153 sequences (148 sequences from Reunion Island and 5 from Madagascar). BLAST searches of these sequences to the nucleotide GenBank revealed that 79% of the isolates showed sequence similarity to *F. oxysporum.* The remaining isolates were identified to be *F. solani* (16%), *F. proliferatum* (3%), F. *mangiferae* (1%) and *F. concentricum* (1%). For the phylogenetic studies additional sequences *EF1a* of *F. oxysporum* isolated from vanilla were incorporated, which includes 19 sequences from Indonesia (Pinaria et al. 2009), 10 from French Polynesia. An additional of 21 sequences from GenBank was also incorporated in the study. The Maximum Likelihood (ML) tree constructed (Fig. 2) identified 22 haplogroups (named from A to V) with a bootstrap threshold of 65% for the *EF* sequences. Unfortunately, there was no correlation between the haplogroups and geographic origin of the tested isolates.

Figure 2: Phylogenetic tree based on partial *EF1a* sequences of *Fusarium* isolates



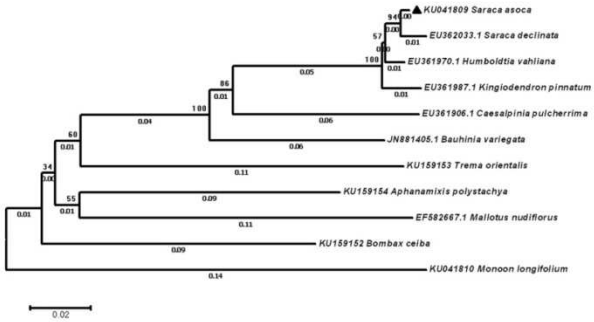
PRIM assay

The *in-vitro* root inoculation method used 103 (79 *V. planifolia,* 15 accessions of 11 other species and 9 interspecific hybrids) *in-vitro* vanilla accessions. The inoculation with Fo072 resulted in symptoms on susceptible plants from 4 days post inoculation (dpi). The well known susceptible accessions *V. planifolia* and *V. tahetensis* displayed early symptoms of RSR as soon as 4 dpi and reached a total collapse at 15 dpi (Fig. 3). The AUDPC values comprise of the tested accessions ranges from 0 to 30. The rpart analysis split the accessions into 5 distinct classes based on the AUDPC values, namely i) highly susceptible, ii) susceptible, iii) moderately susceptible, iv) slightly resistant and v) resistant accessions.

Among the accessions tested 19 occupied the resistant class with AUDPC ranging from 0 to 7. These 19 resistant accessions comprise of eight *V. planifolia* accessions, four *V. bahiana* accessions, four other species (*V. costaricensis*, *V. crenulata*, *V. phalaenopsis* and *V. pompona*) and three interspecific hybrids containing *V. pompona* and *V. phaeantha* as a parent. The highly susceptible class is occupied by the accessions with the AUDPC values ranging from 24.5 to 30. Both the cultivated vanilla species, *V. planifolia* and *V. tahitensis* are included in this class. The accessions with AUDPC scores ranging from 10.5 to 24.5 occupy the intermediate classes.

Figure 3: Image showing susceptible vanilla plants infected with Fo072

Figure 4: NJ tree (unrooted tree) showing the genetic relationship of *S. asoca* and its adulterants with bootstrap test (2000 replicates). The bootstrap values are shown above branches. Branch lengths are also shown.



Discussion

The present study focused on the aetiology of RSR of vanilla in the main vanilla growing areas in the south West Indian Ocean region. The survey and sampling conducted in main vanilla growing cities revealed the severity of the disease, which is responsible for the major economic loss. The sampling, isolation, molecular and pathogenicity (data not shown) characterization led to the conclusion that *F. oxysporum* is the main agent responsible for RSR in vanilla. This is in agreement with the previous studies conducted on RSR on vanilla in Indonesia (Pinaria et al. 2010), China (Xia-Hong 2007) and India (Vijayan et al. 2012). The molecular characterization of *F. oxysporum* causing RSR in Reunion Island and Madagascar was studied for the first time, which showed a high level of diversity. This level of diversity points out that the chances of multiple introductions or a long evolutionary history of the pathogen in the soils of Reunion Island. The well characterized isolates in the study can be used further in pathogenicity related studies in vanilla.

The study developed a rapid and simple screening assay (PRIM) to screen vanilla accessions for resistance to RSR. The screening results with *in-vitro* plants are in correlation with the field performance of vanilla plants in the naturally infested soil. The PRIM assay will help to speed up the process of breeding for *Fusarium* resistant vanilla accession. Considerable difference in the susceptibility of accessions was observed in the phenotypic assay conducted with a Fo072, a highly pathogenic isolate. The assay confirmed the resistance of species such as *V. pompona* and hybrids of *V. planifolia* with *V. pompona* and *V. phaeantha* (Tucker 1927; Tonnier 1960). The study demonstrated for the first time that some level of resistance exists in accessions obtained from auto-pollination of *V. planifolia* showing resistance to RSR is a major output of the study. Additionally 4 other species and hybrids were also identified to

be resistant, which may be useful to include in the breeding programme for resistance to *Forv*.

In conclusion, the study has described the aetiology of RSR of vanilla in the Reunion Island and Madagascar. It also describes the rapid screening method for resistance and new vanilla accessions showing resistance to RSR. These results will open new avenues in the vanilla breeding, and help to find the genes involved in resistance to *Forv* using new genomic tools.

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Utility of using a single locus *Matk* barcode to differentiate adulterants of *Saraca asoca* in ayurvedic industry

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ABSTRACT: *The bark of Saraca asoca is a key ingredient in the manufacture of important Ayurvedic formulations like "Asokarishtam" and "Asokaghritham" and it has been reported that, it is widely adulterated with bark of Polyalthia longifolia. The other adulterants reported by different researchers included Aphanamixis polystachya, Trema orientalis, Bombax ceiba, Bauhinia variegata, Caesalpinia pulcherrima, Humboldtia vahliana, Mallotus nudiflorus, Saraca declinata, Kingiodendron pinnatum. The present study intends to use DNA based method of identification of adulterants, as it is unaltered by age, storage or tissue type of plants, when compared to traditional methods of identification like macroscopic, organoleptic or microscopic and chromatographic techniques which are influenced by age or environmental factors. The present study proves the practicality of using single loci of matk for the identification of all the adulterants of S. asoca wherein, most of the adulterants typically belonged to different genera. The primers matk 390F/1326R was used to amplify the maturase K (matK) loci in S. asoca, P. longifolia, A. polystachya, T. orientalis and B. ceiba and their sequences submitted in NCBI. The sequences of other species were retrieved from NCBI and were analyzed using MEGA 6.06 software. The major take aways of the study are (i) the ability of the matK loci to differentiate between all the adulterants and S. asoca, (ii) 100% success rate in amplification/sequencing reaction using the matk 390F/1326R primers and (iii) sequences having average quality value (QV) >47, with average trimmed read length >800bp. The study recommends using single side sequencing during routine screening of adulterants as a means to reduce cost.*

Key words: *matK, Quality value, ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit, Kimura-2-parameter.*

Introduction

The dried stem bark of *Saraca asoca* (Rosc) DC Willd is the official drug of "AŚOKA" (Ayurvedic Pharmacopoeia of India, 1986) used in important Ayurvedic formulations like "Asokarishtam" and "Asokaghritham". They are mainly prescribed for treatment of gynecological disorders and also used for treating leucorrhoea, dyspepsia, blood disorders, burning sensation, pimples etc. (Warrier et al. 1996). As per the raw material requirements of Ayurvedic Medicine Manufacturers Organization of India (AMMOI), Kerala (nmpb.nic.in), the potential requirement for asoka bark is 820 mt in 2013-14 in the state of Kerala. The estimated annual requirement of Asoka bark in the Indian herbal industry is >2000 mt making it a high volume traded species, and procured from wild populations of tropical forests (Begum et al. 2014; Ved and Goraya 2007).

However, the wild presence of this species have been reported from only a few scattered areas in Western Ghats of Maharashtra, Goa, Karnataka, Tamil Nadu, Kerala, the Eastern Ghats of Odisha and Meghalaya (Begum et al. 2014) and sporadically distributed in the states of Meghalaya, Assam and Manipur, in addition to limited planting throughout the tropical regions of the country in home gardens, parks and around temples (Begum et al. 2014). These sources accounted for only a limited quantity available for trade, which cannot correlate with the huge annual requirements of the Ayurvedic industry.

The International Union for Conservation of Nature (IUCN) has listed *S. asoca* as vulnerable species. This means that either the available wild sources from remote locations are completely being stripped of bark, putting the species to threat of future extinction or a substantial portion of bark being traded is that of some other species (Begum et al. 2014). In addition to adulteration due to non-availability of genuine drugs, mistaken identity due to use of same vernacular name for different plants in different states of India can also lead to substitution with those species as in case of *Monoon longifolium* (Sonn.) B. Xue & R.M.K.Saunders (Xue et al. 2012) and *Saraca declinata* that are also known by the name "ashoka" (Khatoon et al. 2006; Mehrotra et al. 2001). *Monoon longifolium* previously known as *Polyalthia longifolia,* abundantly available avenue tree planted along the fence is a common adulterant of *S. asoca* in India. In addition to above confusions, many researchers working on pharmacology, Ayurvedic physicians and herbal industry and several publications continue to wrongly refer to the Indian plant *S. asoca* as *S. indica* instead of its proper identity. In India *S. indica* can be seen only in the botanical gardens. Its natural distribution is in Thailand, Laos, S. Vietnam and Malaysia (Sumatra, Malay Peninsula, Java), while *S. asoca* is present in India, Sri Lanka, Bangladesh and Myanmar (west of Irrawaddy River), introduced and cultivated in Malaysia (Begum et al. 2014).

The other adulterants reported by different researchers included *Aphanamixis polystachya, Trema orientalis, Bombax ceiba, Bauhinia variegata, Caesalpinia pulcherrima, Humboldtia vahliana, Mallotus nudiflorus, Kingiodendron pinnatum* (Anon 2014; Nanalal 2014; Pradhan et al. 2009; Sasidharan et al. 2010). The phytochemical studies on the bark of many of these plants, by different researchers have shown that chemical constituents present in them are very different from that of *S. asoca* (Katkar et al. 2010; Khatoon et al. 2009; Mathew et al. 2005; Nanalal 2014; Pradhan et al. 2009). Thus the substitution of *S. asoca* with any other plant can prove not only ineffective for the treatment of the particular disease, but may cause unintended clinical effects, which must be prevented at all cost.

The identification by macroscopic and organoleptic characters forms the first line of defense against adulteration, which is followed by microscopy and chromatographic techniques (Khatoon et al. 2009; Beena and Radhakrishnan 2012; Mathew et al. 2005; Nanalal 2014). However, the microscopic characters and chromatographic studies are

expected to give variations depending upon the age of the plant, chemical complexity and amount of secondary metabolites which are influenced by environmental factors. In contrast, the DNA is a stable macromolecule unaffected by the age, storage or tissue of the plants, thus an ideal molecule to study adulteration. Can a single locus barcoding strategy be used for checking adulteration in *S. asoca*? Can *matk* sequence conclusively identify *S. asoca* from its common adulterants? Are the *matk* primers robust enough to amplify in all the adulterants? This study intends to answer all these questions.

Materials and Methods

The *S. asoca* used in the present study was planted by our beloved former President of India (late) Dr. A.P.J. Abdul Kalam during the inauguration of the centre in 2003 (Fig. 1). The young leaves of *P. longifolia, A. polystachya, T. orientalis* and *B. ceiba* were obtained from the Herb garden of Arya Vaidya Sala, Kottakkal (Kerala, India). The total genomic DNA from the leaves was extracted using the CTAB method (Doyle and Doyle 1987). The extracted DNA was subjected to RNase treatment followed by purification**.** The DNA concentration was estimated using UV-spectrophotometer (Biophotometer plus, Eppendorf, Germany) and quality confirmed by electrophoresis in 0.8% (w/v) agarose gels.

PCR amplification was performed for each of the 5 samples in a 20 µl reaction mixture consisting of 20-50 ng genomic DNA, 1x PCR buffer (2mM $MgCl_2$), 200 µM each of dATP, dCTP, dGTP & dTTP, 0.5µM each of *matk*390F- 5'-CGATCTATTC ATTCAATATTTC-3' and *matk*1326R- 5'-CTAGCACACG AAAGTCGA AGT-3' primers (Cuenoud et al. 2002) and 1U of Taq DNA polymerase. DNA amplification was performed in a thermal cycler (Eppendorf, Germany) with the following program: - (i) 1 cycle of 95°C for 2 minutes, (ii) 35 cycles of 95°C for 45 Sec for denaturation, 48°C for 30 Sec for annealing the primers and 72°C for 50 sec for extension; and (iii) a final extension at 72°C for 8 min. The relative position and orientation of the primers used in the present study is shown on the entire length of the *matk* gene of *Arabidopsis thaliana* (1515 bp) in Fig. 2 (Yu et al. 2011).

A 2% routine agarose gel (with 0.5 µg/ml ethidium bromide) in 1X TAE buffer system was used for resolving the PCR products in electrophoresis unit at 70v for 2 h. The gels were documented using MultiImage II Alpha Imager-HP (Alpha Innotech, USA) (Fig. 3). The PCR products (~900 bp) from all the 5 species were, purified using the PureLink PCR purification kit (Invitrogen, Germany) and were bi-directionally sequenced at SciGenom labs (Kerala, India) using *matK*390F and *matk*1326R primers respectively. The software BioEdit (Hall 1999) was used for trimming the low quality reads at the ends of forward and reverse sequences. The overlapping regions were joined together to obtain the contigs. All the sequences were deposited in the NCBI database (see Table

1 for Accession numbers). The *matk* sequences of *Saraca indica* (EU 362034.1), *Bauhinia variegate* (JN 881405.1), *Caesalpinia pulcherrima* (EU 361906.1), *Humboldtia vahliana* (EU 361970.1), *Mallotus nudiflorus* (EF 582667.1), *Saraca declinata* (EU 362033.1), *Kingiodendron pinnatum* (EU 361987.1) were retrieved from the NCBI, and aligned using MEGA 6.06 software (Tamura et al. 2013) using MUSCLE alignment (MUltiple Sequence Comparison by Log-Expectation) (Edgar 2004). The 5′ and 3′ end sequences not having query coverage for all the 12 sequences were trimmed to 828 & 818 positions (with-gaps and without-gaps respectively) in *S. asoca* before proceeding to analysis. Due to absence of any transitions/transversions between sequences of *S. indica* and *S. asoca*, the *S. indica* was excluded from analysis. The two sequences were visually observed for any differences after pairwise alignment. The genetic distances were calculated using the Kimura 2-parameter (K2P) method in MEGA 4 (Kimura 1980) for the 828 positions for the 11 species including *S. asoca* (Table 2).

Figure 1: *Saraca asoca* (a) Habit, (B) Inflorescense (inset) and (C) Bark (inset)

Results

The maturase K specific primers *matk*390F/*matk*1326R used in the present study gave a robust amplification of a 900bp fragment from all the 5 tested species which were available at Arya Vaidya Sala, Kottakkal (Fig. 2). For the rest of the 7 species retrieved from NCBI, the primer annealing capacity was confirmed by *in silico* analysis. The quality parameters of the sequencing reactions and the NCBI accession numbers of the

submitted sequences are shown in Table 1. The genetic distance between the *S. asoca* and *S. indica* was zero based on Transitions/Transversions between the sequences. However pairwise alignment between the two sequences showed a single nucleotide difference in nucleotide position 547, where an additional nucleotide "T" was present in *S. indica.* The pairwise genetic distances calculated using the K2P method is presented in Table 2. The phylogenetic tree derived from the sequence alignment of all the 11 species is shown in Fig. 3. The average pairwise Jukes-Cantor (JC) distance is 0.186 (Jukes and Cantor 1969), which is <1 making the data suitable for Neighbor joining (NJ) tree. The NJ tree was calculated with bootstrap method with 2000 replications.

Figure 2: The relative position and orientation of the primers used in the present study is shown on the entire length of the matk gene of *Arabidopsis thaliana* (1515 bp)

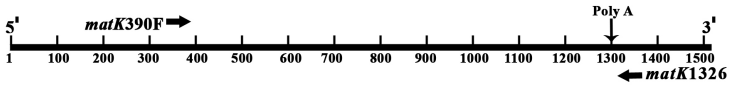
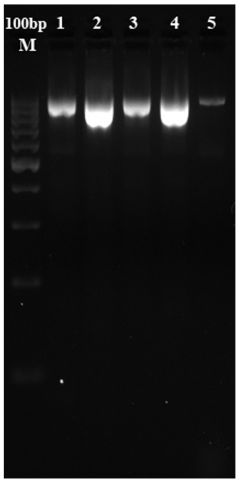


Figure 3: The agarose gel image showing PCR products amplified using matk390F/1326R primers and resolved using 2% agarose gel. Lanes : 100bp M- DNA ladder, 1- *S asoca*, 2- *M. longifolium*, 3- *T. orientalis,* 4- *B. ceiba*, 5- *A. polystachya.*



Discussion

Although the DNA barcoding using single loci is not typically used in evolutionary studies or phylogenetic conclusions drawn based on a single locus, the use of a single locus is hereby justified, as it has been proven conclusively that *matK* sequence can identify *S. asoca* from all its common adulterants. The only species that is highly identical was *S. indica* giving a pairwise identity of zero, as the calculation was based on transition+transversion. Although *S. indica* has not been reported as an adulterant till now, they could still be differentiated based on a single nucleotide polymorphism at position 547, due to insertion of the nucleotide "T" in that position in *S. indica*. As said earlier most of the researchers have been using the name of *S. indica* to refer to the *S. asoca* in many research publications, therefore doubts remain regarding the provenance of submitted sequence of *S. indica*. The original taxonomic description of *S. indica* is incomplete, and the few distinguishing features to identify them, provided by Begum et al. (2014) are very limited and may be inadequate. As *S. indica* is not reported to occur naturally in India, the possibility of using it as an adulterant remains remote, and have not been reported till now.

All the reported adulterants have been clearly distinguished in the phylogenetic tree analysis and the most commonly reported adulterant *M. longifolium* has been grouped as very distant from that of *S. asoca.* The closest match of *S. asoca* was with *S. declinata* and *H. vahliana* with a distance of 0.011 and 0.012 respectively. Different researchers have used different barcode loci in developing specific methods suited for detection of adulterants as per their specific requirements. For example, the *psbA-trnH* loci was used in detection of chilli adulteration in black pepper, *rbcL*, *trnH-psbA* and *matK* was used in detection of East Indian sandalwood adulterants, *rbcL* and ITS2 was used for detection of substitution in North American herbal products, *rpoC1, psbA-trnH* and ITS in identification of medicinal Plants in Southern Morocco (Dev et al. 2014; Kool et al. 2012; Newmaster et al. 2013; Parvathy et al. 2014, 2015).

The present study recommends using amplification of maturase k loci using the primer pair *matk*390F and *matk*1326R, as it has given robust amplification of expected products in all the adulterants of the study. The average trimmed read lengths of the sequenced samples were >800bp with average quality value (QV) >47 (Table 1). This QV is the *de facto* standard of sequencing read base quality and QV=40 means 1 in 10,000 chance of base call being wrong (Ewing et al. 1998; Ewing and Green 1998). Based on the above par values in sequencing and as a cost reducing measure in raw materials testing a single direction DNA sequencing using either one of the primers, is recommended for testing adulteration in *S. asoca* for the Ayurvedic industry. Although the DNA barcoding has been used as a tool for detection of adulterants in plants for in different industries, this will be the first instance where we have proved the utility of using the DNA barcode in detection of adulterants for the Ayurvedic industry.

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Table 1: The quality parameters of the sequencing reactions and NCBI GenBank accession numbers

S. No.	Plant Name	Forward or Reverse Sequence	Quality Value (QV)	Trimmed read length	Forward primer lengths	Reverse primers lengths	Submitted sequence length	GenBank Accession Numbers
1	*Saraca asoca*	Forward	52	768	768	873	874	KU041809
		Reverse	40	873				
2	*Polyalthia longifolia*	Forward	54	821	821	868	860	KU041810
		Reverse	52	868				
3	*Bombax ceiba*	Forward	47	805	805	792	805	KU159152
		Reverse	50	792				
4	*Trema orientalis*	Forward	50	834	834	747	839	KU159153
		Reverse	32	747				
5	*Aphanamixis polystachya*	Forward	51	815	815	781	831	KU159154
		Reverse	50	781				
		Average =	**47.8**	**810.4**	**808.6**	**812.2**	**841.8**	**-**

Table 2: Estimates of genetic distances between Sequences (*matK*) of *S. asoca* and its adulterants. The number of base substitutions per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. Analyses were conducted using the Kimura 2-parameter model (Kimura 1980). The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 828 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

	KU041809_Saraca_asoca	EU361987.1_Kingiodendron_pinnatum	KU041810_Monoon_longifolium	KU159152_Bombax_ceiba	KU159153_Trema_orientalis	KU159154_Aphanamixis_polystachya	JN881405.1_Bauhinia_variegata	EU361906.1_Caesalpinia_pulcherrima	EU361970.1_Humboldtia_vahliana	EF582667.1_Mallotus_nudiflorus	EU362033.1_Saraca_declinata
KU041809_*Saraca_asoca*		0.005	0.020	0.017	0.017	0.017	0.013	0.013	0.004	0.020	0.004
EU361987.1_*Kingiodendron_pinnatum*	0.021		0.020	0.017	0.018	0.018	0.013	0.013	0.005	0.020	0.006
KU041810_*Monoon_longifolium*	0.264	0.263		0.020	0.021	0.020	0.020	0.022	0.020	0.020	0.020
KU159152_*Bombax_ceiba*	0.208	0.215	0.240		0.018	0.016	0.017	0.017	0.017	0.018	0.017
KU159153_*Trema_orientalis*	0.212	0.220	0.268	0.218		0.016	0.018	0.017	0.018	0.019	0.017
KU159154_*Aphanamixis_polystachya*	0.226	0.230	0.256	0.178	0.201		0.017	0.017	0.018	0.017	0.018
JN881405.1_*Bauhinia_variegata*	0.121	0.127	0.253	0.205	0.209	0.209		0.013	0.013	0.019	0.014
EU361906.1_*Caesalpinia_pulcherrima*	0.120	0.122	0.274	0.210	0.213	0.225	0.128		0.013	0.019	0.013
EU361970.1_*Humboldtia_vahliana*	0.012	0.019	0.264	0.208	0.212	0.223	0.117	0.119		0.020	0.005
EF582667.1_*Mallotus_nudiflorus*	0.235	0.234	0.272	0.214	0.232	0.196	0.224	0.233	0.232		0.020
EU362033.1_*Saraca_declinata*	0.011	0.030	0.264	0.211	0.216	0.231	0.129	0.129	0.021	0.237	

Molecular screening of actinomycetes from soil of Kulathupuzha sacred grove for Lipase production and its application

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ABSTRACT: In the present study we describe the attempt of obtaining potential lipase (EC 3.1.1.3) producing actinomycetes from soil sample collected from Kulathupuzha sacred grove of southern Kerala. The isolates were identified as *Streptomyces* spp. through morphological and biochemical characterisation as well as by molecular analysis. The lipase production was optimised using different carbon sources and nitrogen sources by submerged fermentation. The lipase production was carried out using media containing heterogeneous specific substrate as carbon sources. Olive oil has shown production of 45.8 U/ml at 48 hour of incubation. Sunflower oil was also observed to be as a potent substitute to costly olive oil it has given about 44.6 U/ml of activity at 48 hour of incubation and maximum activity was observed to be at 72 hour of incubation. Coconut oil (42U/ml) and palm oil (36.5U/ml at 72 hour of incubation) also has resulted in high yield which observed to be potent sources for lipase production. Presence of surfactant was favouring better lipase production. Other isolates from this region has shown potential sources for other enzymes like chitinase, asparginase, protease, amylase, xylanase etc, indicating that sacred grove are promising spring of diverse group of organism so it is our necessity to conservation and preserve these region for future generation.

Key words: *Glycerol aspargine agar (GAA) and Starch casein agar (SCA), Sprit Blue agar (SBA), Tributyrin agar (TBA), p-nitrophenylacetate (pNPA), Dinitrosalysilic acid (DNS)*

Introduction

Sacred groves are considered as untouched region on earth for centuries and they observed to have diverse group of microbial population, so these region where selected for bioprospecting studies. *Streptomyces* are a group of filamentous Gram-positive bacteria found in soil with G+C content. They differentiate on solid media; in submerged cultures, after the exponential phase of growth, many strains produce antibiotics and a wide variety of other secondary metabolites. Approximately 4000 enzymes are known, about 200 are in commercial which are mainly of microbial origin.

Lipases hydrolyze triacylglycerols to fatty acids, diacylglycerols, monoacyl glycerols and glycerol whatever under certain conditions, catalyze reverse reactions such as

esterification and transesterification (Niyonzima and More 2015; Park and Mori 2005). A true lipase will split emulsified esters of glycerine and long-chain fatty acids such as triolein and tripalmitin. Lipases are serine hydrolases and it displays little activity in aqueous solutions containing soluble substrates. Although *Streptomyces* strains were recognized through their high exogenous lipolytic activity, lipases of this genus were not studied as intensively as were those from some other bacteria (Jaeger et al. 1994). Applications of lipase range from organic chemical processing, detergent formulations, synthesis of biosurfactants, the oleochemical industry, the dairy industry, the agrochemical industry, paper manufacture, nutrition, cosmetics, and pharmaceutical processing (Aravindan et al. 2007). Because of vast difference in applications, the availability of lipases with specific characteristics is still a limiting factor. Thus to search for new lipases with different characteristics and improve lipase production continue to be important topic of research. One limiting factor is a shortage of lipases having the specific required processing characteristics. In this paper we discuss about several *Streptomyces sp.* isolated from Kulathupuzha sacred grove. The strain possessing the highest lipase activity was identified both biochemically and sequencing of 16SrRNA gene. Media optimisation for increase in lipase yield was attempted by using different oil as carbon sources in production media. Also, lipase from selected strain was preliminarily characterized for use in industrial application.

Materials and methods

Isolation of actinomycetes by serial dilution of soil

Soil samples were collected from different region of Kulathupuzha sacred grove (2acres), near Kulathupuzha Sastha temple. Soil samples were enriched and bacterial contaminants avoided by heating at 50^0C for 1 h and fungal contamination by incorporating Nystatin in medium. Serial dilution prepared and 0.1ml aliquots of each dilution plated on GAA and SCA plates (Shirling and Gottlieb, 1966) and incubation at 28 ±2°C for 7 days and plates were observed for growth. Morphologically different types of colonies were picked up separately.

Primary screening for lipase activity

Primary screening for lipolytic activity was done using SBA plates of composition (g/L) Casein enzyme hydrosylate-10, YE-5, Sprit blue-0.15, Agar-17(pH-6.8). TBA plates (g/L) Peptone-5, BE-3, Tributyrin -10 ml, Agar -20 and Tween 80 agar plates (g/L) Peptone -10, NaCl-5, $CaCl_2.2H_2O$-0.1, Agar-agar -20g (pH-6). The plates were incubated at 28 ± 2^0C and strains with lipolytic activity produces zone of clearance on SBA and TBA plates whereas in Tween 80 shows precipitation around the lipolytic organism indicating lipase activity.

Lipase assay

The lipases were estimated by Vorderw¨ulbecke et al. (1992) method with modification. 30mM pNPA was dissolved in 10ml isopropanol. Reaction mixture was prepared by adding emulsion (100mg gum arabic and 0.4% Triton X -100) to 90 ml phosphate buffer (50Mm, pH-7). 1.8ml of emulsion mixed with 0.1ml of substrate solution and 0.1ml of enzyme solution and incubated at 37°C for 30min at 180rpm for mixing and the reaction was terminated by adding 0.2M NaOH, and read at 410 nm.

Production of lipase in liquid media

The basal medium for lipase production contained (g/L) olive oil 1% (v/v), sodium citrate 5, yeast extract 5, calcium chloride 0.05, pH 7. After sterilization, about 2ml of culture suspension, previously grown in nutrient broth medium for 2 days at 28°C, were inoculated into each flask and kept at 150 rpm (28°C) for 5 days. Samples were taken at every 24h interval and centrifuged at 10,000 rpm for 10 min, the cell free supernatant was used for lipase assay. All steps were carried out under aseptic conditions.

Effect of different carbon sources on lipase production

Streptomyces sp. which has given maximum activity with olive oil been subjected for production optimisation with other edible oil in substitution to olive oil in the above mentioned media. In substitution to olive oil other oil like sunflower oil, palm oil and coconut oil (1% (v/v), were used for lipase production. These oil were autoclaved separately and added into autoclaved production medium and experiment repeated as above.

Extraction of genomic DNA and PCR amplification -16s rDNA and sequencing

Spores from SDA culture were inoculated to 50ml YEME broth and DNA extracted using standard procedure and diluted in TE buffer and concentration was estimated. Primer for 16s rDNA 8-27F,5'-AGAGTTTGATCCTGGCTCAG-3, 1495R,5'-CTACGGCTACCTTGTTACGA-3' and PCR run 94°C-3min 1 cycle, Denaturation-94°C-30sec, Annealing 58°C-30sec, extension 72°C-1min. 30sec 35 cycle, Final extension- 72°C-7min, 1cycle and hold at 4 °C.

Results

Soil samples collected from sacred grove was enriched and serial dilution was carried out and 12 were purified. These isolates were subjected to primary screening for lipase by spreading inoculum of each colony on plates on SBA and TBA. Out of the 12 isolates, 4 isolates hydrolyzed the lipid material producing a clear zone around the

growth (Fig. 1). The activity in lipase production for each isolate was measured by the diameter of the clear zone. Lipase producing isolates were grown on agar medium containing Tween 80 here the presence of white precipitate around the colonies indicated lipase production.

Figure 1: Primary screening on SBA plates

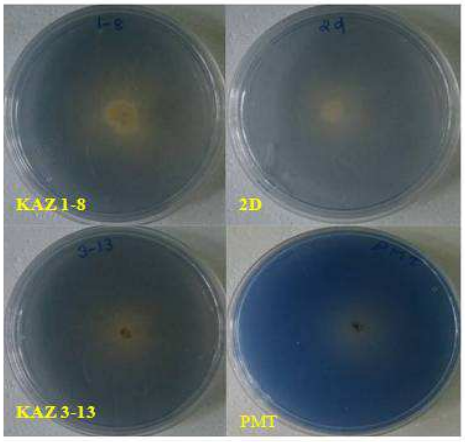
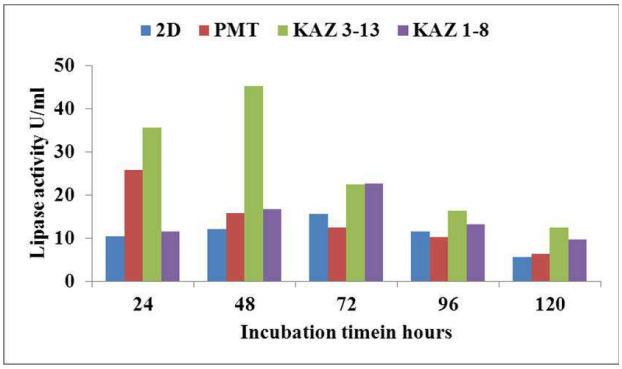
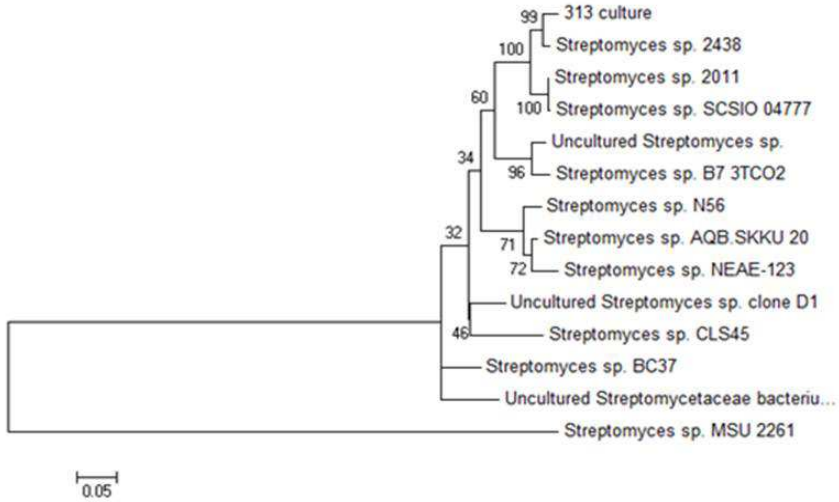


Figure 2: Secondary screening of 4 isolates for lipase production in liquid medium with olive oil as carbon source.



Streptomyces. sp, (KAZ 3-13) was subjected to lipase production by submerged fermentation in media containing olive oil as carbon sources has given maximum yield of 45.8U/ml at 48 hours (Fig. 2) during secondary screening of four cultures for evaluation of potent producer. KAZ 1-8 has given 22.6U/ml at 72h, 2D -15.6U/ml at 72h and PMT-15.8U/ml at 24h. Of these four strains *Streptomyces. sp,* (KAZ 3-13) which has given maximum production was taken for further analysis.

Figure 3: *Streptomyces* (KAZ-313) isolated from Kulathupuzha observed to have 99% similariy to Streptomyces sp. 2438 strain



Being olive oil very expensive and the use of this will increase the production cost, so we tried with other oil as carbon sources in substitution to olive oil. Sunflower oil has given 44.6 U/ml at 48 hour of incubation and 45.3 U/ml at 72 hour. With Coconut oil 42 U/ml at 48h and palm oil 36.5 U/ml at 72h respectively. Presence of surfactant was favouring better lipase production. Little enzyme activity was observed in the absence of olive oil even after prolonged cultivation.

The 16SrDNA sequence was compared to the GenBank database in the NCBI using the BLAST program. The 16SrDNA sequence of this isolate showed high levels of sequence similarity with members of the genus *Streptomyces*. The comparison of 16SrDNA sequence can show evolutionary relatedness among microorganisms. Evolutionary tree constructed by neighbourhood joining method using MEGA 4 software. It was observed that the sequence has 99% similarity report *Streptomyces* sp. 2438 (Fig. 3).

Discussion

The biotechnologically important *Streptomyces* genus contains the largest number of species within the order *Actinomycetales*. Taxonomic status and phylogenetic analysis of *Streptomyces* is based on a polyphasic approach, including description and analysis of pigmentation, morphology, biochemical and physiological properties (Annaliesa et al. 2001). Molecular-biological techniques have utilized for refining or extending classifications, especially those techniques targeting 16SrRNA genomic regions and it is a rapid method (Mohamed et al. 2013). The rRNA gene is the most conserved (least variable) DNA in all cells. Portions of the rDNA sequence from distantly related

organisms are remarkably similar. For this reason, genes that encode the rRNA (rDNA) have been used extensively to determine taxonomy, phylogeny, and to estimate rates of species divergence among microbes.

Tributyrin is a triglyceride naturally present in butter and can be described as a liquid fat. Plates containing lipid substrate on lipolysis produce either clear halos or opaque zones around culture. *Streptomyces rimosus* R6-554W was identified as a lipase producer after culturing on agar plates containing tributyrin (Abramic et al. 1999). A simple and reliable method for detecting lipase activity in microorganisms is by using surfactant Tween 80 (polyoxyethylene sorbitan monooleate) indicated by formation of opaque zones around the colonies (Nair and Kumar 2007). There are modifications of this assay with different Tween in various combinations with Nile blue or neet's foot oil and Cu^{2+} salts. Wang et al. (1995) used plates of a modified Rhodamine B a chromogenic substrates in agar to screen lipase activity in a large number of microorganisms and it exhibited orange colour under UV.

All the isolates used in this study were identified as *Streptomyces* sp. according to Bergey's Manual of Systematic Bacteriology (Williams et al. 1989) and the results was confirmed using 16S rDNA as recommended by Weisburg et al. (1991). *Streptomyces* KAZ 3-13 observed to be a potent producer of lipase in olive oil medium and selected for further analysis. Lipase from *S. griseus* was reported to have lipase coding gene and also reported the factors affecting lipase activity like incubation period and effect of pH (Vishnupriya et al. 2010). The maximum lipase activity was achieved at 24 and 48 h of incubation and the enzyme activity was 51.9 and 51.9U/ml by using sunflower oil and palm oil as a substrate. So far, only few *Streptomycetes* lipases have been described mainly *S. albus, S. coelicolor, S. exfoliates, S. cinnamomeus, Streptomyces rimosus, S. lividans S. clavuligerus, S. diastatochromogens* (Abramic et al. 1999, Mishra and Gupta 2014). Generally, the enzymes of industrial interest were produced in the presence of inducers like triacylglycerols, surfactants (Tween), vegetable oils (Soybean oil, olive oil, etc.), oil industry wastes, or their hydrolysis products in the culture medium (Li et al. 2004). Tween 80 did not stimulate the growth but is the best inducer for lipase production (Hasan et al. 2006). It has double effect on lipase production; it acts as surfactant and inducer, because of its chemical nature that was similar to some natural lipid substrate, stimulating the enzyme release. A mangrove isolate *Streptomyces halstedii* strain ST 70, reported to produce 26.1U/ml lipase in presence of starch, glycine, ammonium chloride and tween 80 as inducer (Mishra and Gupta 2014). 28-kDa lipase from *Streptomyces exfoliatus* M11 (Perez et al. 1993), and the similar-sized lipases from *Streptomyces albus* G (Cruz et al. 1994) and *Streptomyces coelicolor* A3 (2) (Valdez et al. 1999) exhibited 82% sequence identity. *Streptomyces cinnamomeus* (Sommer et al. 1997); has a 27-kDa protein share no sequence similarity with any of these three *Streptomyces* lipase sequences. Considering the rate of olive oil, which is highly expensive, the work also aimed for substitution of it with other cheap sources of

oil. So, in an industry increasing the contents of sunflower oil and palm oil, the lipase yields also increase two folds with low cost, because they were the cheapest substrate and available in higher amount.

Actinomycetes are potent producers of secondary metabolites used commercially as antibiotics and other novel drugs. The present study shows Sacred groves are region of rich microbial diversity and all isolates are observed to be potent producers of various enzymes like chitinase, amylase, asparginase, protease, etc. The isolated *Streptomyces* spp. are novel strains, so protection and conservation of this region very important. Further research is required to optimise the culture condition for maximisation of enzyme yield and to develop a technology for biodiesel production using lipolytic enzyme.

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Biotechnological Applications of Tannase: A Review

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ABSTRACT: *Tannin Acyl Hydrolase (EC. 3.1.1.20) commonly referred to as tannase is an enzyme which catalyzes the hydrolysis of ester bond and depside bond present in hydrolysable tannins to form glucose and gallic acid. Tannase is widely used in the leather, pharmaceutical, beverage, and food industries. The main applications of tannase are in the production of instant tea, acorn liquor and gallic acid. Tannase is also used as clarifying agent in juices and flavoured coffee soft drinks. Gallic acid is used in the pharmaceutical industry as an important intermediate compound in the synthesis of trimethoprim. It is used in the chemical industry as a substrate for chemical or enzymatic synthesis of propyl gallate and other antioxidant compounds, cosmetics, hair products, adhesives, and lubricants. Gallic acid is also used in fabrication of semiconductors, dyes, and in photographic revelation. Tannase helps to reduce the adverse effects of tannins in beverages and foods. In the manufacture of instant tea, tannase is used to remove insoluble precipitates that are formed when tea is cooled to temperatures below 4°C. These precipitates are formed by polymerization of phenolic compounds and their interaction with caffeine. The chemical processes to remove the tea precipitates can eliminate a large amount of aromatic compounds; however, by enzymatic treatment, it is possible to obtain a soluble tea in cold water with a high content of aromatic compounds and an appropriate colour. Tannase cleaves ester bonds of certain tannins and thereby prevents its polymerization and complex formation with caffeine. The enzymatic treatment to reduce the bitter taste of fruit juices has certain advantages that enhance the quality of these drinks. The high concentration of tannins in fruits like blueberries, pomegranate, and raspberry causes the formation of sediment, colour, and bitter taste during its storage and tannase can eliminate this problem. This enzyme has been proposed for use in environmental biotechnology, e.g. for the treatment of tannery effluents that are rich in polyphenols.*

Key words: *Tannase, environmental, applications*

Introduction

Tannase or tannin acyl-hydrolase (E.C. 3.1.1.20) is an extracellular, inducible enzyme produced in presence of tannic acid by plants, animals and microbes (Aguilar and Gutíerrez-Śanchez 2001). It is classified under the family of hydrolases, especially acting on carboxylic ester bonds (Brahmbhatt and Modi 2015). It catalyzes the hydrolysis of ester bonds present in gallotannins, complex tannins and gallic acid esters releasing of glucose, gallic acid and some galloyl esters. Although tannase is present in plants, animals and microorganisms, it is mainly produced by the latter. Microorganisms have been the most important source for the production of industrial enzymes due to their biochemical diversity and their technical and economic advantages. Filamentous fungi of the genus *Aspergillus* and bacteria of the genus *Bacillus* have been widely used for tannase production (Mondal et al. 2001; Pinto et al. 2001). Tannase has applications in food and beverages processes; however, the

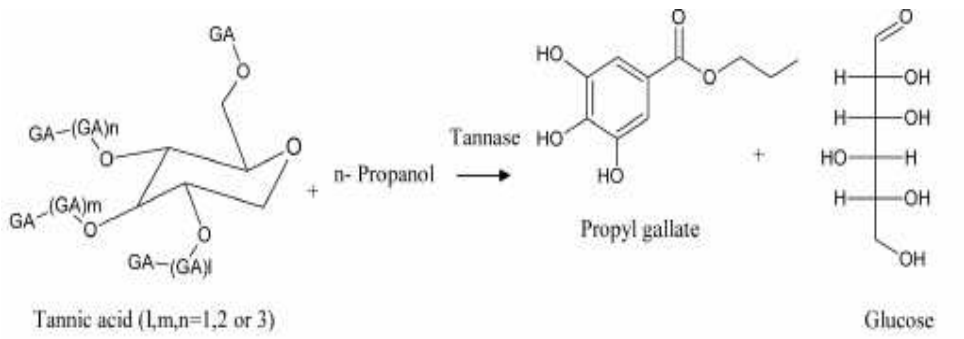
application is limited because of the little knowledge about its properties, optimal expression and scale up (Aguilar et al. 2007). In beverage industry application of tannase resides in the production of acorn liquor, instantaneous tea and production of gallic acid (Belmares et al. 2004). Microbial tannases are amongst important industrial enzymes which also find immense applications in food, feed, leather and pharmaceutical industries (Lekha and Lonsane 1997). The current review focuses on some of the recent progress in industrial application of microbial tannase.

Applications of Tannase

Instant tea production

Tea is the most popular beverage worldwide. Instant tea is stored at or below temperatures of 4°C. During this storage certain insoluble precipitates are formed which results in haze (tea cream) formation (Niehaus and Gross 1997). The formation of this haze is due to the interaction between caffeine and tea flavonoids mainly epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate. When reconstituted as a hot beverage, chemically synthesised instant tea powder reacts very badly on addition of milk by forming a blackish, unpleasant colouration (Coggon et al. 1975). Removing the insoluble compounds helps to produce instant tea with good colour, solubility and good yield (Sanderson and Coggon 1974). Tannase treatment cleaves the ester bonds of flavanoids avoiding its complex formation with caffeine (Aguilar and Gutíerrez-Śanchez 2001). It also enhances the levels of epicatechin and gallic acid, which in turn favours the formation of epitheaflavic acid, which gives bright reddish colour to tea. Tannase treatment yields tea with a good cold-water solubility and colour. The reaction that follows is a de-esterification between galloyl groups and various compounds in tea leaves. Tannase treatment increases the gallic acid content which favours cold water solubility.

Figure 1: Trans-esterification of tannic acid to propyl gallate in presence of n-propanol (Natarajan 2009)



Pharmaceutical industry

Gallic acid (3, 4, 5-trihydroxy benzoic acid), is an intermediate used in the synthesis of an antifolic antibacterial drug trimethoprim (Lekha and Lonsane 1997; Kar and Banerjee 2000). Conventionally gallic acid is produced by acid hydrolysis of tannic acid but it is expensive, and also the yield and purity was low. The hydrolysis product of tannic acid upon action by tannase is gallic acid (Iibuchi et al. 1972). Therefore tannase can be applied in pharmaceutical industry for producing gallic acid. Gallic acid is also used as a synthetic intermediate for the production of pyrogallols and gallic acid esters. Propyl gallate is used as an anti oxidant in foods, cosmetics, hair products, adhesives and lubricant industries (Gaathon et al. 1989; Haadi et al. 1994).

Beer and wine production

Haze formation in beer is due to the complex formation by certain wort phenolics with other chemicals is beer mixture. Tannase treatment can hydrolyse these complexes and prevent haze formation (Giovanelli 1989). Tannase hydrolyses the chlorogenic acid present in beer mixture to caffeic acid and quinic acid which favourably influences the taste of wine (Chae and Yu 1983). The main tannins present in wines are catechins and epicatechins. The colour of wine is mainly due to the presence of these tannins. Tannins are oxidized to quinines by contact with air which results in undesirable turbidity and poor quality. The high protein content of beer causes undesirable turbidity when they interact with these tannins. Tannase treatment helps to avoid such problems and enhances the quality of these beverages.

Animal feed improvement

Tannins are present in a variety of plant materials which can be used as feed (Bate-Smith and Rasper 1969). But its anti nutritional effect limits its application as animal feed. Pre-treatment of tannin containing feed using tannase removes undesirable compounds present and improves digestibility. The plant cell wall comprises polymers of diferulic acid which is a major obstacle that limits the accessibility of main chain degrading enzymes resulting in the reduced digestibility of cell wall (Bunzel et al. 2001). The breakage of these dehydrodimer crosslinks between plant cell wall polymers is essential for degradation of plant cell wall resulting in the improvement of its digestibility. Conesa et al. 2001 used tannase produced from *A. oryzae* to hydrolyse synthetic ethyl esterified substrates such as diethyl 8-5-benzofuran diferulate, ethyl 8-5-benzofurandiferulate, diethyl 5-5-diferulate, diethyl 8-O-4-diferulate to ferulic acid which suggests its application in cleaving some of the cross links existing between cell wall polymers, increasing the digestibility of animal feed. Garcia-Conesa et al. 2001 used tannase from *Aspergillus oryzae* for animal feed improvement by hydrolysing diethyl diferulates. Sharma and Saxena 2002 found that tannase from *A. niger* and *P.*

variable were able to hydrolyze xylan and pectin present in fodder producing ferulic acid, thereby increases feed digestibility.

Debittering of fruit juices

Bitterness of fruit juices is due to the haze formation by the tannins present in it. Enzymatic treatment to reduce the bitterness enhances juice quality by lowering haze formation. Recently juices of cranberry, raspberry, pomegranate etc have gained importance for their health benefits, mainly its antioxidant activity. Presence of high tannin content in these juices is responsible for haze and sediment formation as well as for colour, bitterness and astringency of the juice upon storage. Rout and Banerjee 2006 used tannase obtained by the co-culture of *Aspergillus foetidus* and *Rhizopus oryzae* in the debittering of pomegranate juice. Results suggest that tannase treatment resulted in 25% degradation of tannin content in the juice. de Lima et al. 2014 used tannase from *Penicillium montanense* URM 6286 for the clarification of grape Juice (*Vitis vinifera* L.). After incubating the juice with tannase for 120 m there was a reduction in tannin content of about 46%. A recent study by Sharma et al. 2014 evaluated the efficiency of tannase from *Aspergillus niger* for removing tannin from guava (*Psidium guajava*) juice. The authors added 2% tannase to the juice and observed a 53.23% reduction in tannin content. From the above literatures it is evident that tannase can be efficiently used to clarify and stabilize fruit juices thereby it improves the quality.

Tannery Effluent treatment

Tannery effluents contain high amounts of tannins, mainly polyphenols. These polyphenols can cause serious environmental problems as they are dangerous pollutants (Van de Lagemaat and Pyle 2001). Tannase can be potentially used for the degradation of tannins present in these effluents and thus removes of these pollutants.

Conclusion

Tannase (E.C 3.1.1.20) is an inducible enzyme that catalyses the hydrolysis of ester bonds present in hydrolysable tannins and gallic acid esters. They are extensively used in food, feed, pharmaceutical, beverage, brewing and chemical industries as well as environmental depollution. The major commercial applications of the tannases reside in the elaboration of instantaneous tea or of acorn liquor and in the production of gallic acid. In the pharmaceutical industry, gallic acid is an important intermediary compound in the synthesis of the antimalarial drug, trimethroprim. In food industry tannase is used for the synthesis of propyl gallate, a potent antioxidant. Moreover, tannase is used as a clarifying agent in some wines, beers, fruit juices, and in refreshing drinks with coffee flavour. From this it is clear that tannase have the potential to be used as industrial catalysts with its ability to perform chemical reactions both under mild and harsh conditions.

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Beta Glucanases- Microbes are the potential source of production considered unusual as of cellulases.

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ABSTRACT: *β- glucan is the natural abundant resource broadly disseminated in microbial or fungal cell walls and in plants. The enzyme systems responsible for the degradation of β- glucans are β- glucanases (EC 3.2.1.x), group of glycosyl hydrolyses which breaking down the beta linked glucose polymers that is uable to digest by humans easily. Extracellular β- glucanases exhibits both exo- and endo-hydrolases are produced by variety of microorganisms. Thus helps for digesting grains such as barley, oats, wheat, soya bean meal etc. Based on the type of hydrolysis they are further classifies as β- 1, 3-Glucanase, β- 1, 6-Glucanase and β- 1, 4-Glucanase having different substrate specificity. They have significant role in the structural analysis of polysaccharides. β-glucanases produced by microbial systems have great ecological importance since they are different from cellulases, further stable and have a potential role in biotechnological processes for many industrial applications, as a part of brewing industry, feed enzyme industry, textile industry, coffee processing, vinification and along with it have some plant growth promoting activities and curing properties against some food and environmental allergies. Participation of β- glucanases in the morphogenetic events of yeast cell is most prominent. Complete exhaustion of β- glucan requires the synergic action of different β- glucanases. The role and synergism of different β- glucanases will allow better understanding of some metabolic processes as well as explain their roles in depolymerization of β- glucans.*

Key words: *β- glucans, exo and endo hydrolases, applications, fungal β-glucanase.*

Introduction

β- glucans are polysaccharides, present in microbial or fungal cell walls and a chief component of cell walls of cereals such as oats and barley (Demirbas 2005). Major source of nutrients for animals are constituted by cereals. β- glucans occur at different levels in various cereals (Genc et al. 2001). However β- glucans have several adverse effects, it increases the energy metabolism in poultry when it is introduced in feed particularly for monogastric animals and is responsible for poor nutritive value of some cereals (Annison et al. 1991). In brewing industry high molecular weight β-glucans causes severe problems such as increased brewer mash viscosity and turbidity which impairs pumping and filtration, reduced yield of extracts, formation of gels and hazes and leaving residues in beer (Plans 2000). These problems can be effectively solved by the sensible use of β- glucan degrading enzymes typically known as β-glucanases, glycosyl hydrolases responsible for the hydrolysis of insoluble β- glucan substrates. The hydrolysis of β- glucan molecules by the action of β- glucanases leading to the production of D- glucose, thus serving as carbon sources. β-D- glucan hydrolases

can act as both exo and endo hydrolases. Endo-β-glucanases cleave inside a β- glucan chain in a more or less random fashion while exo- β- glucanase release glucose residues from the non-reducing end. On the basis of type of glycosidic linkage cleaved, they are further classified as 1,3-β- glucanases, 1,4-β- glucanases and 1,6-β- glucanases (Dake et al. 2004).

A wide range of β-glucanases comprise bacterial or fungal β-glucanases are active on β- glucan substrates and cleave within the main chain of mixed linkage β-glucan with different point of action. This is significant in choosing specific substrates for the assay of these groups of enzymes (McCleary 2001). The known specificity and mode of action of β- glucanases within the enormous substrate range would allow us to understand these are different from cellulases. Enzymes diversified in the β- glucanases complexes are endo-1,3- β- glucanase (EC 3.2.1.39), endo-1,4- β- glucanase (EC 3.2.1.4), endo -1,3(4)- β- glucanases, termed as laminarinases (EC 3.2.1.6), endo-1,6- β- glucanases (EC 3.2.1.75), endo- β-1,3-1,4- glucanases or lichenase (EC 3.2.1.73), endo-1,6- β- glucanases (EC 3.2.1.75), exo-1,3- β- glucanases (EC 3.2.1.58), exo-1,4- β- glucanases (EC 3.2.1.91) and exo-1,6- β- glucanases (EC 3.2.1.70). These enzymes are believed to be considerable ecological significance and have commercial usage in several industrial productions.

In biotechnological industries β-glucanases application is well established such as preparation of fungal protoplasts for fungal cell wall structure analysis, in beer production, in wine extract clarification, barley β- glucan degradation for animal feed enzyme industry, used as an addictives in detergents, saccharification of agricultural and industrial wastes, coffee processing and exhibits antifungal activity for disease protection in plants.

Substrates for β- glucanases

Curdlan is a linear polysaccharide containing mainly 1,3 -β-glycosidic bonds having average degree of polymerization of upto 455 (Takahashi et al. 1986). This polymer is produced by many strains of *Agrobacterium* and some strains of *Rhizobium*. Curdlan produced by *Alcaligenes faecalis* is a commercially available substrate for 1, 3- β-glucanases assay, perhaps it consist of few intra or inter chain 1,6- linkages and heating on the aqueous suspension creates elastic gels (McIntosh et al. 2005). Curdlan conformation by X-ray analysis and NMR studies shown that when heating in water triple helix formed from single helix (Kanazawa et al. 1989). Linear mixed link 1,3-1,4-β-glucan, barley β-glucan is a significant component of barley endosperm made up of cellotriosyl and cellotetraosyl residues separated by 1,3- β-linkages. This active component has significant outcome in industrial exploitation of barley grain. High viscosity solutions formed by barley glucans adversely affect brewing industry; can be used as a substrate for β- glucanase assays. (Poppitt et al. 2007).

Laminarin, a polysaccharide from brown sea weeds, exploit as a substrate for endo -1,3(4)- β- glucanases. These low molecular weight β- glucans comprises (1,3)- β-D-glucopyranose residues with some β- 1,6-intrachain links. Laminarins from different sources have been showed different structure and linkage composition (Kadam et al. 2015). A 1,6- linked β- glucans, pustulan from a variety of lichens is a gel forming and showing some degree of acetylation, is a good substrate for assaying 1,6-β- glucanase activity. The acetylation level within and between the species can vary and even based on the storage conditions of lichens (Narui et al. 1999). Lentinan is a 1,3- β- glucan having two 1,6- β-glucopyranoside branching for every five 1,3- β-glucopyranoside linkage. It is an accessible substrate for 1,3-β-glucanase isolated from Japanese edible mushroom, *Lentinus edodes*. It has various immunomodulator activities and exerts some inhibitory action on tumors and prevents chemical and viral carcinogenesis (Bielecki et al. 1991).

Microbial β- glucanases

β- glucanases with non-cellulolytic activity has been widely reported in different microorganisms like bacteria, fungi and archaea. The first report of these enzymes is from bacteria (Bass et al. 1952).Enzymes with β- glucanases activities are produced by diverse microorganism having different substrate and product specificities. *Bacillus* species is the most well known producer of these enzymes. β- glucanases from microbes are classified in glycosyl hydrolase family 16 (GHF 16) (Hong et al. 2002). 1,3-β- glucanases from *Bacillus circulans* and *Arthrobacter* sp. hydrolyses laminaran into laminarapentose (Bielecki and Galas 1991). Studies have been done for the purification and characterization of endo-1,3-β- glucanases from *Arthrobacter* species, hydrolyses curdlan and lichenan (Pang et al. 2004). β- glucanases from *Pyrococcus furiosus* showed the ability to hydrolyses β- 1, 3 and β- 1,4 bonds (Lieshout et al. 2004). β- glucanases in *Actinomyces* are less characterized in comparison with fungal and other microbial glucanases. β-1,6- glucanases from *Streptomyces* sp. EF-14 (Fayad et al. 2001), β-1,3-glucanases from *Streptomyces* sp. Mo. (Kurakake et al. 2013), 1,3-*β*-D-glucanase from *Streptomyces torulosus* PCPOK-0324 (Park et al. 2012) are few of the recent reports available.

Fungi can also produce β- glucanases with wide activities. Fugal β- glucanases are not always produced exocellularly, most of them are cell –wall associated and having both hydrolase and glycosyl transferase activity, suggesting that they play important roles in cell wall glucan metabolism that leads to several morphogenetic processes particularly in yeasts. Structure and functions of these exo-cellular and cell wall associated β- glucanases are not well understood and very less is known about the fermentative culture conditions of exo-cellular fungal β- glucanases (Martin et al. 2007). Most of the knowledge related with wall associated β- glucanases comes from the works related with yeasts *Saccaromyces cerevisiae, Candida albicans and*

Schizosaccharomyces pombe (Adams 2004). *Schizosaccharomyces* 1,3-β- glucanases hydrolyses 1,3-β- glucan in random manner and produces laminarabiose and glucose (Reichelt and Fleet 1981).

Conclusion

Currently β- glucanases have been considered to be great importance and have potential applications in food, beer and wine industries as well as an elegant tool for β-glucan structural analysis. Production costs of the enzymes are very high, because only a few kinds of pure β- glucan offered by some companies can be used as the substrate for β- glucanase with too high price. It also increases the cost of β- glucanase leading to the limited application of β- glucanase in large scale industries. As a part of extensive production some of the β- glucanase gene have been effectively cloned and expressed in heterologous host cells, this also facilitated the better understanding of the regulation of synthesis. In addition genetic engineering approach is a powerful tool to construct recombinant expression cassette encoding different β- glucanase genes and production of enzymes with commercially reasonable scale. At present only a few organisms are exploited for screening β- glucanase enzyme systems. Further research should be carried out for the purification and characterization of more enzymes will be necessary for better understanding of their properties.

Acknowledgments

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Diversity of phylloplane fungi affecting selected plants and evaluation of its position using molecular tools

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ABSTRACT: *Phylloplane fungi are strictly obligate biotrophs, infecting the leaves of many plants. Fungi are the important component of the biodiversity in tropical forests. As a major contributor to the maintenance of the earth's ecosystem, biosphere and biochemical cycles, fungi perform unique and indispensable activities on which humans depend. Fungi inhabiting the leaves are known as phylloplane or foliicolous fungi. Members of Meliolaceae are the major group of phylloplane fungi. These fungi are commonly known as black mildews and are obligate parasite symbionts. The plant infected with phylloplane fungi were collected from selected botanic gardens, examined microscopically and herbarium specimens were prepared. The microscopic slides were prepared using nail polish technique and mounted on DPX. The slides were observed under microscope and drawings were made as per scale. Genomic DNA was extracted by modified CTAB method, Wizard genomic DNA extraction method (Pinho et al. 2012) and modified Wizard genomic DNA method. Among this modified Wizard genomic DNA extraction method was found to be ideal and 28S, ITS regions were amplified using universal primers and the successful amplicons were sequenced. The sequenced data were analyzed and compared to distinguish different species of Meliola infecting plants.*

Key words*: Phylloplane fungi, obligate biotrophs, meliolaceae, CTAB,DNA, ITS*

Introduction

Foliicolous fungi are ectophytic obligate biotrophs infecting wide range of flowering plants and produce black colonies on the leaf surface. Black mildews are the abundantly available type of foliicolous fungi. These are one of the important pathogens among this group. These fungi infect most of the plants distributed in the tropics and sub-tropic regions. The black mildew fungi includes the genus *Meliola* in having hyphal setae on the mycelium and lacking vermiform appendages and setae on perithecial surface (Hosagoudar and Kapoor 1985). *Meliola* is the largest genus of the family *Meliolaceae*. As these fungi are strictly obligate biotrophs and must interact with living plant cells for growth and reproduction, they are usually host specific or have a narrow host range. The molecular phylogenetic analysis would allow the taxonomic validation and diversity analysis of black mildew species and might reveal discriminatory differences previously overlooked.

Materials and Methods

Sample Collection

Representative samples are the *Vallaris solanaceae, Millettia ovelifolia, Gmnelina asiatica, Jasminum nitidum,* etc. were collected from JNTBGRI site, Palode, Trivandrum. The specimens were air-dried and preserved in standard size herbarium packets. Fungal herbariums were deposited on Herbarium TBGT and HCIO, New Delhi.

Nail Polish technique

Infected plant parts collected along with its twig, preferably with flowers and fruits, to facilitate identify of the plant. Infection pattern, date of collection, altitude, type of forest, additional information regarding host plant, etc. are recorded in the field. Collections pressed in between the blotters, changed to fresh blotters everyday so as to ensure their dryness. In the laboratory, nail polish technique (Hosagoudar and Kapoor 1985) used for ectophytic fungi to study them insitu, while, sections were made for innate fungi.

Nail polish technique was used for the preparation of permanent slides to study the structural and morphological characters of ectophytic black mildew fungi. A drop of high quality natural coloured or well transparent nail polish was applied to the selected colonies with the help of a fine brush without disturbing the colonies. Colonies with hyperparasites (wooly nature) were avoided. As the nail polish dries (in 2-5 minute), a thin colourless "film" or "flip" is formed with the colonies firmly embedded in it. For soft host parts, flip was lifted up with a slight pressure on the upper side of the leaves or just below the colonies. In case of hard host parts, the flip was eased-off with the help of a razor or scalpel. A drop of DPX was smeared on clear slide and the flip was spread properly on it. Care was taken to avoid air bubbles while mounting. One or two more drops of DPX were again added on the flip and clean cover glass was placed over it. A gentle pressure over the cover glass oozes out the excess DPX. These slides were labeled and placed in the dust free chamber for one to two days for drying. The excess DPX on the slide was removed after drying. The slides were observed under LEICA DM 2000 compound microscope for further details and the line drawings were made by using Camera Lucida of Mirror type.

DNA extraction:

To obtain a representative fungal DNA, the plants *Vallaris solanaceae, Millettia ovelifolia, Gmnelina asiatica, Jasminum nitidum,* etc. were collected in a microcentrifuge tube and stored at -20^{0}C for later use.

Fungal samples were frozen by using liquid nitrogen and grinded in washing buffer. The crushing continued after adding lysis buffer and incubated at 60^{0}C in water bath

for 5 minutes. The solution was cooled at -20^{0}C for 5 min. Then the samples were vortexed in equal amount of chloroform:isoamylalcohol for 20 seconds, and centrifuged for 10 min at 12000 rpm. Equal amount of choloroform were added and centrifuged 12000rpm for 10 min. DNA was precipitated by using 100% ethanol -20^{0}C for 30 min. The DNA was pelleted at 14000 rpm for 10 min at 4^{0}C. The pellet was washed with 70% ethanol at 10000rpm for 10 min at 4^{0}C . The pellet was resuspended with 50µl TE buffer.

The samples were treated with RNase solution at 370C for 1 hour. The sample was washed with phenol:chloroform:isoamylalcohol and precipitated using alcohol and sodium acetate. The pellet was resuspended into 50µl TE buffer.

PCR amplification and DNA sequencing:

For each 25 µl PCR reaction we used 12.5 µl of Master mix, 1 µl each of 10 µM forward and reverse primers, 1 µl dimethyl sulfoxide 5 µl 100× (10 mg/mL) Bovine Serum Albumin (BSA), 2 µl genomic DNA, and nuclease-free water to bring the total volume to 25 µl. The primers LR0R (5′-acccgctgaacttaagc-3′) and LR5 (5′-tcctgagggaaacttcg- 3′) and ITS1 (5′-tccgtaggtgaacctgcgg–3′) and ITS4 (5′-tcctccgcttattgatatgc- 3′) were used to amplify the partial 28S rDNA and ITS, respectively (Vilgalys and Hester 1990, White et al. 1990). Amplifications began with an initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 45s, extension at 72°C for 2 min and a final extension of 7 min at 72°C. PCR products were analyzed on 2% agarose electrophoresis gels in a 1x TAE buffer and visualized under UV light to check for amplification size and purity. PCR products were purified and sequenced by Scigenom Labs Pvt.Ltd.,Cochin.

Phylogenetic analysis

Sequence result were blasted against nucleotide database and the sequences with 94% similarity were downloaded in FASTA format and aligned using the multiple alignment was carried out using CLUSTALW. The CLUSTALW result used to construct the phylogenetic tree using mega4.

Results and Discussion

Samples Collected

Infected leaf samples

Vallaris solanaceae* leaves are infected with *Meliola vallaridis

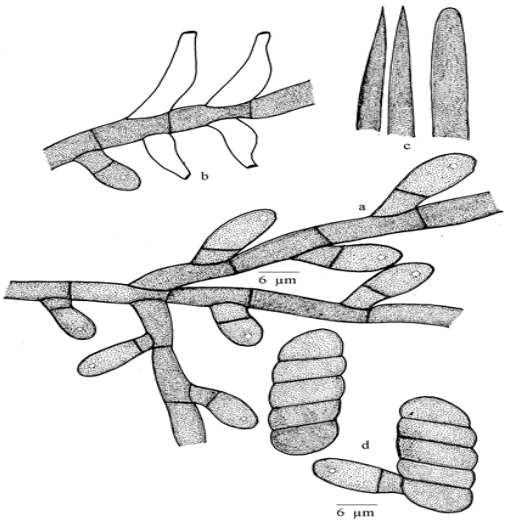
Millettia ovelifolia* leaves are infected with *Meliola pequensis

Gmnelina asiatica* leaves are infected with *Meliola clerodendricola

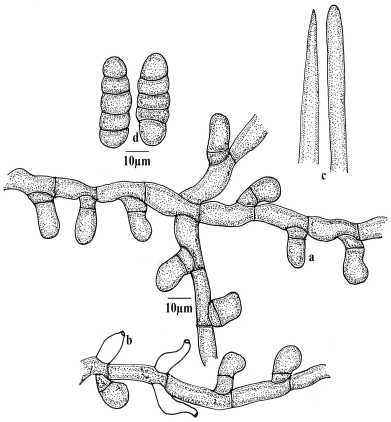
Jasminum nitidum* leaves are infected with *Meliola jasmini

The leaves and twigs of host plants of *Vallaris solanaceae, Millettia ovelifolia, Gmnelina asiatica, Jasminum nitidum,* etc. infected with black mildews were collected from

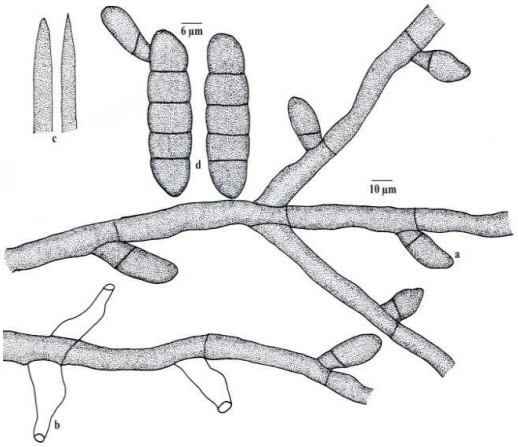
JNTBGRI campus during winter (2013–2014). And their line drawings and descriptions are given.



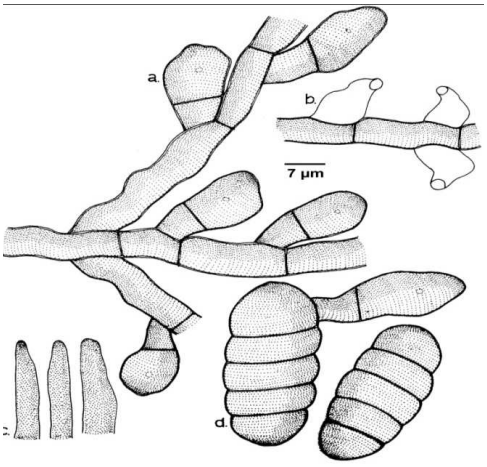
Meliola vallaridis



Meliola pequensis



Meliola clerodendricola



Meliola jasmini

Colonies amphigenous, mostly epiphyllous, caulicolous, ramicolous, dense, crustose to velvety, up to 3 mm in diameter, confluent. Hyphae straight, substraight to flexuous, branching mostly opposite at acute to wide angles, loosely to closely reticulate, often form solid mycelial mat, cells 12-18 x 4-7 µm. Appressoria alternate, less than 1% opposite, antrorse to subantrorse, 16-23 µm long; stalk cells cylindrical to cuneate, 4-8 µm long; head cells ovate, oblong to cylindrical, often narrowed towards the apex, entire, 11-17 x 6-10 µm. Phialides numerous, mixed with appressoria, alternate to opposite, ampulliform, 12-22 x 4-7 µm. Mycelial setae numerous, scattered to grouped around perithecia, simple, straight, acute at the tip, up to 452 µm long. Perithecia closely scattered, verrucose, up to 168 µm in diam.; ascospores oblong to cylindrical, 4-septate, deeply constricted at the septa, 30-37 x 11-16 µm. *Meliola vallaridis* Hosag., Sabeena, Archana & Jacob, J. Scient. Trans. Environ. Techn. 1: 69, 2007; Hosag., J. Threatened Taxa 5(6): 4054, 2013.

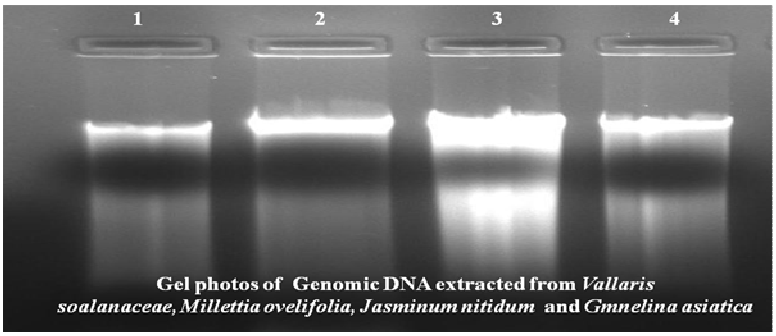
Materials examined: On leaves of *Vallaris solanacea* (Roth) Kuntze [*V. heynei* Spreng.] (Apocynaceae), JNTBGRI campus, Palode, February 8, 2007, A. Sabeena & al HCIO 48049, TBGT 2832; May 13, 2010, A. Sabeena HCIO 50582, TBGT 4499; December 30, 2010, TBGT 5618. *Meliola pequensis* Hosag., Abraham & Crane, Mycotaxon 69: 394, 1998; Hosag.,Meliolales of India. Vol. 2: 301, 2008; Hosag. & Agarwal, Taxonomic studies of Meliolales. Identification Manual, p. 210, 2008. Colonies epiphyllous, subdense to dense, surrounded by yellow haloes up to 4 mm in diameter, rarely confluent. Hyphae straight, substraight, flexuous to slightly crooked, branching alternate, opposite to irregular at acute to wide angles, loosely to closely reticulate, cells 12-20 x 6-8 µm. Appressoria alternate, about 5% opposite, antrorse, subantrorse to recurved, straight to curved, 12-20 µm long; stalk cells cylindrical to cuneate, 3-7 µm long; head cells ovate, oblong, globose, straight to curved, rounded to truncate at the apex, 8-14 x 7-10 µm. Phialides few, mixed with appressoria, alternate to opposite, ampulliform, 14-20 x 7-10 µm. Mycelial setae, scattered, simple, straight, acute at the tip, up to 267 µm long. Perithecia scattered, up to 148 µm in diameter; ascospores oblong to cylindrical, 4-septate, slightly constricted at the septa, 29-32 x 10-13 µm.

Materials examined: On leaves of *Millettia pequensis* Ali (Fabaceae), JNTBGRI Campus, Palode, November 11, 2005, Sabeena.A HCIO 47002, TBGT 2219. *Meliola clerodendricola* Henn. var. *micromera* (Sydow & Sydow) Hansf., Sydowia Beih. 2: 694, 1961; Hosag. & Goos, Mycotaxon 37: 227, 1990; Hosag., Meliolales of India, p. 170, 1996. *Meliola micromera* Sydow, Ann. Mycol. 12: 552, 1914. Colonies epiphyllous, subdense, velvety, up to 2 mm in diameter, confluent. Hyphae straight to sinous, branching opposite at acute angles, loosely to closely reticulate, cells 18-34 x 6-8 µm . Appressoria alternate, antrorse, rarely spreading, mostly straight, 16-20 µm long; stalk cells cuneate, 5-8 µm long; head cells ovate, globose, entire, 11-14 x 10-12µm. Phialides numerous, mixed with appressoria, opposite to alternate, ampulliform, 12-20 x 6-10 µm. Mycelial setae few, grouped around perithecia, straight, simple, obtuse at the tip, up to 190 µm long.

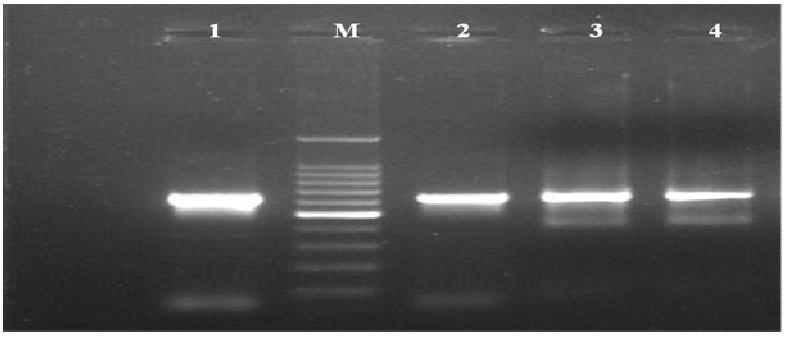
Perithecia scattered, up to 189 µm in diam.; ascospores oblong, 4-septate, constricted at the septa, 30-32 x 10-14 µm.

Materials examined: On leaves of *Gmelina asiatica* L. (Verbenaceae), JNTBGRI campus, Palode, November 2, 2007, A. Sabeena HCIO 50860, TBGT 4777; JNTBGRI campus, Palode, September 5, 2008, A. Sabeena HCIO 50958, TBGT 4875; Palode, October 5, 2009, A. Sabeena HCIO 50685, TBGT 4602; July 9, 2010, A. Sabeena HCIO 50862, TBGT 4779; January 4, 2012, A. Sabeena TBGT 5924. *Meliola jasmini* Hansf. & Stev., J. Linn. Soc. London 5: 273, 1937; Hansf., Sydowia Beih. 2: 235, 1961; Hosag., Indian J. Bot. 11: 185, 1988; Hosag. & Raghu, New Botanist 20: 70, 1993; Hosag., Meliolales of India, p. 226, 1996. Colonies amphigenous, mostly epiphyllous, dense, velvety, up to 2 mm in diameter, confluent. Hyphae straight to substraight, branching opposite at acute to wide angles, loosely to closely reticulate, cells 18.5-25 x 6-8 µm. Appressoria alternate, straight, antrorse, 15.5-22 µm long; stalk cells cuneate, 4.5-6 µm long; head cells ovate, entire, 12.5-15.5 x 9-12.5 µm. Phialides borne on a separate mycelial branch, opposite to alternate, conoid to ampulliform, 31-37 x 9-15.5 µm. Mycelial setae fairly numerous, scattered, straight, simple, acute to obtuse, up to 500 µm long. Perithecia scattered, verrucose, up to 124 µm in diam.; ascospores obovoidal, 4-septate, 31-34 x 12-18 µm.

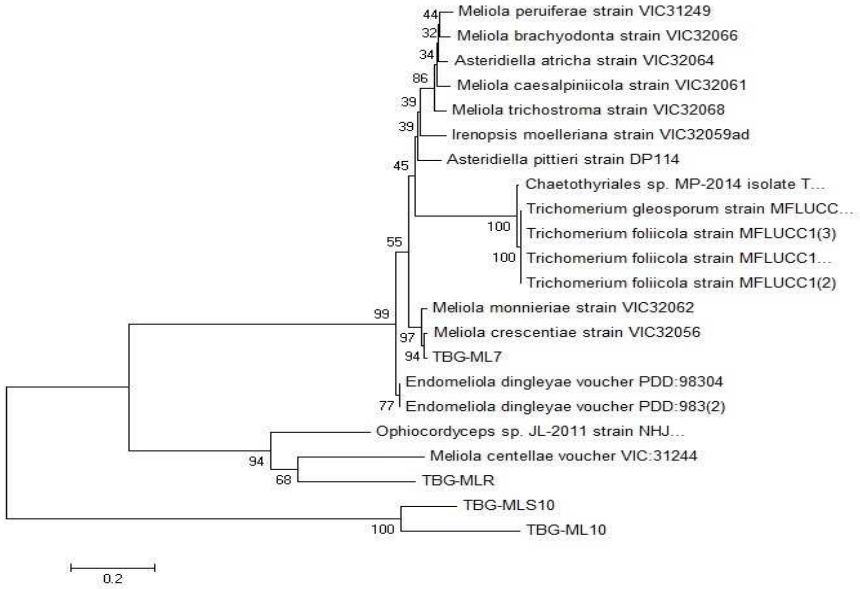
Materials examined: On leaves of *J. nitidum* Skan (Oleaceae), HCIO 44479, TBGT 769; December 30, 2010, A. Sabeena TBGT 5610.



Genomic DNA were extracted from the plants of *Vallaris solanaceae, Millettia ovelifloia, Gmnelina asiatica and and Jasminum nitidum*,etc.



PCR photos of samples of *Vallaris solanaceae*, *Millettia ovelifolia*, *Jasminum nitidum* and *Gmnelina asiatica*



Evolutionary Relationships

The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Phylogenetic analyses were conducted in MEGA4.

In *Gmnelina asiatica*, *Jasminum nitidum, Vallaris solanaceae* and *Millettia ovelifolia* the Meliolaceous fungus reported were *Meliola clerodendricola, Meliola jasmini, Meliola*

vallaridis and Meliola milletiae respectively. But the sequence analysis revealed the following results.

In *Gmnelina asiatica* the infected meliolaceous fungus showed 98% similarity with *meliola crescentiae* and 96% similarity with *meliola monnieriae*. In *Jasminum nitidum* the infected meliolaceous fungus showed 98% similarity with *Trichomerium folicola*. In *Vallaris solanaceae* the infected meliolaceous fungus showed 99% similarity with endomeliola dingleyae and 96% similarity with *meliola centellae.* In *Millettia ovelifolia* the infected meliolaceous fungus showed 95% similarity with *meliola peruiferae* and 94% similarity with *meliola brachyodonta.*

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A study on the pteridophyte flora of man-made habitats of Thalssery

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ABSTRACT: *Pteridophytes are the first vascular plants ever occurred on land in the history and evolution of plant life. They are highly adapted to survive on longitudinally placed substrata and habitats, though most of them are terrestrial. With the progressive evolution of the humans towards highly modernized and sophisticated homes and apartments, the natural habitats of the pteridophytes have faced severe levels of destruction and thereby forcing them towards habitat loss. The newly constructed buildings, compound walls and cemented surfaces give much challenge for the survival of the pteridophytes. The present study is carried out to enumerate the pteridophyte flora of selected man-made habitats of Thalassery. Some of the ferns have shown high resilience from the destructed natural habitats to the newly made artificial or man-made habitats, while some others have disappeared.*

Key words: *Pteridophytes, Man-made habitat, Buildings, Compound walls, Habitat destruction, Adaptability.*

Introduction

The evolution of human beings inevitably reached the advancement of technology leading to adverse impact on the natural habitats of plants. The destruction of natural habitats is the primary reason for the loss of biodiversity (Ehlrich 1988). Rocks were crushed and buildings were made. Conversion of natural habitats to artificial ones became a challenge for the plants. The evolution of plants in man-made habitats was detailed in the IOPB 7h International Symposium in 1998.

Pteridophytes are vascular cryptogams, the first plants on the earth with established tissues for conducting water and solutes. They are of two types, the ferns and the lycophytes, according to the recent systems of classification (Smith et al. 2006). India has a rich vegetation of pteridophytes with more than 1200 species (Dixit 1984; Chandra 2000). We felt interesting to enumerate the Pteridophytes occurring on the man-made habitats after observing several ferns on cemented compound walls and buildings. Thalassery Taluk was conducted within the boundary of Thalassery Taluk. Thalassery is a coastal town, one of the early settlements of the British East India Company, located at Kannur District, Kerala, with paddy fields and estuaries as common habitats. Thalassery has undergone drastic phases of development after year 2000 with the emergence of several shopping malls, speciality hospitals and houses. Most of the houses have compound walls made of red-stone or brick, cemented or non-cemented. The cemented compound walls are nutrient-deficient for plant growth. The present study aims to enlist pteridophytes occurring on the cemented compound walls within the geographical boundary of Thalassery Taluk.

Materials and Methods

Field visits were conducted at various places at Thalassery during June 2014 to October 2015. Photographs were taken using Nikon Coolpix camera and specimens occurring on cemented substrata were collected for laboratory analysis. Microscopic studies were carried out using Magnus Compound Light Microscope at Government College Kasaragod and spores were analysed using Leica microscope at SVNSS College, Wadakkanchery. Herbaria of collected specimens were deposited at the Department of Botany, Government College Kasaragod. Plants were identified with available literatures and by experts in the field. A comparison of species occurring in natural and man-made habitats was also made. The number of plants was also counted per meter square of area.

Results

The common Pteridophytes occurring in the natural habitats of Thalassery are *Adiantum philippense, Selaginella delicatula, Pteris vittata, Pteris confusa, Drynaria quercifolia, Acrostichum aureum, Lygodium flexuosum*, *Athyrium hoheneckarianum, Cheilanthes tenuifolia, Christella dentata* and *Pityrogramma calomelanos.* The pteridophytes found on man-made cemented surfaces are *Adiantum philippense, Pteris vittata, Pteris confusa, Selaginella delicatula, Drynaria quercifolia, Pityrogramma calomelanos, Christella dentata*, and *Acrostichum aureum* (Table 1). A comparison of relative number of plants per area and adaptability of each plant to man-made habitat is represented in Table 2.

Table 1: A comparison of pteridophytes on man-made and natural habitats of Thalassery

Sl No.	Name of the plant	Family	Natural habitat	Man-made habitat
1	*Adiantum philippense*	Pteridaceae	+	+
2	*Selaginella delicatula*	Selaginellaceae	+	+
3	*Pteris vittata,*	Pteridaceae	+	+
4	*Pteris confusa*	Pteridaceae	+	+
5	*Drynaria quercifolia*	Polypodiaceae	+	+
6	*Acrostichum aureum*	Pteridaceae	+	+
7	*Lygodium flexuosum*	Lygodiaceae	+	-
8	*Athyrium hoheneckarianum*	Athyriaceae	+	-
9	*Cheilanthes tenuifolia*	Pteridaceae	+	-
10	*Christella dentata*	Athyriaceae	+	+
11	*Pityrogramma calomelanos.*	Pteridaceae	+	+

+ shows presence, - shows absence.

Table 2: Comparison of number of pteridophyte species/area in man-made and natural habitats.

Sl. No.	Name of species	Number of plants/m²		Adaptability
		Natural habitat	Man-made Habitat	
1	*Adiantum philippense*	20	12	60%
2	*Selaginella delicatula*	10	3	30%
3	*Pteris vittata,*	1	1	100%
4	*Pteris confuse*	2	1	50%
5	*Drynaria quercifolia*	5	2	40%
6	*Acrostichum aureum*	1	1	100% (?)
7	*Lygodium flexuosum*	1	0	0
8	*Athyrium hoheneckarianum*	25	0	0
9	*Cheilanthes tenuifolia*	3	0	0
10	*Christella dentate*	2	1	50%
11	*Pityrogramma calomelanos.*	1	1	100%

Discussion

As human populations grow at ever-accelerating rates, an increasing proportion of the land which supports ecological communities is converted for agriculture, urban development or other human activities (King 1998). The destruction of one habitat actually creates a new habitat though it eradicates the previously existed vegetation. These man-made habitats offer very few soluble nutrients for plant growth. The solid surfaces are incapable of storing water for the luxuriant growth of plants. The presence of plants on these difficult substrata requires special remark that plants possess inherent ability to survive and adapt during the threats caused by anthropogenic reasons. Pteridophytes are one of the earliest land plants which survived and evolved during the early times of plant evolution on land. The pteridophytes showing good adaptability to man-made habitats are *Pteris vittata, Adiantum philippense*, and *Pityrogramma calomelanos*. The occurrence of *Acrostichum aureum* was found only at a single location; hence the result needs further confirmation though the large size of *Acrostichum aureum* theoretically gives the plant 100% adaptability in man-made habitats. *Selaginella delicatula* and *Drynaria quercifolia* also has commendable adaptability. The observations show that the pteridophytes can survive on cemented man-made habitats.

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The species diversity of the genus *Rotala* L. (Lythraceae) in Kerala, India

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***ABSTRACT**: The genus Rotala L. is represented by more than 55 species distributed in tropical and subtropical regions of the world. It has greatest diversity in tropical Asia. South Asia is considered as the centre of origin of the genus Rotala. Twenty nine species were reported so far from India, many of which are endemic to South India. Among them, eight species are recently described from Peninsular India including six species from Kerala (Prasad et al. 2012; Sunil et al. 2013; Prasad & Raveendran 2013a; Gaikwad et al., 2013; Yadav et al. 2013; Prasad & Raveendran 2013b; Anto et al. 2014; Ratheesh et al. 2014). As a part of biosystematic studies on closely similar genera Ammannia L., Rotala L. and Nesaea Kunth, a field exploration, collection and documentation of species diversity of three genera is progressing at this laboratory. The present paper is focused on morphological diversity and distribution pattern of the genus Rotala in Kerala.*

***Key words:** Rotala, Lythraceae, Species diversity, Kerala, India*

Introduction

Kerala has the largest proportion of land area under wetlands among all the states of India. By virtue of its unique location, Kerala provides a wide variety of aquatic habitats, harboring unique types of vegetation of their own. Major wetland types in the Kerala are rivers, lagoons and reservoirs, beaches, ponds, waterlogged area in lateritic hills. These are treated as sites of exceptional biodiversity of aquatic or amphibious angiosperms that are characterized by several endemic species. Manilal and Sivarajan (1975) had initiated studies concentrating on the aquatic angiosperms. Subsequently, Joseph (1991) studied taxonomy and diversity of the aquatic angiosperms of Malabar. Cook (1996) listed 352 aquatic and wetland plants from Kerala, of which I5 species were endemic to Kerala. The present study highlighted the taxonomy and species diversity of aquatic or amphibious species in the genus *Rotala* (Lythraceae) that are distributed in the wetlands of Kerala.

The genus *Rotala* L. belongs to the loosesstrife family Lythraceae which contains approximately 600 species placed in 31 genera, worldwide. This genus comprises more than 55 species of aquatic and amphibious plants within tropical and subtropical region of the world. Generally these show greater generic adaptability and vegetative plasticity than any other herbaceous genera of this family. In Africa and Asia, these are represented by equal number of species, while species of Southern Asia display greater morphological diversity than the African taxa (Graham 2011).

The genus *Rotala* was initially closely allied with genus *Ammannia* in having remarkable degree of similarity in habit. Clarke (1879) in his account of Indian species of this group for Hooker's *Flora of British India* considered *Ammania* as a larger, more inclusive taxon including *Rotala* as a subgenus, in it. Currently *Rotala* is treated as distinct genus based mainly on the dehiscence of fruits and structure of pericarp. Cook (1979) in his monograph of this genus recognized 44 species whole over the world which includes 20 Indian species. He considered, southern Asia to be the probable Centre of Origin of the genus. Joseph and Sivarajan (1989) revised the genus for Peninsular India and reported 16 species. Hitherto, 29 species are reported so far from India which includes eight new species, reported from Peninsular India (Prasad et al. 2012; Sunil et al. 2013; Prasad & Raveendran 2013a, Gaikwad et al. 2013; Yadav et al. 2013; Prasad & Raveendran 2013b; Anto et al. 2014; Ratheesh et al. 2014). Among them, six species are known only from Kerala.

Materials and Methods

The present investigation is the outcome of several field trips conducted during the year 2012 – 2014, covering all the seasons of the year in maximum area of wetlands of Kerala. All the species found were recorded in the field note book with their necessary information. The freshly collected specimens were dissected and examined in laboratory during flowering period. Measurements were taken using a stereo microscope (Leica M80). Specimens were identified and authenticated with the help of local floras and monographs**.** Type specimens were consulted to confirm the identity of each taxon. Herbarium specimens were prepared by standard methods.

Results and Discussion

Diagnostic morphological features of the genus *Rotala*

Aquatic, amphibious or terrestrial, annual or perennial, glabrous herbs. Stems simple or branched, creeping, ascending, erect or floating. Leaves decussate or whorled, sessile or rarely shortly petiolate, simple, entire. Bract is leaf like or scale like, Bracteoles 2, Flowers actinomorphic, Monomorphic or dimorphic, sometimes cleistogamous, solitary in the axils of bracts, borne along the main axis or on lateral or terminal racemes. Calyx tube campanulate or tubular or urceolate, free from but often enclosing the ovary, hypanthial; calyx lobes 3-6, valvate, persistant, calyx appendages occasionally present between calyx lobes; nectar glands may be often present at the base of the calyx tube. Petals large 0-6, minute or large and showy, inserted in between the calyx lobes, entire or erose. Stamens 1- 6, episepalous, less than or equal to the number of calyx lobes, located on the inner surface of the calyx tube on the lower half or occasionally at the base of the calyx tube. Ovary superior, 2-4 locules; placentation axile, becoming free central at maturity; style simple, stigma capitate; fruit septicidal

dehiscent capsule opening by 2- 4 valves; valves with microscopic, horizontal striations; seeds numerous or few, semi-ovoid to ellipsoidal; germination epigeal; cotyledons simple.

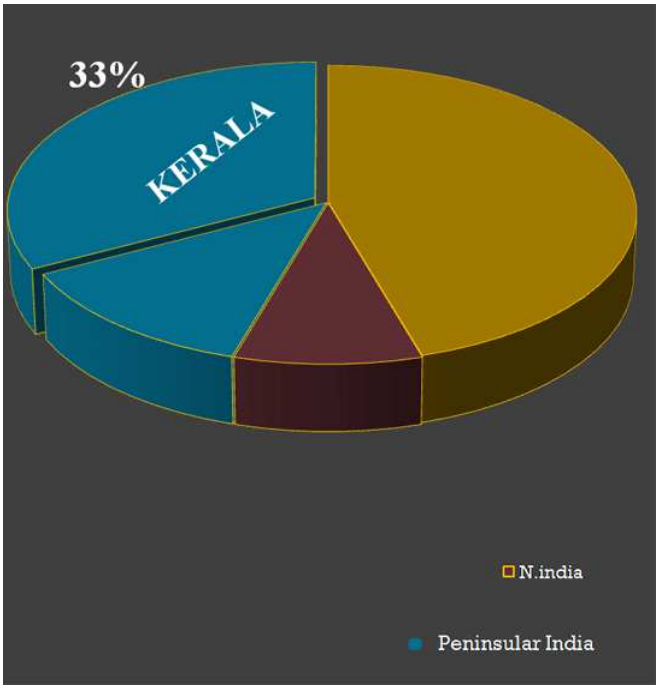
The genus *Rotala* is widely distributed in almost all districts of Kerala and up to date, eighteen species are reported that includes recently described six species from 2012 onwards. Most of the new species are described from Northern region of Kerala. The present documentation reveals that, of the total world distribution percentage of this genus, 33% is represented from Kerala (Fig. 1).

R. cookii and *R. vasudevanii* are obligate aquatic species while others are amphibious in nature. Common apetalous species are *R. mexicana* and *R. occultiflora*, which are also considered as smaller in size in comparison to other species. *R. malampuzhensis* is very common and is widely distributed to all type of habitat within entire Kerala especially in the shallow pools in depressions on lateritic rocks. *R. cooki, R. vasudevanii*, *R. malampuzhensis* and *R. malabarica* are characterized with the presence of nectar glands. In *R. rotundifolia* and *R. macrandra* flowers are usually in spikes or terminal racemes while the former is usually seen in higher altitude and the latter is restricted to wet lowlands. *R. roseae* and *R. densiflora* are rather similar in habit but can be differentiate by their relative length of bracteoles and calyx appendages. *R. indica* is very common weedy species and probably, the most variable of all species (Joseph and Sivarajan 1989). *R. ritchiei* is an aquatic or amphibious plant, which is now considered as a rather vulnerable species. *R. cookii*, *R. vasudevanii* and *R. malabarica* are endemic to Kerala, however these are included in the IUCN red list as endangered. From the year 2012 onwards, six more species were added to this genus by various authors from Peninsular India (Prasad et al. 2012; Sunil et al. 2013; Prasad & Raveendran 2013a; Prasad & Raveendran 2013b; Anto et al. 2014; Ratheesh et al. 2014), which are listed in Table1.

Table 1: Six species added to the genus *Rotala* by various authors from Peninsular India

Sl No	Name of species	Type locality	References
1.	*Rotala tulunadensis*	Permude, Kasargode (DT)	Prasad et al. 2012
2.	*Rotala khaleeliana*	Kanayikanam, Kannur (DT)	Sunil et al. 2013
3.	*Rotala meenkulamensis*	Meenkualam, Kannur (DT)	Prasad & Ravi 2013
4.	*Rotala kasaragodensis*	Mugu, Kasargode (DT)	Prasad & Raveendran 2014
5.	*Rotala cheruchakkiensis*	Cheruchakki Dam, Thrissur (DT)	Anto et al. 2014
6.	*Rotala dhaneshiana*	Muthanga, Wayanad (DT)	Sunil et al. 2014

Figure 1: A map showing distribution of *Rotala* in Kerala.



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A study of the physico chemical parameters and aquatic macrophytes of Aruvikkara reservoir, Thiruvananthapuram District, Kerala

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***ABSRACT**: The present attempt is to analyze the physico chemical parameters and to study the diversity of aquatic macrophytes in the Aruvikkara reservoir. Aruvikkara reservoir is located on the banks of the Karamana River 15 km away from Thiruvananthapuram city. The reservoir is one of the main sources of drinking water in the capital city. The analyzed physico chemical parameters included Water Temperature, pH, Total Hardness, Alkalinity, Nitrate, Silicate, Phosphate, Dissolved Oxygen (DO), Biological oxygen demand (BOD) and Chemical Oxygen Demand (COD). Aquatic macrophytes including aquatic angiosperms, pteridophytes, bryophytes, macro algae that are found growing in or very near surface waters. They provide food, habit and shelter for fish, aquatic insects, water fowl and other aquatic organisms. The major aquatic plants founded were Nymphoides indica (L.) Kuntze., Nymphaea micrantha Guill. & Perr., Cabomba caroliniana Gray., Cyperus species, Eriocaulon species, Monochoria vaginalis (Burm. f.) Presl., Marsilea quadrifolia L., Colocasia esculenta (L.) Schott., Salvinia molesta D.Mitch., Ceratophyllum demersum L. Three out of the eight stations were fully flourished with aquatic plants; three stations with sparse vegetation and the remaining two stations without aquatic plants. The results indicated that the water present in the reservoir was non-polluted and can be used for domestic and irrigation purposes but the stations in the reservoir were flourished with aquatic macrophytes which can lead to cultural eutrophication.*

***Key words**: Aruvikkara reservoir, Physico chemical parameters, Aquatic macrophytes*

Introduction

Water is one of the most essential item needed by man, plants and other living beings for their survival. It maintains the ecological balance between various groups of living organisms and their environment. India has a great potential of water resources, both fresh and marine and a great variation in environmental conditions provide a wide range of habitats and contrasts with its varied physical and meteorological features. Ponds, pools, reservoirs, etc. are water locked ecosystems i.e. bodies of standing water with plenty of submerged floating or rooted plants along with phytoplankton. Aquatic plants can only grow in water or in soil that is permanently saturated with water. They are therefore a common component of wetlands and rivers (Keddy 2010).

The proposed study area is of great significance as the water in the dam is the source of drinking water to the whole metropolis of Thiruvananthapuram. As the water is used by quite a number of people, the assessment of water quality parameters is highly significant. The present attempt is to analyze the physico chemical parameters and to study the diversity of aquatic macrophytes in the Aruvikkara reservoir.

Materials and Methods

Aruvikkara located in Kerala, India with coordinates 8.5677800°N 77.018890°E is a village in Thiruvananthapuram district in the state of Kerala, India. It is on the banks of the Karamana river, 15 km from Thiruvananthapuram, the capital city of Kerala, South India. Aruvikkara dam is one of the main sources of water for distribution in Trivandrum city.

Over the years the quality of water in the reservoir has been influenced by the physicochemical factors of the environment. The present study is to assess the diversity of aquatic plants present in the Aruvikkara reservoir. Water quality exhibits a close relation with the micro and macro flora present in the reservoir because the occurrence, abundance and chemistry of related flora get changed with a change in the physicochemical factors. In the present study eight different stations were selected at different places of the Aruvikkara dam for sample collection and analysis. The stations selected for the study are Koovakudy, Vembanni, Mundela, Kalian kuzhi, Kanchikkavila, Mulilavinmoodu, Mailamoodu and Temple side.

The Water Samples from Aruvikkara were collected from eight different stations between 8.00 AM to 12.00 PM in sterilized Polythene bottles regularly for every month. The Water samples were immediately brought in to Laboratory for the estimation of various Physico chemical parameters, Temperature and pH were recorded at the time of sample collection by using Thermometer and portable digital pH meter. While other parameters such as DO, Hardness, Alkalinity, Phosphate, Silicate and Nitrate were estimated in the Laboratory by using Standard methods (Trivedy and Goel 1986; APHA 1992).

The aquatic macrophytes from Aruvikkara reservoir were collected from eight different stations in Polythene bags regularly from May 2014 to May 2015. The plants were collected and identified using different literatures (Gamble 1967; Sasidharan 2011).

Results and Discussion

Physico chemical parameters

Temperature of water depends upon water depth besides solar radiation, climate and topography. It is the most important factor as it has profound direct or indirect

influence on physico chemical, biological, metabolic and physiological behavior of aquatic ecosystem (Welch 1982). The maximum seasonal average of temperature was 31° C at the station 8 during the pre monsoon and the minimum was 25.5° C at the station 1 during the post monsoon.

Water pH is one of the very significant chemical characteristic of all waters, which explains certain significant biotic and abiotic ecological characteristics of aquatic systems in general. Seasonal average values of pH ranged between 6.6 at station 3 in the pre monsoon and 7.3 at station 3 in the post monsoon. The pH was within the limits of standard values (WHO 1985- 7 to 8.5). Alkalinity of water is its capacity to neutralize acid and is characterized by the presence of hydroxyl ions capable of combining with hydrogen ions in solution. The maximum seasonal average of alkalinity was 20.79 mg/l at the station 6 in the pre monsoon and the minimum alkalinity was 11.34 mg/l at the station 3 in the post monsoon. The permissible value of Alkalinity ranges a maximum of 600mg/l.

Hardness to water is imparted by alkaline earth metal cations mainly calcium and magnesium present in it. The maximum total hardness was 24 mg/l at the station 1 during the pre monsoon and the minimum total hardness was 7 mg/l at station 4 in the monsoon. The hardness of water observed at all stations was within the limits – 100 mg/l and 250 mg/l respectively. Sawyer (1960) classified water on the basis of hardness into three categories i.e., soft (0-75 mg/l), moderately hard (75-150 mg/l) and hard (151-300 mg/l). According to this classification, Aruvikkara reservoir falls in the category of soft water body with hardness ranging from 7 mg/l to 24 mg/l.

The nitrogen in water occurs as bound forms like nitrate, nitrite, ammonia and organic nitrogen viz. urea, amino acids, etc. The seasonal data on nitrate varied between 1.79mg/l at the station 6 in the pre monsoon and 0.43mg/l at the station 2 in the post monsoon season. The amount of nitrate found in the water was less than the accepted drinking water standards. Silica (SiO_2) usually occurs in moderate abundance in fresh water. The silicate concentration in the water varied between 4.2 mg/l at the station 1 in the pre monsoon and 1.3 mg/l at the station 4 in the monsoon. The concentrations of silicate detected in the reservoir were within the limits of worldwide average of silicate for rivers (13.1 mg/l). Phosphorous is a nutrient which has a great role in the productivity of the water body and can influence the growth of aquatic biota. The phosphate concentration in the water varied between 0.079 mg/l at the station 1 in the post monsoon and 0.028 mg/l at the station 1 in the monsoon. The amount of phosphate found in the stations was mostly lower than the prescribed levels.

Dissolved oxygen provides valuable information about the biological and biochemical reactions going on in waters. The DO concentration varied between 7.5 mg/l at station 5 during monsoon and 6.1 mg/l at the station 1 during the pre monsoon. The values of

dissolved oxygen ranged between 7.7 mg/l to 12.5 mg/l in Mhaswad reservoir at Maharashtra (Lubal et al. 2012). These DO values were above the standard values prescribed by various authorities. Biological Oxygen Demand is the amount of oxygen utilized by microorganisms in consuming the organic matter in waters. Seasonal values of BOD ranged between 3.5 mg/l at the station 1 during the pre monsoon and 1.7 mg/l at the station 1 in the monsoon. The results are in agreement with the results in Ramsagar reservoir which was in the range of 0.93mg/l to 4.68mg/l with low values in monsoon and high values during pre monsoon (Garg et al. 2009). The average values of BOD during all seasons were generally not greater than the international standards – 6 mg/l prescribed by WHO. The chemical oxygen demand is a measure of the oxygen equivalent to the organic matter content of water that is susceptible to oxidation by a strong oxidizing agent (APHA et al. 1995). COD values in Aruvikkara reservoir varied between 12 mg/l at station 3 during monsoon and 60mg/l at station 5 during pre monsoon.

Aquatic macrophytes

By this study, a total of 10 plants including aquatic and wetlands belongs to different families were collected periodically. The collected plant specimens were identified with standard references. The dominant aquatic species include *Nymphoides indica* (L.) Kuntze., *Nymphaea micrantha Guill. & Perr., Cabomba caroliniana* Gray., *Cyperus species, Eriocaulon species, Monochoria vaginalis* (Burm. f.) Presl., *Marsilea quadrifolia* L., *Colocasia esculenta* (L.) Schott., *Salvinia molesta* D. Mitch., *Ceratophyllum demersum* L. were spread throughout the reservoir. Some regions were seen in the reservoir as islands with bushy grasses, plants and other organisms. The aquatic and wetland plants identified from the study area possess potentiality in the accumulation of water damaging compounds like trace metals and plays a keen role in maintaining the water quality status. At the water same time noxious weeds which are restricted flourishes in the water body and leaded to the destruction of balance of the ecosystem and deterioration of the water quality. Some stations were seen in the reservoir as islands with bushy grasses, plants and other aquatic organisms.

Table 1: Physico chemical parameters of Aruvikkara reservoir (Pre Monsoon season)

Parameter	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8
Temperature	28	28	29	29	30	30	31	31
pH	7.1	6.7	6.6	7.1	7.2	6.7	7.2	7.1
Total Alkalinity, (mg/l)	17.9	16.1	17.01	19.8	15.12	20.79	17.01	13.23
Total hardness, (mg/l)	24	14	14	22	12	17	10	12
Nitrate, (mg/l)	0.57	0.59	0.88	0.53	1.21	1.79	0.89	0.87
Phosphate, (mg/l)	0.046	0.042	0.034	0.037	0.037	0.052	0.032	0.017
Silicate, (mg/l)	4.2	3.2	3.6	3.4	2.7	3.5	3.1	2.2
Dissolved oxygen, (mg/l)	6.1	6.5	6.2	7.1	6.9	6.3	5.2	7.2
Biological oxygen demand, (mg/l)	3.5	2.1	2.5	3.1	3.3	2.1	1.9	2.3
Chemical oxygen demand, (mg/l)	50	36	38	34	60	38	34	32

Table 2: Physico chemical parameters of Aruvikkara reservoir (Monsoon season)

Parameter	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8
Temperature	26.5	26.5	27.5	28	28.5	28.5	29	29
pH	7.1	7.2	7.2	6.8	6.9	7.4	7.1	7.4
Total Alkalinity, (mg/l)	15.12	16.1	12.28	12.28	15.12	16.1	13.23	10.39
Total hardness, (mg/l)	10	9	10	7	12	19	10	12
Nitrate, (mg/l)	0.54	0.82	0.55	0.67	0.89	1.11	0.35	0.36
Phosphate, (mg/l)	0.028	0.041	0.051	0.043	0.044	0.029	0.032	0.041
Silicate, (mg/l)	3.3	2.1	1.8	1.3	2.5	2.2	2.9	3.1
Dissolved oxygen, (mg/l)	6.3	7.3	7.3	6.5	7.5	6.9	7.1	7.3
Biological oxygen demand, (mg/l)	1.7	2	2.2	2.5	2.1	0.7	1.7	1.9
Chemical oxygen demand, (mg/l)	14	22	12	20	16	26	22	20

Table 3: Physico chemical parameters of Aruvikkara reservoir (Post Monsoon season)

Parameter	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8
Temperature	25.5	25.5	26.5	26.5	27	28	28.5	29
pH	7.2	6.7	7.3	6.8	6.7	6.8	7.1	6.7
Total Alkalinity, (mg/l)	17.01	14.17	11.34	17.55	15.12	22.68	16.1	15.12
Total hardness, (mg/l)	21	13	11	15	10	17	9	10
Nitrate, (mg/l)	0.61	0.43	0.98	1.1	0.76	1.32	0.45	0.44
Phosphate, (mg/l)	0.079	0.065	0.073	0.066	0.051	0.063	0.042	0.034
Silicate, (mg/l)	3.4	3.6	3.7	3.9	2.2	3.0	2.8	1.8
Dissolved oxygen, (mg/l)	6.8	6.7	6.7	7.4	6.8	7.2	6.7	7.8
Biological oxygen demand, (mg/l)	2.5	1.8	2.5	3.2	2.2	2.9	2.3	2.4
Chemical oxygen demand, (mg/l)	24	28	34	32	18	38	30	20

Table 4: Different aquatic plant species recorded in the Aruvikkara reservoir

Sl. No	Name of plant species	Family
1	*Cabomba caroliniana* Gray.	Cabombaceae
2	*Ceratophyllum demersum* L.	Ceratophyllaceae
3	*Marsilea quadrifolia* L.,	Marsileaceae
4	*Nymphoides indica* (L.) Kuntze	Nymphaeaceae
5	*Nymphaea micrantha Guill. & Perr.*	Nymphaeaceae
6	*Salvinia molesta* D.Mitch.	Salviniaceae
7	*Cyperus species*	Cyperaceae
8	*Eriocaulon species*	Eriocaulaceae
9	*Monochoria vaginalis* (Burm. f.) Presl.	Pontederiaceae
10	*Colocasia esculenta* (L.) Schott	Araceae

Conclusion

The study of physical and chemical characteristics of water present in the reservoir provides an insight into the quality of water. The increasing concentration of various nutrients released from the neighboring agriculture fields as well as the domestic water can cause adverse effects on human health and environment. The results from the present study clearly pointed out that most of the parameters studied were within the permissible limits given by WHO except chemical oxygen demand. This study also revealed the presence of aquatic macrophytes in the form of weeds on the water surface. The trophic status of reservoir warrants a proper conservation and management strategy. In order to have proper management and best possible use of the reservoir, the macrophytes will have to be controlled.

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Granite quarrying leads to biodiversity loss – A case study from two granite quarries in Palghat gap of Western Ghats

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ABSTRACT: *Mining in granite hillocks are posing serious challenges to the survival of many plant species. The case study from two granite quarries of Palghat gap of Western Ghats could identify many rare species of plants which are facing threat of local and global extinction. The study points out the need of proper EIA before granting permission for granite quarrying.*

Key words: *Granite quarries, Biodiversity loss, Environment impact assesment*

Introduction

Mining in granite hillocks has become a routine activity in South India. The number of legal and illegal mining operations are increasing at an alarming rate. Improper mining operations on hilly terrains will lead to unprecedented changes in the local microclimate and adversely affect the socio economic and cultural wellbeing of the villages (Darwish et al. 2011). In addition to the disturbance caused by the dust and noise created by these quarrying operations the loss of hills will results in lowering of the ground water level and results in water scarcity (Boulton et al. 2003). Other than these obvious effects there are much unapparent effects of these destructive activities on the biodiversity. Palakkad Gap Region of Western Ghats is a plain with intermittent hills. These hills are under the tremendous pressure of destruction from the quarry mafia. Most of the hills in this area are already partially or completely lost due to quarrying (Krishnan and George 2009). The impact of such unscientific quarrying on the local and global climate will be unpredictable (Gadgil 2014). Hills are the local biodiversity hubs where a large number plant species are growing in abundance and many of these species are restricted to the hills and not elsewhere. The destruction of hillocks by mining activities will lead to biodiversity loss and may even result in species extinction.

This paper deals with case study of two such quarries of Palakkad gap region of Kerala and the status of plants naturally growing in these hills and the risk of extinction encountered by these plants. The selected granite quarries were of Vengappara, Kollengode, Palakkad (10°35'24.44"N 76°34'55.73"E) and Gangothri Hill, Nemmara, Palakkad (10°34'34.45"N 76°42'46.37"E).

Materials and Methods

The mined areas of the both quarries were analyzed through Google earth professional version. The earth data from the year 2006 has been used to compare the area under destruction. The surface area of the quarries has been quantified using Google Earth Pro measure tool along different time periods. The images of these quarries in different time periods have also been taken. The use of google earth tool in aerial survey and studies are reported earlier (Clarke et al. 2010). The plants growing in these hills were identified using available literature and enlisted. The conservation status, distribution and endemism ifany, were also discussed based on digital database of Kerala Forest Research Institute, Peechi (Sasidharan 2013) and other relevant literatures.

Results

The survey on the plants growing on the two granite hills under study could enumerate 76 species of angiosperms and 4 Species of Pteridophytes (Table 1). Many of them are ephemerals and confined to such habitats.

Table 1: Survey on the plants growing on the two granite hills

Sl.No	Name of plant species	Family	Vengappara Hillock	Gangothri Hillock	RET Category	Endemic status
1	*Abrus precatorius* L.	Fabaceae	N	Y	-	-
2	*Acanthospermum hispidum* DC.	Asteraceae	Y	N	-	-
3	*Aerva lanata* (L.) Juss. ex. Schult.	Amaranthaceae	N	Y	-	-
4	*Allmania nodiflora* (L.) R. Br. ex Wight	Amaranthaceae	Y	N	-	-
5	*Anisochilus carnosus* (L. f.) Wall. ex Benth. in Wall.	Lamiaceae	Y	Y	-	-
6	*Anisomeles indica* (L.) O. Ktze.	Lamiaceae	N	Y	-	-
7	*Apluda mutica* L.	Poaceae	Y	N	-	-
8	*Asparagus racemosus*Willd.	Liliaceae	Y	N	-	-
9	*Boerhavia diffusa* L.	Nyctaginaceae	Y	Y	-	-
10	*Centranthera indica* L. Gamble	Scrophulariaceae	Y	Y	-	-
11	*Chamaecrista kleinii*	Fabaceace	Y	Y	-	-

	(Wight & Arn.) Matthew					
12	*Cleome burmannii* Wight &Arn.	Capparaceae	Y	Y	-	-
13	*Cleome viscosa* L.	Capparaceae	Y	N	-	-
14	*Commelina diffusa* Burm. f.	Commelinaceae	Y	Y	-	-
15	*Croton hirtus* L. Herit.	Euphorbiaceae	N	Y	-	-
16	*Curcuma decipiens* Dalz.	Zingiberaceae	Y	Y	En	Peninsular India
17	*Cyanotis axillaris* (L.) D. Don	Commelinaceae	N	Y	-	-
18	*Cyanotis papilionacea* (Burm. f.) Schult. f.	Commelinaceae	Y	-	-	Peninsular India
19	*Cynodon dactylon* (L.) Pers.	Poaceae	Y	Y	-	-
20	*Dentella repens* (L.) J. R. & G. Forst.	Rubiaceae	N	Y	-	-
21	*Digitaria ciliaris* (Retz.) Koeler	Poaceae	Y	Y	-	-
22	*Dipcadi montanum* (Dalzell) Baker	Hyacinthaceae	Y	N	Rare	-
23	*Dopatrium junceum* (Roxb.) Buch.-Ham. ex Benth.	Scrophulariaceae	Y	N	-	-
24	*Drosera indica* L.	Droseraceae	N	Y	-	-
25	*Eclipta prostrata* (L.) L.	Asteraceae	N	Y	-	-
26	*Eragrostis tenella* (L.) P. Beauv. ex Roem. &Schult.	Poaceae	Y	Y	-	-
27	*Eriocaulon thwaitesii* Koernicke	Eriocaulaceae	Y	Y	-	-
28	*Eriocaulon xeranthemum* Mart. in Wall.	Eriocaulaceae	Y	Y	-	-
29	*Euphorbia hirta* L.	Euphorbiaceae	N	Y	-	-
30	*Evolvulus nummularius* (L.) L.	convolvulaceae	N	Y	-	-
31	*Glinus oppositifolius* (L.) A. DC.	Molluginaceae	Y	Y	-	-
32	*Gmelina asiatica* L.	Verbanaceae	Y	N	-	-
33	*Gomphrena celosioides* Mart.	Amaranthaceae	Y	N	-	-
34	*Gymnema sylvestre* (Retz.) R. Br. ex Schult.	Asclepiadaceae	Y	Y	-	-
35	*Heliotropium indicum* L.	Boraginaceae	Y	Y	-	-
36	*Heliotropium rottleri* Lehm.	Boraginaceae	Y	Y	-	Peninsular India
37	*Hemidesmus indicus* (L.) R. Br.	Periplocaceae	Y	Y	-	-

38	*Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult.	Poaceae	Y	N	-	-
39	*Holostemmaada kodien* Schult.	Asclepiadaceae	N	Y	-	-
40	*Hoppea fastigiata* (Griseb.) Clarke	Gentianaceae	N	Y	-	-
41	*Hybanthusen neaspermus* (L.) F.v. Muell.	Violaceae	N	Y	-	-
42	*Hygrophila schulli* (Buch.-Ham.) M. R. & S. M. Almeida	Acanthaceae	N	Y	-	-
43	*Hyptissu aveolens* (L.) Poit.	Lamiaceae	Y	Y	-	-
44	*Ichnocarpus frutescens* (L.) R. Br	Apocynaceae	Y	Y	-	-
45	*Indigofera uniflora* Buch.-Ham. ex Roxb.	Fabaceae	Y	Y	-	Peninsular India
46	*Kyllinga brevifolia*Rottb.	Cyperaceae	Y	Y	-	-
47	*Leucas aspera* (Willd.) Link	Lamiaceae	Y	Y	-	-
48	*Lindernia crustacea* (L.) F.v. Muell.	Scrophulariaceae	Y	Y	-	-
49	*Lobelia alsinoides* Lam.	Lobeliaceae	Y	N	-	-
50	*Melochia corchorifolia* L.	Strculiaceae	N	Y	-	-
51	*Merremia hederacea* (Burm. f.) Hall. f.	Convolvulaceae	N	Y	-	-
52	*Mimosa pudica*L.	fabaceae	Y	Y	-	-
53	*Mitrasacme indica* Wight	Loganiaceae	N	Y	-	-
54	*Mollugo pentaphylla* L.	Molluginaceae	Y	Y	-	-
55	*Murdannia semiteres* (Dalz.) Sant.	Commelinaceae	Y	N	-	Peninsular India
56	*Oldenlandia corymbosa* L.	Rubiaceae	Y	Y	-	-
56	*Oldenlandia dineshii* Sojan & V. suresh	Rubiaceae	N	Y	Cr. En	Palakkad Gap
57	*Oldenlandia herbacea* (L.) Roxb	Rubiaceae	Y	Y	-	-
58	*Parasopubia delphiniifolia* (L.) H.-P. Hofm. & Eb. Fisch.	Scrophulariaceae	Y	Y	-	-
59	*Phyllanthus amarus* Schum. & Thonn.	Euphorbiaceae	Y	Y	-	-
60	*Polycarpaea corymbosa* (L.) Lam.	Caryophyllaceae	Y	N	-	-
61	*Polygala bolbothrix* Dunn	Polygalaceae	Y	Y	-	SWG
62	*Rhamphicarpa longiflora*	Scrophulariaceae	Y	Y	-	India

	(Arn.) Benth.					
63	*Richardia scabra* L.	Rubiaceae	Y	N	-	-
64	*Scoparia dulcis* L.	Scrophulariaceae	Y	Y	-	-
65	*Sesamum laciniatum* Klein. ex Willd	Pedaliaceae	N	Y	-	Peninsular India
66	*Sesamum prostratum* Retz	Pedaliaceae	Y	Y	-	India
67	*Sida acuta* Burm. f.	Malvaceae	Y	Y	-	-
68	*Sida cordata* (Burm. f.) Borss.	Malvaceae	N	Y	-	-
69	*Sida cordifolia* L.	Malvaceae	Y	N	-	-
70	*Striga angustifolia* (D. Don) Saldanha	Scrophulariaceae	Y	Y	-	-
71	*Strychnos potatorum* L.f.	Loganiaceae	Y	N	-	-
72	*Tephrosia purpurea* (L.) Pers.	Fabaceae	N	Y	-	-
73	*Utricularia graminifolia* Vahl	Lentibulariaceae	N	Y	-	-
74	*Xenostegia tridentata* (L.) Austin & Staples	Convolvulaceae	Y	Y	-	-
75	*Ziziphus mauritiana* Lam.	Rhamnaceae	Y	N	-	-
76	*Zornia diphylla* (L.) Pers.	Fabaceae	Y	N	-	-
77	*Ophioglossum costatum* R. Br.	Ophioglossaceae	Y	N	-	-
78	*Selaginella wightii* Hieron	Selaginellaceae	Y	N	-	-
79	*Parahemionitis cordata* (Roxb. ex Hook. & Grev.) Fraser-Jenk.	Hemionitidaceae	Y	Y	-	-
80	*Isoetes coromandeliana* L. f.	Isoetaceae	Y	N	-	-

En. – Endangered, Cr. En.- Critically Endangered SWG – Southern Western Ghat

The progression of quarrying and other destructive operations in both sites are given in table 2 below. The Fig. 1 A – C showing Vengappara quarry during the 3 different time periods viz 20006, 2012 and 2014 respectively and Fig. 1 D - F shows the same of Gangothri.

Table 2: Progression of quarrying and destructive operations

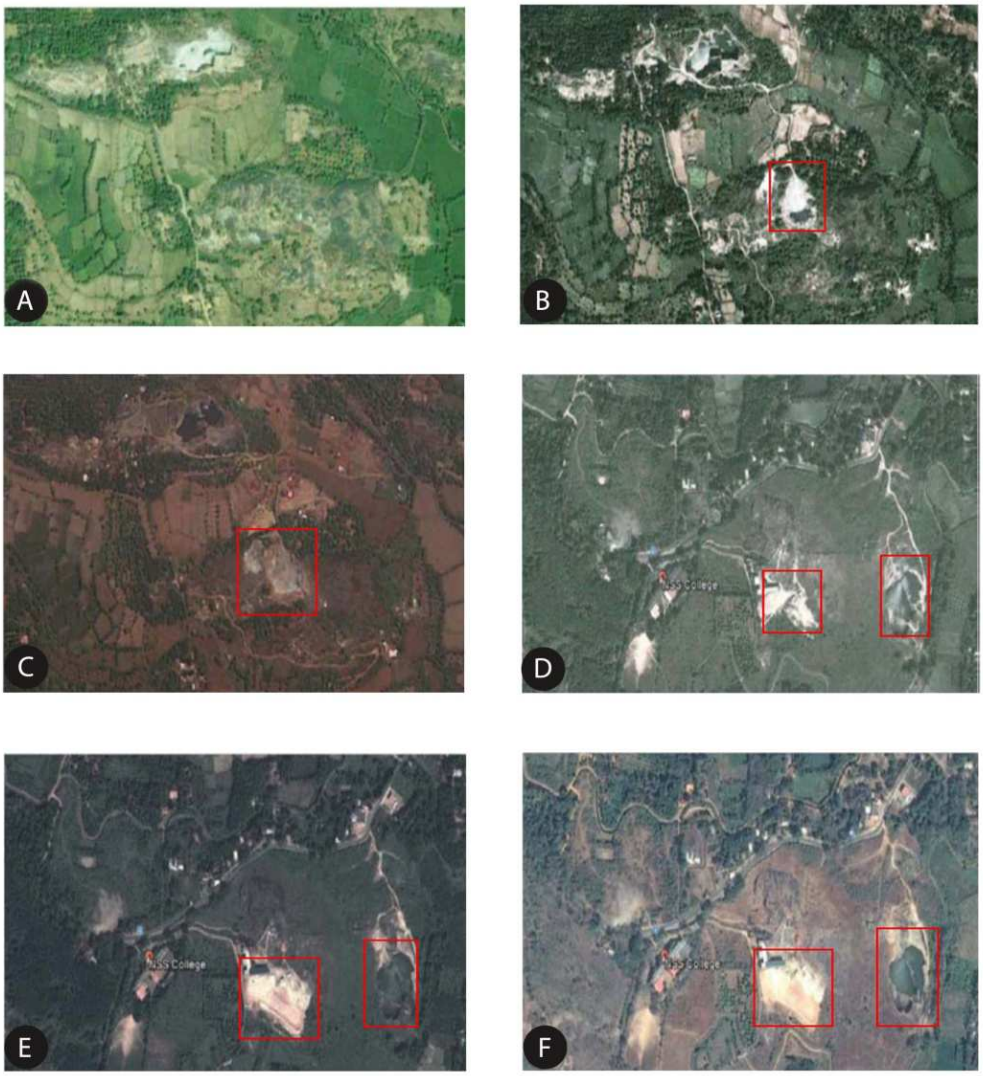
Year	Total Area of the Hill (sq. m)	Area (sq.m) of Quarry in different years		
		2006	2012	2014
Vengappara	92592.07	0	5363	7870
Gangothri	214491.48	17401	25119	27877

Discussion

The granite quarries are home for many plants which normally cannot grow in other habitats. The survey could locate a few species which are important in terms of their distribution and conservation status. The Vengappara granite hills is the only location in Kerala where *Dipcadi motanum* L. is reported (Jose et al. 2013) so far. Recently Deepa et al. (2015) has reported a critically endangerd bryophyte (*Exormotheca ceylonensis* Meijer) from these hillocks. The Gangothri hills in Nemmara is the type locality of the critically endangerd and recently discovered *Oldenlandia dineshii* Sojan & V. Suresh (Jose et al. 2015). The destruction of these hillocks will result in the local extinction of many species.

On the areal measurement of the destructed area using Google earth it has been found that the quarrying has not been started in Vengappara in the year 2006 but it reached an area of 5363 m^2 in 2012 which increased drastically to 7870 m^2 within a short span of 2 years showing alarming rate of mining operations (Table 2 & Fig. 1 A- C). In case of Gangothri, 2 different operations can be observed from the aerial view (Fig. 1 D - F) which includes quarrying on one side of the hill and levelling on other side for construction purpose. The initial phase of destruction covered an area of 17401 m^2 which drastically increased to 25119 m^2 in 2012 and has been reached 27877 m^2 in 2014 (Table 2, Fig. 1 D - F). The status quo of these sites is still worse. This clearly shows that both these hills are in a state of rapid destruction. The unsustainable mining in such pockets of biodiversity, if continued uncontrolled, may lead to the reduction in the local populations of many rare species and eradication of critically endangered endemic species. This warrants the need of urgent measures to document and conserve the biodiversity of hilly terrains of Western ghat region and to conduct proper Ecological Impact Assessment (EIA) before permitting quarries in granite hillocks.

Figure 1: Vengappara quarry during the 3 different time periods (A). Vengappara 2006, (B). Vengappara 2012, (C). Vengappara 2014, (D). Gangothri 2006, (E). Gangothri 2012 and (F). Gangothri 2014.



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An analysis of the physico chemical charactersitcs and phytoplankton to assess the pollution status of Vellayani lake, Thiruvananthapuram, kerala.

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***ABSTRACT**: Lakes, rivers, ponds and streams are the reservoirs of fresh water that plays a significant role in the existence and survival of the entire flora and fauna on our earth. The present study is aimed to analyse the physico-chemical parameters and pollution indicating phytoplankton of Vellayani Lake. Phytoplankton were collected using plankton net. The algal genera were identified referring monographs and journals. The physico chemical parameters such as pH, temperature, dissolved oxygen, Biological Oxygen demand, silicate, phosphate, nitrate and nitrites, alkalinity and total hardness in the water were studied during the year 2014 - 2015. The physico-chemical parameters were analysed according to the guidelines of APHA (American Public Health Association 2005). Dissolved oxygen, phosphate, silicate were found to be higher in pre-monsoon and the Biological oxygen demand was high during the monsoon period. The phosphate level in the water was always below or near to detectable limit. Palmer's Algal Pollution Index was calculated from the phytoplankton data and the score obtained above 20 indicated organic pollution in the lake.*

***Key words:** APHA, Physico-chemical, Algae, Pollution index*

Introdution

Freshwater scarcity is one among the most challenging environmental issues of this century. To improve water management and measure the achievement of internationally agreed goals on water and sanitation, countries and organizations need access to relevant information. Fresh and pure water in limited quantity indicates the need for comprehensive water management. So researche on the impacts of anthropogenic and technical factors on fresh water resources are imperative and also such studies provide us with information of our limits in nature (Ray 1992). The quality of water is characterized by various physico-chemical parameters. These parameters change widely due to many factors like source of water, type of pollution, seasonal fluctuations and adjacent human intervention (Parikh 2012). The alteration of physico-chemical parameters of water affects the biota, its number and diversity. As physico-chemical parameters plays a major role in ascertaining the distributional pattern and quantitative abundance of organisms inhabiting a particular ecosystem (Singh et al. 2009), the constant discharge of sewage into the aquatic system enriches the organic content, leading to eutrophication and deterioration of the quality of water (Sukumaran 2002). The phytoplankton in a reservoir is an important biological indicator of the water quality**.** Phytoplankton study provides a relevant and convenient point of focus for research on the mechanism of eutrophication and its adverse impact

on an aquatic ecosystem. Much work has been carried out through out Kerala regarding the diversity and water quality of fresh water. The present investigation is focused on the water quality and phytoplankton diversity of Vellayani lake, which is the only fresh water lake in Thiruvananthapuram district, Kerala. The lake is located about 7 km away from Kovalam. The lake water is extensively used for drinking and irrigational purposes. It supports a variety of flora and fauna which begins from microscopic forms to macroscopic ones which have ecological as well as economic significance.

Materials and Methods

Vellayani Lake lies between $8^0$24'09" -$8^0$26'30" N Latitude and $76^0$59'08"- $76^0$59'47"E Longitude. The lake is bordered by Thiruvallom and Nemom villages of Neyyatinkara Taluk. Major part of the lake is stagnant but a small portion flows to Karamana River. It is the main source of water supply to four nearby panchayats. Water samples were collected from 8 sites of Vellayani Lake during the pre-monsoon and monsoon and post monsoon period of 2014-2015. The collected samples were brought to the laboratory for the analysis of various physico chemical parameters like pH, temperature, TDS, Conductivity, Dissolved oxygen, Biological Oxygen Demand, alkalinity, silicate, nitrate, nitrite and phosphate in the water. The pH was determined electrometrically using digital pH meter, TDS and Conductivity by TDS – Conductivity meter. Alkalinity, silicate, phosphate, etc. were determined by the method suggested by APHA. Estimation of Sodium and Potassium were done be Flame photometric method. Phytoplanktons were collected using plankton net ones. The specimens were preserved at the site itself using 4% Formaline and brought to the laboratory and observed under advanced Research microscope. The algal genera were identified referring various monographs (Anand 1980) and journals. The phytoplankton settled at bottom were diluted to a desirable concentration in such a way that they could be easily counted individually under compound binocular microscope and phytoplankton were measured and multiplied with the dilution factors using Sedgwick Rafter cell (Smith 1950; APHA 2005).

Palmer (1969) prepared a list of 60 genera tolerant to organic pollution and also generated Algal Genus Index based on the algal data, for the rating of organic pollution of a water body. From the phytoplankton collected from the study site, the Palmer Algal Genus Index was calculated.

Results and Discussion

pH is one of the most important single factor which influences aquatic production. In the present study pH value remains alkaline throughout the study period and it ranged from 6.5 to 7.9. pH is an important parameter in water body since most of the

aquatic organisms are adapted to an average pH and do not withstand abrupt changes (Mini et al. 2003). Total Dissolved Solids shows an average value of about 88 mmho where as the conductivity was above the detectable limit. According to Trivedy et al. (1989) the variation in the conductivity values seasonally is mostly due to increased concentration of salts because of evaporation; the dilution resulted from precipitation brings down its values.

A fair amount of dissolved oxygen is always essential to support aquatic life. In Vellayani Lake, the amount of dissolved oxygen recorded ranges from 4.05 mg/l to 14.5 mg/l. the maximum value of DO was recorded during monsoon and minimum value was recorded in the pre- monsoon period. Singh et al. (1991) also reported that the low dissolved oxygen value may be due to higher water temperature. Concentration of silicate in the water ranged from 0.6 mg/l to a maximum of 6.4 mg/l. The decreased amount of silicate may occur when there is an abundance of diatoms.

Indigenously formed nitrate and the oxidation of organic substances in the water contribute to the presence of nitrate ions in the aquatic body. The value of nitrate in the present study ranged between 3.17- 0.7 mg/l. Usually unpolluted, natural water contain minute amount of nitrate (Shinde et al. 2011). The value of nitrite ions in the lake ranged from 0.03-0.6 mg/l. Phosphate is the nutrient considered to be the critical limiting nutrient causing eutrophication of fresh water systems (Rabalais 2002) and it usually occur in organic as well as in organic forms. During the present study the phosphate showed a minimum value especially during rainy season. According to Patel and Ragothaman (2005), the decrease in phosphate values in monsoon was due to absorption by plankton. Alkalinity is a measure of buffering capacity of water and is important for aquatic life in a fresh water system because it equilibrate the pH ranges that occur as a result of photosynthetic activity of plants in water (Kaushik and Saxena 1999). In the present study, the value of alkalinity ranges from 8 mg/l to 21.1 mg/l.

Hardness of water is mainly due to the presence of calcium and magnesium ions and is an important indicator of toxic effect of poisonous elements present in water (Tiwari 2001). Total hardness of the lake, during the study period ranges from maximum of 92mg/l to minimum of 48 mg/l. High values of hardness may be probably due to regular addition of sewage and detergents to the lake from nearby residential areas (Kaur et al. 1996). The detailed examination of the phytoplankton in the sample revealed the abundance of certain algal genera whose Pollution index was calculated using Palmer's Algal index (Table 1).

Table 1: Pollution index of algal genera (Palmer 1969)

Genera	Pollution Index	Abundance of Phytoplnkton in the sample
Anacystis	1	+
Ankistrodesmus	2	+
Chlamydomonas	4	+
Chlorella	3	++
Closterium	1	++
Cyclotella	1	++
Euglena	5	+
Gomphonema	1	+
Lepocinclis	1	++
Melosira	1	++
Micractinium	1	+
Navicula	3	++
Nitzschia	3	++
Oscillatoria	4	+
Pandorina	1	+
Phacus	2	++
Phormidium	1	+
Scenedesmus	4	++
Stigeoclonium	2	+
Synedra	2	++

(++ indicates abundance of a phytoplankton genera in the lake)

As per the Palmer's Index, abundance of *Synedra, Phacus, Ankistro desmus, Melosira, Euglena, Navicula, Nitzschia, Scenedesmus* and *Closterium* was observed in the sample collected from the lake during the study period. The score assigned for each genera were counted and the Index Value obtained was 23, which indicates organic pollution in the lake. In the present study a score above 20 for the Palmer Index was observed and this confirms organic pollution in the lake. Although several ecological studies have been performed in the Vellayani Lake, it is under constant threat of urbanization and anthropogenic influences. The lake is under threat of pollution, encroachment and sand mining. Hence a continuous monitoring of the water quality status is essential in order to create awareness about the value and relevance of aquatic bodies.

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A study of certain physico-chemical characteristics of Polachira wetland, Kerala

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ABSTRACT: *Wetlands are probably earth's most important fresh water resource and are also the most threatened. The continued degradation of wetlands, and more specifically the continued decline in water quantity and quality, will result in further impoverishment of human health especially for vulnerable people in developing countries. Study of water quality is an indicator of health of any water body. Polachira, is a wetland located in Kollam district. It is one of the most important wetland in Kerala because of its rich biodiversity especially fishes and wide variety of migratory birds. To evaluate the water quality of the wetlands and to identify the pollution sources, random sampling was done during the month of April 2013 to March 2014. The analyzed variables are temperature, pH, dissolved CO_2, nitrate, phosphate, TDS and total hardness. Temperature showed positive correlation with pH, total hardness, phosphate and CO_2. pH showed negative correlation with nitrate, TDS and phosphate. The studies revealed that the certain water quality parameters were satisfactory with exceptions like pH, nitrate, TDS and total hardness.*

Key words: *Polachira, Wetland, Temperature, pH, Hardness, Nitrate, Phosphate, TDS, Dissolved CO_2.*

Introduction

Wetlands are transition areas between land and water, characterized by shallow water overlying waterlogged soil as well as interspersed submerged or emergent vegetation (Prasad et al. 2002). Wetlands are the most productive important part of global ecosystem, which because attention has been turned recently to using the wetland systems and the plant species occurring there in as bio-energy sources and also for use in pollution abatement projects to filter sewage, agricultural run-off, leachate from landfills and acid mine drainage mitigation (Brooks 1989; Oliver and Hill 1998). Wetlands are fragile ecosystem that is susceptible to damage even with only a little change in biotic and abiotic factors. They are threatened due to inadequate water holding capacity, excessive withdrawal, pollution due to sewage and sullage, eutrophiction, leached fertilizers and insecticides (Ramana 2007). The removal of wetland systems because of urbanization or other factors typically cause, its water quality worsens. In addition, wetlands are important feeding and breeding areas for many organisms.

Conservation of wetlands is very much essential as wetlands are one of the most threatened habitats of the world. The most important step for conservation of wetland is to maintain a proper water quality (Smitha et al. 2013). The water quality is to directly relate to the health of the water body. Hydrologic conditions of a wetland can

directly modify or change chemical and physical properties such as nutrient availability, degree of substrate anoxia, soil salinity, sediment properties and pH. These modifications of the physiochemical environment, in turn, have a direct impact on the biotic response in the wetland (Gosselink and Turner 1978). Therefore proper water quality analysis is necessary for the conservation of wetlands.

Materials and methods

The study has been conducted in the Polachira wetland ($8^0$50'26.89"- N latitude, 76^0 42' 0.3" longitude). Polachira is a wetland spread over 600 hectares located in Kollam district. As a result of the biodiversity of fish and mussels, Polachira is a favourite destination of migratory birds. The wetland formed in the estuaries of the Ithikkara River and Paravur backwaters is encircled by small rivulets and is thickly vegetated.

Five different sampling stations of Polachira wetland were selected (station 1- Thalachira, station 2- Polachira Nadappalam, station 3- Mannathipara, station 4- Manalmukku, station 5- near Meenadu bridge). After selecting the permanent sampling stations, the surface water samples were collected systematically from the sampling sites. The surface water samples were collected on monthly interval for a time period of five months (April 2013 to March 2014). The water quality investigations were carried out according to the standard methods **(**APHA 2012). The water quality parameters which have taken for analysis are temperature, pH, dissolved carbon dioxide, nitrate, phosphate, total dissolved salts (TDS) and total hardness. Of the aforesaid water quality parameters, temperature, pH and dissolved carbon dioxide were recorded on-site, whereas rest of the parameters was analyzed in laboratory using standard literatures. Water temperature is measured by using hand mercury thermometer. pH of samples was determined by pH meter. The dissolved carbon dioxide was determined by titration using phenolphthalein. The nitrate was determined by brucine method. The phosphate was determined by using spectrophotometry. TDS was measured with the help of Digital TDS meter. The hardness was determined by titration Erichrome black *T* indicator and standard (0.01 N) EDTA solution.

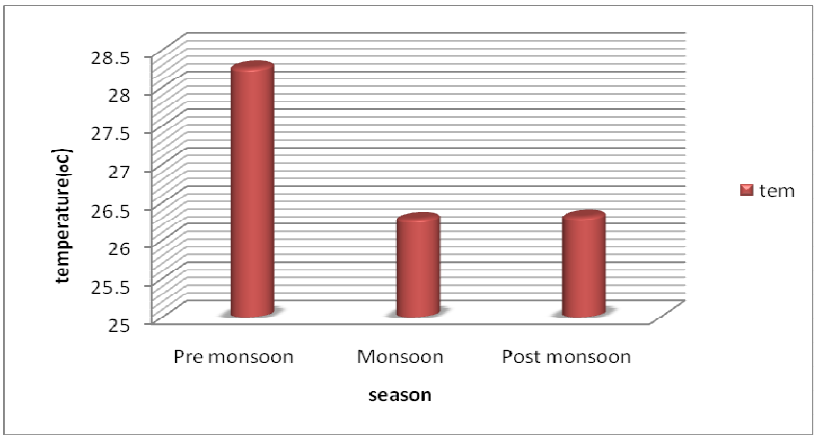
The correlation between various physico-chemical parameters of water samples were analyzed statistically conducting Pearson correlation analysis using PAST version 1.32.

Results

Water temperature is one of the essential parameter, since it influences the growth and distribution of flora and fauna. Oxygen status of any water body is greatly influenced by temperature (Ruttner 1953; Wetzel 1966). The higher value of temperature (30^0 C) was recorded in the month of March 2014 at station II and minimum value (24.2^0 C) was observed in the month of August 2013 at station V. Temperature showed positive

correlation with pH, total hardness, phosphate and CO_2 (r=0.28871, r=0.47764, r=0.53121 and r=0.16461 respectively) (Table 1). Seasonal variation is showed in Fig. 1.

Figure 1: Showing seasonal variation of temperature



pH that maintain the acidic or basic property, is a vital characteristic of any aquatic ecosystem, since all the biochemical activities and retention of physico-chemical attributes of the water are greatly depend on pH of the surrounding water (Jalal and Kumar 2013**).** The monthly pH ranged from 9 during July and august 2013 at station I to 5.1 during October 2013 at station III. Seasonal variation is showed in Fig. 2. pH showed negative correlation with nitrate (r=-0.16863), TDS (r=0.43361) and phosphate (r=-0.04629) (Table 1).

Figure 2: Showing seasonal variation of pH

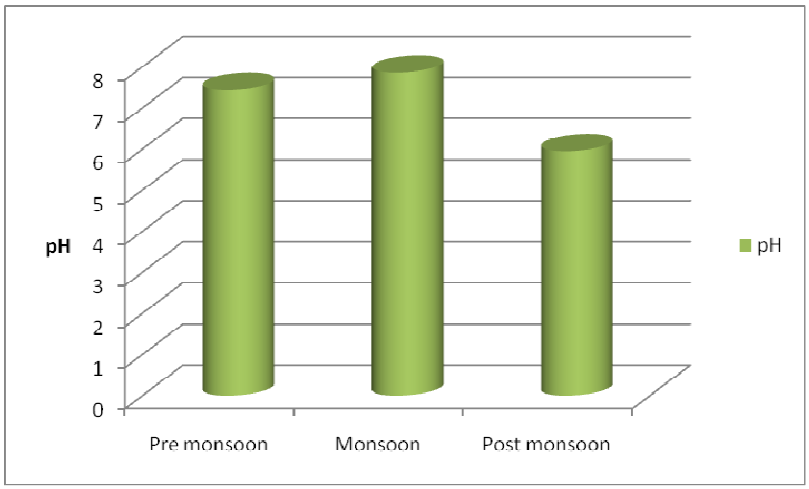


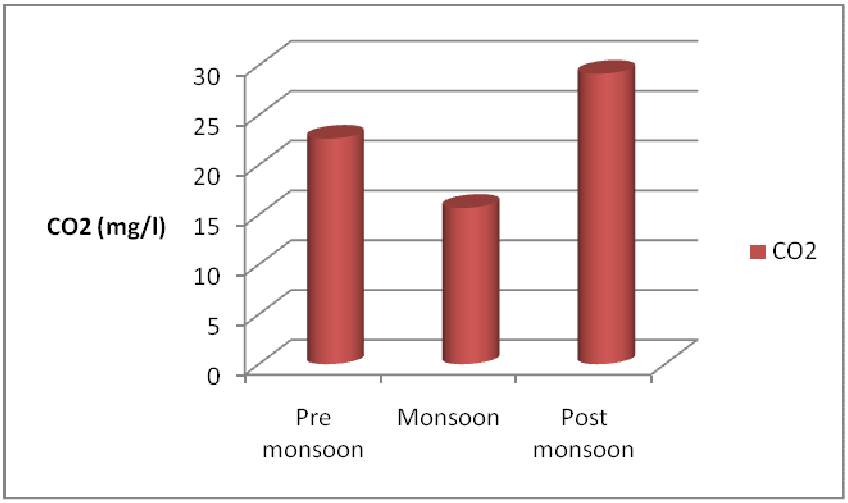
Table 1: Showing Correlation between different parameters

	Temp	pH	CO_2	NO_{3-}	Phos	TDS	TH
Temp	1						
pH	0.28871	1					
CO_2	0.16461	-0.62388	1				
NO_{3-}	-0.73888	-0.16863	-0.04607	1			
Phos	0.53121	-0.04629	0.51018	-0.34616	1		
TDS	0.79029	0.43584	-0.042819	-0.56051	0.59963	1	
TH	0.47764	0.11397	0.15382	-0.27915	0.42055	0.73545	1

(NO_3 - Nitrate; Phos- Phosphate; TDS- Total Dissolved Solids, TH- Total Hardness)

Carbon Dioxide is present in water in the form of dissolved gas. Aquatic plant life depends upon carbon dioxide and bicarbonates in water for growth. The higher value of CO_2 (81.4mg/l) was recorded in the month of December 2013 at station III and lower value (4.4 mg/l) was recorded in the months of July 2013 at station IV. CO_2 showed positive correlation with temperature (r=0.16461), total hardness (r=0.15382) and phosphate (r=0.51018) (Table 1). Seasonal variation is showed in Fig. 3.

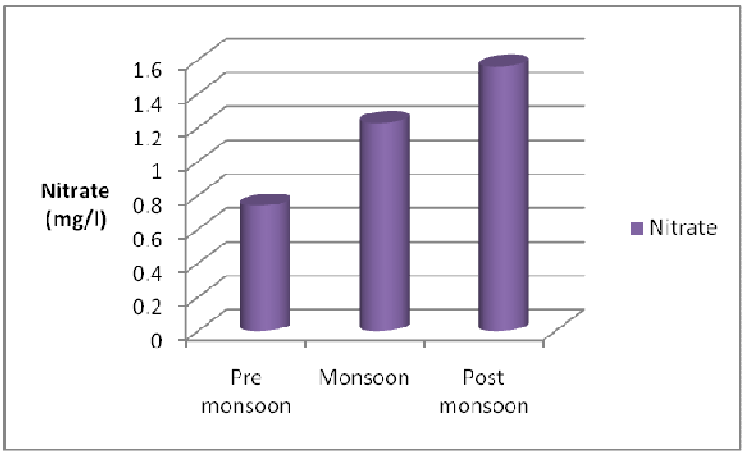
Figure 3: Showing seasonal variation of dissolved CO_2



All organisms require nitrogen for the basic process of life to synthesize protein for growth and reproduction. The presence of nitrates in the water is suggestive of some bacterial actions and growth (Majumder et al. 2006). The main sources of nitrate in water are human and animal waste, industrial effluent, use of fertilizers and chemicals, silage through drainage system (Golterman 1975). The higher value of nitrate (5.675 mg/l) was recorded in the month of July 2013 at station I and lower value (below 0) was recorded in the month of April 2013 at station I, II and III. Nitrate showed negative

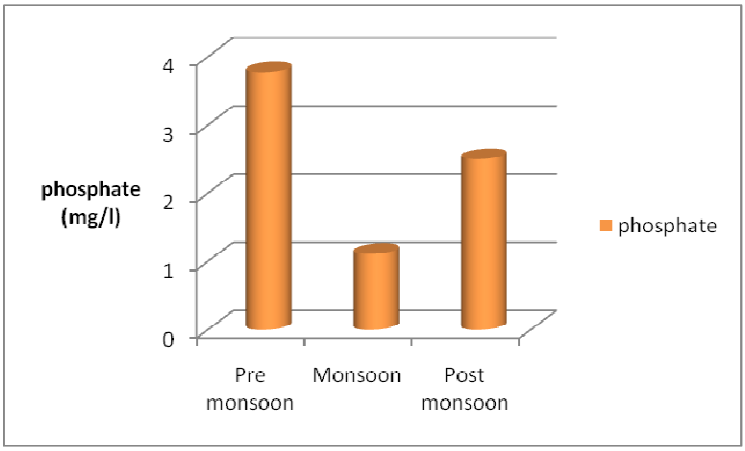
correlation with temperature (r= -0.739), pH (r= -0.169), total hardness (r= -0.279), phosphate (r= -0.346) and CO_2 (-0.046) (Table 1). Seasonal variation is showed in Fig. 4.

Figure 4: Showing seasonal variation of nitrate



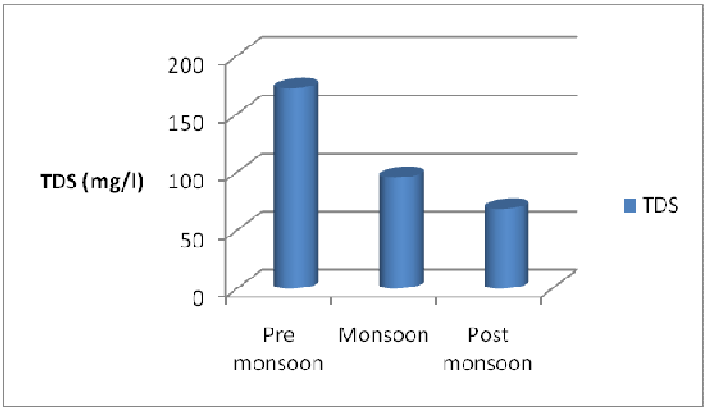
Phosphate is an important nutrient maintaining the fertility of water body (Sahni and Yadav 2012). The increased application of fertilizers, use of detergents and domestic sewage greatly contribute to the heavy loading of phosphorous in the water (WRC 2003). Seasonal variation is showed in Fig. 5. During this investigation station II had the highest phosphate content (29.44 mg/l) and station I, II & III had the lowest phosphate content (BDL) in the months of February 2014 and April 2013 respectively. Phosphate showed positive correlation with temperature (r=0.53121), total hardness (r=0.42055), CO_2 (r=0.51018) and TDS (r=0.59963) (Table 1).

Figure 5: Showing seasonal variation of phosphate



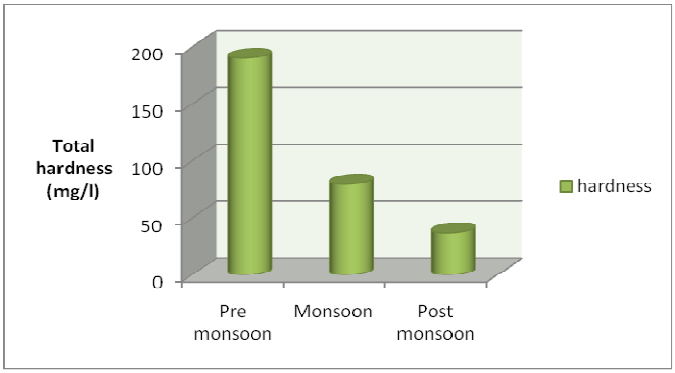
Total dissolved solids are the measure of the combined content of all inorganic and organic substances in a water sample. During the study period, the summer months February to May the value of TDS was recorded as ADL (Above Detectable Limit) at stations II, III and V. The lower value of TDS was recorded as 24.9 mg/l in the month of June at station I. Seasonal variation is showed in Fig. 6. TDS showed positive correlation with pH (r=0.071012), total hardness (r=0.17688), phosphate (r=0.24763) and CO_2 (r=0.1454) (Table 1).

Figure 6: Showing seasonal variation of TDS



Total hardness is the parameter of water quality used to describe the effect of dissolved minerals (mostly Ca and Mg), determining suitability of water for domestic, industrial and drinking purpose attributed to presence of bicarbonates, sulphates, chloride and nitrates of calcium and magnesium **(**Rai 1974). The month wise data ranged from 992 mg/l to 12 mg/l during February 2014 at station V and August 2013 at station I respectively. Seasonal variation is showed in Fig. 7. Total hardness showed positive correlation with temperature (r=0.47764), pH (r=0.11397), phosphate (r=0.42055) and CO_2 (r= 0.15382 (Table 1).

Figure 7: Showing seasonal variation of total hardness



Discussion

In the present study maximum temperature (30^0 C) was recorded in summer and minimum during mosoon (24.2^0 C). The increase in water temperature during summer may be influenced by the increased solar radiation and evaporation. pH vary from 5.1 to 9. The higher range of pH indicates higher productivity of water (Gopalkrushna 2011) because availability of carbonates and bicarbonates in water enhance dissolve carbon dioxide level by dissociation and acts as a raw material for photosynthesis. The higher value of CO_2 (81.4 mg/l) was recorded in post monsoon. When the oxygen concentration in waters containing organic matter is reduced, the carbon dioxide concentration rises. The highest nitrate concentration recorded is 5.7 mg/L, but in some stations it was below detectable limit. Low levels of nitrate are due to its utilization by phytoplankton. Chowdhary and Panigraphy (1991) have supported this fact that assimilation of nitrate by phytoplankton is a key factor in regulating the level of nitrate in water bodies. During the study period higher values of phosphate (29.44 mg/l) were observed in summer. This may be attributed to the huge quantity of domestic sewage, cattle dung, detergents and agricultural fertilizers from surrounding catchment area. The maximum TDS concentration was recorded as ADL (Above Detectable Limit) in pre monsoon and minimum TDS was recorded as 24.9 mg/l during rainy season. The high value of TDS in pre monsoon is due to the high silt content in sediment which causes the accumulation of salts in sediments and water. The water containing excess total hardness (992 mg/l) is due to the use of soap and detergents during bathing, washing of clothes and domestic animals.

Conclusion

The studies revealed that the certain water quality parameters were satisfactory with few exceptions like pH, TDS, total hardness and dissolved CO_2. Moreover, the results manifested the need and prime necessity to restore the physical, chemical and biological integrity with viable and rigorous restoration and management strategies in order to maintain, preserve, conserve and to avert the ecological imbalance and disturbance in hydro-geo-chemical and hydro-biological cycles, which adversely affect the food chain and food web of the significant wetland ecosystem. The study concluded that the Polachira wetland was moderately polluted in respect to analyzed water quality parameters.

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Investigations on phenology of *Celastrus paniculatus* willd.

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ABSTRACT: *Phenology of Celastrus paniculatus, a vulnerable medicinal plant of Western Ghats of South India was investigated, growing in Malabar Botanical Garden and Institute for Plant Sciences located in Kerala. Two different plants of Celastrus paniculatus were compared. Phenological studies indicated that although flowering season of the species occurs once in every year, period (month) of flowering is varying. Flowers are borne on terminal panicles in both plants but the number of flowers on inflorescence differs. The flowers are small, bisexual and open pollinated. Although the flowers are frequented by many species of insects and butterflies, Apis sp. is the major pollinator. Fruit set followed by open pollination was significantly higher. Seeds are enclosed in orange-red aril. The variations between two plants of Celastrus paniculatus are observed in flower size, anther filament length, number of pollen grains, shape of pollen grain, size of gynoecium and fruit shape. Anthers are almost like a reduced staminode in one and in other anthers are functional, pollen grains are abundant. The fruit is a capsule, with prominent ridges in one and the other shows shallow ridges.*

Key words*: Phenology, Celastrus paniculatus, RET, Flower variation*

Introduction

In nature it is often seen that each species has a definite period, month, season in a year during which its seeds germinate, seedlings grow or show maximum vegetative growth, leaves fall (if it is deciduous), flushing of new leaves, flowering and then fruiting. The study of the timing of recurring biological events, among phases of the plant species, which provide a background for collecting and synthesizing detailed quantitative information on rhythms of plant communities called its phenology. In the life cycle of a plant each and every stage is greatly influenced by a number of environmental factors. The different stages of the plant species remain completely embedded in an environmental complex. It is very interesting to note that being fixed at a particular place, the requirement of germination, growth, flowering, fruiting, leaf fall etc of the species are met with at the same place but of course in different times of the year. There is a synchronization of phenological behaviour of the species and the various factors of the environment that plants are spoken of biological clocks. This is mostly regulated by external signals from the environment. But the interactions of each and every species are different at different stages of their life cycle. Thus plant phenological study has great significance because it not only provides knowledge about the plant growth pattern but also provides the idea on the effect of environment and selective pressure on flowering and fruiting behaviour (Zhang et al. 2006).

C. paniculatus Willd. (Celastraceae) is a medicinal woody climber distributed at 1200m altitudes in hilly tracts of India and other countries of South-East Asia. In Indian traditional system of medicine, it is used as an appetizer, laxative, emetic, aphrodisiac and brain tonic. The bark is reported to have abortifacient activity by Sharada et al. (2003). Seeds of this plant are the source of Ayurvedic drug 'Jyothishmati' using in treating rheumatism, gout and neurological disorders. Wild populations of *C. paniculatus* are severely depleted owing to injudicious exploitations in the places where they occur. Destructive harvesting of plants long before flowering has hampered natural regeneration through seeds. While seeds exhibit poor viability and germination, conventional propagation through vegetative cutting is slow and cumbersome (Nair and Seeni 2001).

Materials and Methods

Study site

The study site is located (between 11º14´37.37´´N and 75º49´36.85´´E) in the Malabar Botanical Garden and Institute for Plant Sciences. There are two different wild *C. paniculatus* plants present. Other trees associated with these plants are *Morinda pubescens* J.E. Smith (manjapavatta), *Neolitsea cassia* (L.) Kosterm., (venkana), *Holigarna arnottiana* Hook.f., (cheru), *Hopea ponga* (iyyakutti) and *Ficus sp*.

Phenology

Phenological events (bud break, flowering, fruiting, shedding of leaves, fresh leaf emergence, fruit dispersal and seed germination) were recorded for a period of one year (2014-2015). To obtain the above information, observations were made every day in the morning hours during the flowering period (April-June). Subsequent events, such as fruit maturation and fruit dispersal were noted and recorded once a week.

The average numbers of flowers borne on an inflorescence were recorded from a set of randomly tagged flowering branches (n= 30). For estimating the average pollen grain numbers produced in a flower, mature but undehisced anthers (n=20) were squashed in two or three drops of 25% glycerol (v/v) + 1 drop of 1% acetocarmine. Ovule number was determined by clearing the fresh pistils (n=20) with NaOH (1N) and staining with 1% acetocarmine. Temporal activities of the floral visitors were recorded between 0600 and 0200 h during flower opening days. As fruit set under open pollination in both plants is very high (approx. 90%). Pollen viability is tested using acetocarmine and tetrazolium tests (Shivanna and Rangaswamy 1993). Pollen size was measured with an ocular micrometer under light microscope. Photographs were taken by using a light microscope (Olympus microscope, India) attached to a camera (Olympus).

Results

Phenology

Two marked plants for phenological observations showed variation with respect to leaf fall and flowering. One plant (Plant A) began to shed its foliage in mid December and was completely bare by January. Leaf primordial appeared in the third week of May and leaves attained their maximum size by June. The inception of inflorescence primordia in the leaf axils occurred at the end of May. Flower buds were greenish yellow in colour and commenced opening during the third week of June. Peak flowering period is third week of June; the plant was seen in bloom for only one week. Fruiting commenced from the last week of June, the fruits reaching maturity by the end of October and dispersal starts from the first week of November. Fruit is a loculisidal capsule. Seeds were enclosed in an orange fleshy aril.

The other plant (Plant B) started shedding their leaves from the end of December. Leaf primordial appeared in the first week of April and by the end of April leaves attained their maximum size. The inception of inflorescence primordia in the leaf axils occurred in the end of second week of April. Flower buds were whitish yellow in colour and commenced opening during the end of April. Peak flowering period is first and second week of June; the plant remaining in bloom till the end of third week of June. Fruiting commenced from the last week of June, the fruits reaching maturity by the end of October and dispersing in mid November. Fruit is a loculicidal capsule and were seen to drop in and around the vicinity of the plant on which they were produced. Orange fleshy aril is present around the seeds.

Freshly opened flowers attract a large number of visitors. Maximum visitation occurred between 0800 and 0500 h in both plants. Honeybees and butterflies were the most frequent visitors. *Apis sp.* is the major pollinator in both plants. Butterflies seen are *Euploea core* Cramer (Common Indian Crow), *Cirrochroa thais* (Tamil Yeoman) and *Neptis jumbah* (Chestnut-Streaked Sailer).

Floral biology

Plant A

The leaf size of plant A ranges from 12.25 ± 2.50 x 5.19 ± 0.67 cm. The inflorescence is terminal panicle, 6.39 ± 1.31 cm (n=10) in length. Inflorescences are smaller than the leaves. On average, an inflorescence developed 47.8 ± 9.76 (n=10) flowers that opened in acropetal order. Average size of the flower is 0.81 ± 0.057 cm (n=10) (Fig. 1a). Flower bud opening occurred between 0700 and 0900 h. The petals are greenish yellow in colour and 0.30 cm in length. Five petals are present. Each flower lasts for 2-3 days. A gamosepalous cup-like, green persistant calyx is present. Five free stamens are present

attached to the disk with short filament. Stamens are reduced to staminodes. Ovary 3-celled and 2 ovules per cell. Pistils were 0.5 cm long at maturity and curved at the tip. Stigma is recurved and 6 fringed. Fruits are slightly oblong with shallow ridges (Fig. 1 b). Seeds 1-6 in number, brown in colour.

Plant B

Leaf size ranges from 8.84 ± 1.58 x 3.99 ± 0.63 cm. The inflorescence is terminal panicle, 6.84 ± 2.33cm (*n*=30) in length. Inflorescences both smaller and larger than leaves are present. On average, an inflorescence developed 75.5 ± 37.49 (*n*=10) flowers that opened in acropetal order. Average size of the flower is 0.72 ± 0.042 cm (*n*=10) (Fig. 1 c). Anthesis (flower bud opening) occurred between 0700 and 0830 h. The petals are greenish yellow in colour and 0.25 cm in length. Five petals are present. Each flower lasts for 2-3 days. A gamosepalous cup-like, green persistant calyx is present. Five free stamens are present attached to the disk with long transparent filament. Anther size is 0.1 cm and filament length is 0.2 cm. The average number of pollen grains in an anther is 4098 ± 464. Pollen grains are Suboblate. Pollen viability tests were carried out using acetocarmine and tetrazolium tests. Around 78% of pollen grains were viable at the time of anther dehiscence. Stigma 6 fringed, ovary 3-celled, 2 ovules per cell. Fruits are slightly round like with 3 ridges (Fig. 1 d). Seeds 1-6 in number, brown in colour. The comparison between plant A and plant B are given in Table 1.

Discussion

According to Matthew (1999) *C. paniculatus* is seen in high altitude areas ranging from 800-1300 m. But in this present study the plant is seen in an altitude range of 48 m. Francis et al. (2012) mentioned that inflorescences are born on terminal shoots, twice as long or more than twice as long as the leaves in the species *C. paniculatus*. In our study area both plants A and B produce inflorescence on terminal shoots, but in plant A the inflorescences are smaller than the leaves and in plant B some inflorescences are smaller and clustered and some are larger than the leaves and not clustered.

C. paniculatus flowers are described as unisexual in the study of Arora and Rai (2012). In plant A the stamens are reduced staminodes, only the gynoecium is functional, so the flower can be considered as unisexual (female flower). Bisexual flowers are seen in plant B, both androecium and gynoecium is functional. Deodhar and Shindae (2015) described female flowers as ovary globose, narrowed into a short stout style, glabrous stigma large 3-lobed; stamens inserted on the edge of disc which is larger than disc in male flowers, anthers small without pollen, ovate, acute or subtriangular, less than 1mm long. The anthers are without pollen grains and smaller than 1mm long in plant A. Rekha et al. (2005) collected seeds of *C. paniculatus* from Palampur, Himachal Pradesh during October, 1996 for her study on seeds. The fruits got matured during October in our study area also.

Figure 1: (A). Flower of Plant A, (B). Fruit of Plant A, (C). Flower of Plant B and (D) Fruit of Plant B

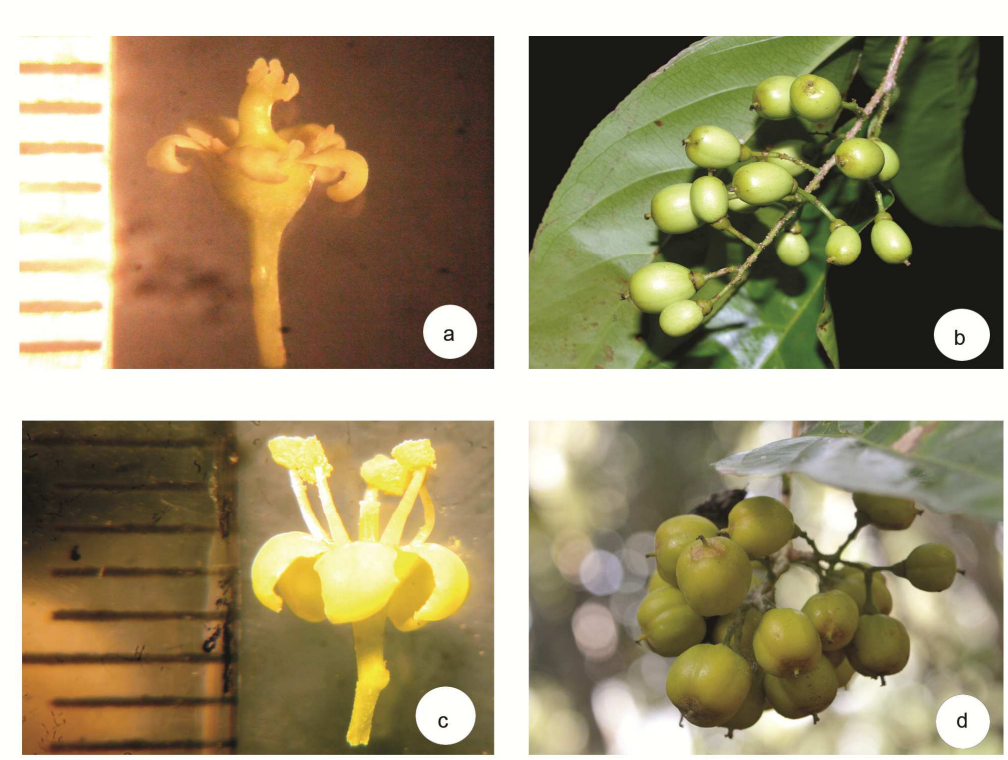

Table 1: Comparison between two *C. paniculatus* plants - A and B

		Plant A	Plant B
1	Leaf type	Average leaf size-12.25 ± 2.50 x 5.19 ± 0.67 cm	Average leaf size-8.84 ± 1.58 x 3.99 ± 0.63 cm
2	Flowering season	May-June	April-June
3	Inflorescence size	Inflorescences are smaller than leaves. Size - 6.39 ± 1.31 cm	1. Some inflorescences are clustered and smaller than leaves 2. Some inflorescence are larger than leaves and not clustered Size-6.84 ± 2.33cm
4	Date of 1st flower	12/6/15	25/5/15
5	Peak flowering period	3rd week of June	1st and 2nd week of June

6	Date of last flower	19/6/15	21/6/15
7	Flower longetivity	2-3 days	2-3 days
8	Flower Opening time	7.00-9.00 am	7.00- 8.30 am
9	Flower colour	Greenish yellow	Whitish yellow
10	No. of flowers/ inflorescence	Average 47.8 ± 9.76 flowers/inflorescence	Average 75.5 ± 37.49 flowers/ inflorescence
11	Flower type	Perigynous , gynoecium is large in size. 50cm, anthers are reduced to staminodes, Stigma recurved, 6 fringed.	Bisexual, perigynous and complete. Filaments of anthers are long, transparent and contains large number of pollen grains, Stigma not curved, 6 fringed.
12	Petals	5 petals, greenish yellow in colour, Petal size – 0.3 cm	5 petals, whitish yellow in colour. Petal size – 0.25 cm.
13	Androecium	Anthers are reduced to staminodes, filament is too short.	5 anthers, pollen grains are numerous, filament is long and transparent (0.2cm)
14	Gynoecium	It is large and stout Stigma recurved and 6 fringed, Size-0.50 cm	It is small in size (.3 cm) Stigma 6 fringed and not curved
15	Flower size	0.81 ± 0.057cm	0.72 ± 0.042 cm
16	Fruit shape	Slightly oblong with shallow ridges	Slightly round like with 3 ridges

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Isolation and axenic culture of nostocales (*Nostoc* spp. and *Anabaena* spp.) of Kerala

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ABSTRACT: *Blue green algae (Cyanobacteria) are the ubiquitous pioneer autotrophic organisms among the Plant Kingdom that represent an interesting group as capable of both Photosynthesis and N2 fixation simultaneously. The aim of the present study was to isolate, identify and characterise the heterocystous cyanobacteria (Nostoc spp. and Anabaena spp.) occurring in the rice fields of the Malabar region of Kerala (Kozhikode, Kannur, Malappuram and Thrissur Dist.) The samples were collected from the field as blue green mass or soil samples. From soil the algae were isolated using serial dilution and streak plate method. The medium used in this study was BG11 medium (Rippka et al. 1979) with the omission of NaNO3. Solid medium was found ideal for the long term maintenance. Unialgal cultures were raised. In the liquid media the cultures were examined regularly to check the purity of the culture. The organisms were identified using standard manuals and by consultation with expert algologists. After identification the isolated colonies were subcultured. 6 pure cultures were raised viz; Anabaena torulosa, A. variabilis, A. oscillrioides, Nostoc muscorum, N. linckia, N. spongiaeformae. The distinction of these genera based on morphological feature is difficult due to morphological plasticity in nature as well as in cultures. Majority of the species were distinguished based on the characters of hormogonia and spore morphology but in culture conditions the sporulation was not very common. To solve such problems molecular tools are being used.*

Key words: *Blue-green algae, Axenic, Hormogonia, BG-11*

Introduction

Algae are the simplest members of the Plant Kingdom, and the Blue Green algae are the simplest among them. Cyanobacteria (until recently being known as blue green algae) owing to its bacterial characters such as peptidoglycan cell wall, prokaryotic nature, etc. are ubiquitous organisms, capable of photosynthesis and nitrogen fixation simultaneously. They are more related to eubacteria in their structure and chemical composition. The division Cyanophyta includes a large number of algae which are characterised by a low state of cell organisation. The cell lacks the well defined nucleus in contrast with other algae which are typically eukaryotic (Desikachary 1959).

Cyanobacteria fix atmospheric nitrogen and convert it into ammonia with the help of nitrogenase enzyme produced in specialised cell called heterocyst. The heterocystous cyanobacteria thus play an important role in enhancing the soil fertility. The role of algal inocula in the rice fields as bio-fertilizer was studied by Venketaraman (1979).

Various methods have been adopted to efficiently isolate and purify the freshwater blue-green algae by Ferris and Hirsch (1991). Among different factors that influence the growth of blue green algae the pH of the medium plays a major role. The growth and nitrogen fixing potential of acid tolerant cyanobacteria were investigated by Madhusoodanan and Dominic (1996). The present study aims the isolation, characterisation and axenic culture of *Nostoc* and *Anabaena* species occurring in the rice fields. The major distinguishing feature of the heterocystous cyanobacteria is the position of spores. But in culture conditions these members do not show sporulation in common. So their species distinction is difficult. To solve such problems the use of molecular tools will be helpful. For such molecular studies, axenic culture is a prerequisite. So this study is a preliminary step towards the molecular characterisation.

Materials and methods

Collection of Samples

The samples were collected from different areas of Thrissur, Malappuram, Kannur and Kozhikode either as soil samples or as blue green (mucilaginous) algal mass. The collected materials were isolated for which BG.11 medium (Rippka et al. 1979) with the omission of nitrogen source was used. The isolation techniques include direct isolation, by inoculating the samples on the medium while that of indirect method include the inoculums prepared by serial dilution method from the soil. In both the cases, they were inoculated on agar plates containing 8 gm/l agar. The growth of algae was visualised by naked eyes by their gliding movement and phototactic migration in the agar plate. Once growth noticed in solid medium, they were inoculated to liquid medium. The liquid media were examined periodically for any contamination. The medium were autoclaved at 121 lbs pressure for 20 minutes before autoclaving the pH were adjusted to 7.2 ± 2 with 1N HCl. The cultures were maintained in conditions of 23 ± 2^0 C under a 12/12 hr photoperiod supplemented from white fluorescent lamps. The unialgal cultures are raised by continuous sub culturing. The purity of the cultures was checked under light microscope. The unialgal stocks were maintained in 250 ml Erlenmeyer flasks with 100 ml $BG_0$11 medium (nitrogen free) and also in agar plates for long term maintenance.

Results

12 samples were collected from different paddy fields of Kerala in which 3 species of *Nostoc* and 3 species of *Anabaena* could be identified upto species level. The *Nostoc* spp. are;

***Nostoc linckia* (Roth) Bornet ex Born.*et* Flah.** (Fig. 1 A)

Thallus varying size, globose later irregularly expanding, blue-green to blackish green, filaments densely entangled. Cells short barrel shaped, heterocyst sub spherical; spores sub spherical, 6-7 µm broad.

***Nostoc muscorum* Ag.ex Born.*et* Flah.** (Fig. 1 B)

Thallus membranous, olive green or brown, trichome 3.5- 5 µm broad, cells variable in shape, spores oblong, many in chains 4-5 µm broad.

***Nostoc spongiaeforme* Agardhex Born.*et* Flah.** (Fig. 1 C)

Thallus gelatinous, globose at first trichome 4-5 µm broad, cells elongate-cylindrical or barrel shaped 5-6 µm long. Spores seen as a chain away from heterocyst. Heterocyst terminal or intercalary. These were distinguished based on position of spores and the nature of trichome.

The three Anabaena species isolated and identified were:

***Anabaena oscillarioides* Bory ex Born. *et* Flah.** (Fig. 1 D)

Thallus dark green, cells barrel shaped end cells rounded, spores on both side of the heterocyst at first oval later rounded- cylindrical, 8-9 µm broad.

***Anabaena torulosa*(Carm.) Lagerh. ex Born. *et* Flah.** (Fig. 1 E)

Thallus mucilaginous, thin, pale blue green, trichome 4-5 µm broad, apical cell acutely conical, cells barrel shaped, heterocyst ovoid, spores on either side of the heterocyst sub-cylindrical with rounded ends.

***Anabaena sphaerica* Bornet. *et* Flah.** (Fig. 1 F)

Thallus soft gelatinous, blue green, trichome straight 4-6 µm broad, cells barrel shaped, end cells rounded, heterocyst sub spherical, spores on either side of the heterocyst, spherical to oval in shape.

The generic identity and distinction of *Anabaena* from *Nostoc* has always been cumbrance owing to their plasticity, which varies according to the habitat characteristics such as temperature, salinity, pH, etc. Hence, an attempt is initiated inorder to distinguish these genera based on all possible taxonomic evidences including the cellular features, hormogonial characters, pigment content, etc.Culture characteristics of the Cyanobacterial species in BG_0-11 medium are given in Table 1.

In the present work three species of *Nostoc* and three species of *Anabaena* isolated from paddy fields of Kerala and 12 more species are yet to be identified owing to obscure characters.

Table1: Culture characteristics of the Cyanobacterial species in BG_0-11 (nitrogen free) culture medium- both solid and liquid

Sl. No.	Cyanobacterial species	Place of Collection	Time taken (days)		Thallus characteristics
			Visible Colony	Complete spreading on agar plate	
1.	*N. linckia* (Roth) ex. Bornet. *et* Flah.	Kozhikode	10-12	30-35	Globose colony, blue green to dark green colour
2.	*N. muscorum* Ag. ex Born. *et* Flah.	Thrissur kole field	6-8	30	Olive green to brownish colonies spreading the entire plate
3.	*N. spongiaeformae* Agardhex. ex Born. *et* Flah.	Payyannur Kannur	7-8	30	Olive green to blue-green colonies
4.	*A. oscillarioides* Bory ex Born. *et* Flah.	Kozhikode	7	28-30	Blue green colonies form as a mat on the agar surface
5.	*A. torulosa* (Carm.) Lagerh. Ex Born. *et* Flah	Malappuram	7	28-30	Blue green colonies on the surface spreading. Distinguished from *A. oscillaroides* by the shape of spores
6.	*A. sphaerica* Bornet. *et* Flah.	Thrissur	5-6	28-30	Mucilaginous blue-green mass formed on the surface of the agar. In the liquid medium the colonies found floating

Discussion

Heterocystous cyanobacteria have been already studied for their diversity in rice fields of Kerala by Umamaheswari (2005) but limited works have been reported. The genus *Nostoc* is being distinguished from *Anabaena* by the coiling or wavy nature of the hormogonia and shape of the heterocysts. *Nostoc* spp. showed more coiling whereas the *Anabaena* hormogonia are rather straight or wavy and with the heterocysts ovoid or elongated which was in accordance with Desikachary (1959). The morphology of strains could not be considered for the distinction of genus because at certain times environmental conditions and culture conditions also affect the morphology.

Morphology of strains may change depending on environmental conditions and the diversity of the strains can be altered by selective culture conditions (Palinska et al. 1996).

Simple thallus organisation of cyanobacteria, except for the presence of unique structures like heterocyst and akinite based on which the classification is done is a limiting factor. The existing classification is inadequate to delimit the species (Roger 1991). In these contexts, the use of molecular markers for the systematic studies becomes important. Molecular studies have undertaken by Rajaniemi et al. (2005) in which they separated *Nostoc* strains from *Anabaena* strains using molecular marker such as 16s rRNA. Prasanna et al. (2006) differentiated *Anabaena* strains by employing molecular markers. For such molecular studies the prerequisite is the unialgal cultures from which DNA could be successfully extracted. Such works are in progress in the laboratory.

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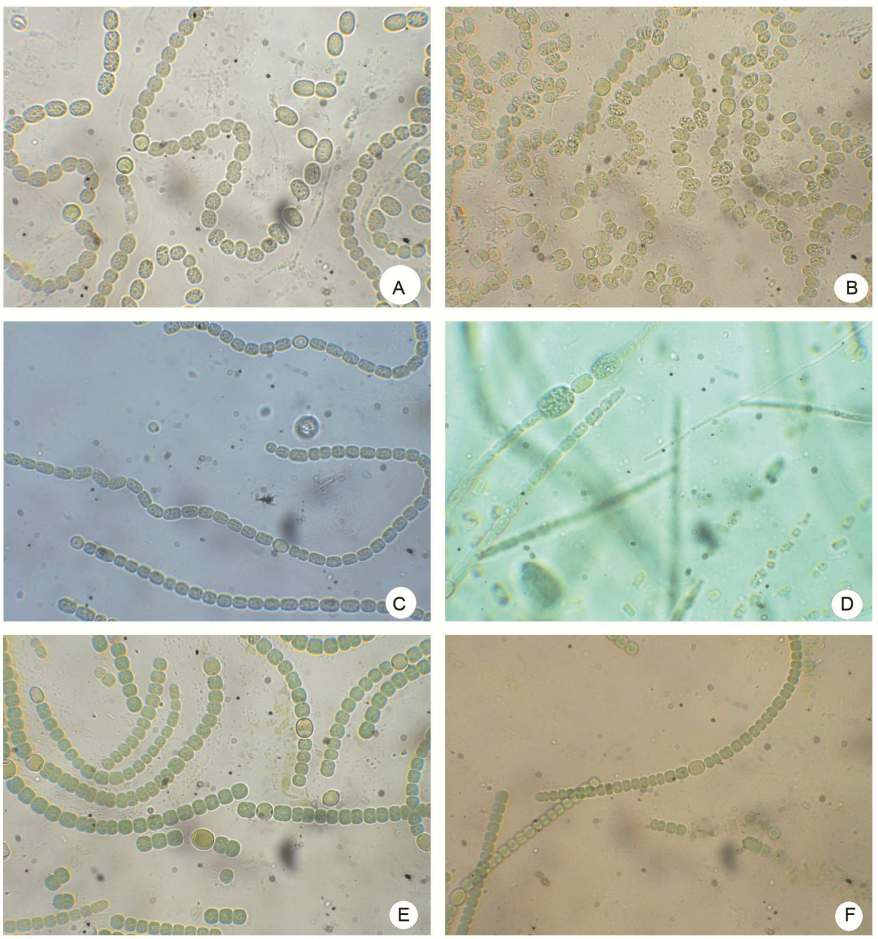
Figure 1: A. *N. linckia,* B. *N. muscorum*, C. *N. spongiformae*, D. *A. oscillaroides*, E. *A. torulosa*, F. *A. sphaerica*



Conservation of RET aquatic plants of South India at MBGIPS, Kozhikode, Kerala

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***ABSTRACT**: The wetlands form specialised ecosystems supporting rich and much diversified life forms, but that are fast diminishing due to continued anthropogenic activities. We have conserved 413 aquatic plants from south India, of which 26 species are found to be under Threat Category. Under the present situations these plants may become extinct in near future. In this context, we have undertaken efforts for the conservation of these plants by propagating them in the aquatic plant conservatory (Aquagene) of Malabar Botanical Garden and Institute for Plant Science and initiated the in vitro multiplication of species.*

***Key words**: Conservation, RET Aquatic plants, Aquagene*

Introduction

Wetlands are considered to be the 'Cradle of biological diversity' providing water and primary productivity upon which countless species of plants and animals depend for survival. They also form important store house for plant genetic resources. It is important to note that most of the wetland ecosystems in Kerala are in the midland and coastal areas which are densely populated and hence are subjected to be great anthropogenic pressure. The wetlands are fast diminishing due to indiscriminate land filling and deteriorating due to the discharge of industrial effluents and city wastes. Thus the rich and specialized biodiversity in those wetlands are fast disappearing. This may lead to a situation that the rare plants of these wetlands may be lost before we know what we have and how useful they are.

Materials and methods

Extensive field exploration trips have been conducted and maximum number of plants were procured in flowering condition. Information on local name, local use, etc. are collected from local people and noted in field book. Water sample have been collected from every aquatic system and its pH values have been recorded. Part of the plant specimens collected has been processed for herbarium and deposited in the Herbarium of Malabar Botanical Garden and Institute for Plant Science. The live seedlings of RET species have been collected for introduction in the Aquatic plant Conservatory (Aquagene) of Malabar Botanical Garden and Institute for Plant Science as an *ex situ* conservation. The delicate aquatic plants collected have been primarily introduced in the special germination pots filled with pH adjusted, for adaptation in the Aquatic

Plant Conservatory (Aquagene). Only those plants fully established in nursery have been shifted to field Conservatory. We have initiated the multiplication of these RET species through natural and *in vitro* methods for the reintroduction in their natural habitat.

All the plants introduced have been labelled depicting their common name, scientific name, family, uses, etc. to provide eco education facility to visitors in the Malabar Botanical Garden and Institute for Plant Science.

Results

The plant specimens collected have been identified by studying under a stereo dissection microscope and with the help of standard floras. Out of the 413 species collected through the field exploration, 25 species were found under RET category. Scientific name, common name, family, conservation status, etc. of each RET species were noted (Table 1).

Discussion

The studied 25 RET species are under threat due to habitat destruction and water pollution due to anthropogenic interference. Under this condition, these plants may become extinct in future. Many of these plants have significant important in human life and a conspicuous role in ecosystem dynamics. Owing to the fast destruction of aquatic habitat we conserved these species by introducing in the Aquatic Plant Conservatory (Aquagege) of Malabar Botanical Garden and Institute for Plant Sciences as *ex situ* conservation. We initiated the multiplication of these RET species through natural or *in vitro* methods for the reintroduction in the natural habitat. However this is not a permanent remedy for extinction of species. The plant should be protected in the natural habitat itself. But, the wetlands which are mostly found in plains where the RET species are often met with, are under serious threats of ecosystem destruction. Conversion of wetlands for other land uses like construction, dumping of city wastes, discharge of industrial and domestic effluents, over exploitation of water for irrigation, mining of sand, etc are some of the reason for declining of wetlands. *In situ* conservation of RET species is possible only by identifying the sites of its occurrence and by protecting those sites from the above deeds of the public. The people should make themselves aware of the significance of conservation of these RET plants and take concerted efforts to protect the original habitat from pollution and deterioration.

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Table 1: RET aquatic plants conserved at MGBIPS

Sl No.	Species	Family	Common name	Conservation status	Uses
1	*Acorus calamus L.*	Araceae	Vayambu	Endangered	Rhizomes have medicinal value
2	*Aponogeton appendiculatus* H. Bruggan	Aponogetonaceae	Chammikaya	Endemic to Kerala, Vulnerable	Rhizomes are edible
3	*Crinum malabaricum* Lekhak & S. R. Yadav	Amaryllidaceae	Kanthanga	Endemic to northern Kerala, Critically Endangered	Bulbs have medicinal value
4	*Cryptocoryne sivadasanii* Bogner	Araceae	Neerthali	Endemic to Malappuram Endangered	As an aquarium plant
5	*Cyanotis burmanniana* Wt.	Commelinaceae	Cyana	Rare	
6	*Eriocaulon cuspidatum* Dalz.	Eriocaulaceae	Chooth	Endangered	
7	*Eriocaulon sivarajanii* Ansari & Balakr.	Eriocaulaceae	Chooth	Endemic to Kerala, Endangered	
8	*Eriocaulon thwaitesii*	Eriocaulaceae	Chooth	Threatened	

	Koernicke				
9	*Heliotropium keralense* Sivar. & Manilal	Boraginaceae	Telkkatta, Venalpacha	Endemic to Kerala, Critically Endangered	The whole plant have medicinal value
10	*Hydrocera triflora* Wt. & Arn.	Balsaminaceae	Jalathumba	Endangered	As an Ornamental plant
11	*Hydrocharis dubia* Bach.	Hydrocharitaceae	Himalayan - kulavazha	Vulnerable	As an ornamental plant
12	*Lagenandra keralensis* Sivadasan & Jaleel.	Araceae	Chuvappu - Kinarvazha	Endemic to Kerala, Endangered	As an ornamental plant
13	*Lagenandra meeboldii* (Engler) Fischer	Araceae	Cheriya - kinarvazha	Endemic to Kerala, Endangered	
14	*Lagenandra nairii* Ramam & Rajan	Araceae	Cheriya - kinarvazha	Endemic to Kerala, Endangered	
15	*Limnopoa meeboldii* (Fischer) C.E. Hubb.	Poaceae	Nelppullu	Endemic to south west India, Endangered	
16	*Lindernia manilaliana* Sivaraj	Scrophulariaceae	Manilalpushpam	Endemic to Kerala, Endangered	As an ornamental plant
17	*Nuphar luteum* (L.) Sm.	Nymphaceae	Yellow pond lily	Vulnerable	As ornamental and medicinal plant
18	*Nymphoides aurantiaca* (Dalzell) O. Kuntze	Menyanthaceae	Swarna - neyyambal	Endemic to Kerala and Karnataka, Endangered	As an ornamental plant
19	*Nymphoides krishanakesara* Joseph & Sivaraj	Menyanthaceae	Krishnambal	Endemic to northern Kerala, Endangered	As an ornamental plant
20	*Nymphoides macrosperma* Vasudevan.	Menyanthaceae	Neyambal	Endemic to Kerala, Critically endangered	As an ornamental plant
21	*Nymphoides parvifolia* O.Kuntz.	Menyanthaceae	Chinnambal	Endemic to northern Kerala	As an ornamental plant

22	*Pogostemon erectus* (Dalzell) Kuntze	Lamiaceae	Bhothachedayan	Endemic to south India, Rare	As an ornamental plant
23	*Rotala malabarica* Pradeep, Joseph & Sivar.	Lythraceae	Rotala	Endemic to south west India, Critically Endangered	As an ornamental plant
24	*Rotala malampuzhensis* Vasudev.	Lythraceae	Rotala	Endemic to south west India, Endangered	As an ornamental plant
25	*Rotala ritchiei* Koehne.	Lythraceae	Rotala	Endemic to south west India, Endangered	As an ornamental plant
26	*Rotala tulunadensis* Prasad, Biju, Ravi & Bhat	Lythraceae	Rotala	Endemic to northern Kerala	As an ornamental plant

Diversity of green algae in Bodinayakanur of Theni District, Tamilnadu

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ABSTRACT: *A total of 56 algal species have been identified. The high relative abundance of Chlorophyceae is an indicator of productive water. It appears that algal diversity to be significant for maintaining fresh aquatic ecosystem.*

Keywords: *Freshwater Algae, Pediastrum, Chlorophyceae, Indicator.*

Introduction

The Chlorophyceae are large and important group of fresh water green algae occur abundance in ponds, lake, streams, rivers, water resources and different habitats on earth such as in the soil and wall, in animal and plants. They are important both economically and scientifically. These are very sensitive to the environment their live in and any alteration in the environment lead to the change in their communities in terms of tolerance, abundance, diversity and dominance in the habitat (Amarsinghe and Viveberg 2002; Elliott et al. 2002).

The Global scenario has dramatically changed on the last few years. The emphasis is on pressuring algal flora and genetic resources which tropical countries are richly endowed with country like India needs to be recognized for the occurrence of a wide range of aquatic forms like alage (Kalita et al. 2015).

Materials and Methods

The present study was done in Bodinayakanur, Theni District situated in the foot hills of Southern Western Ghats of Tamil Nadu which is the "Cardamom Capital of India". Its location is 0.02°N 77.35°E and receives adequate rainfall which provides ideal condition for the growth of freshwater algae. We have chosen two ponds namely Thamarai kulam, surrounded by the paddy field holds beautiful *Nymphaea sp.* Flowers throughout the year and Pudhu kulam, surrounded by *Mangifera indica* and *Bombax ceiba* vegetations. Samples were collected once in every 7 days interval for a period of three months from December 2014 to February 2015 from different

sites place of the ponds. The freshwater algal sample was fixed and settled at the bottom of the container were collected and preserved in bottles containing 4% formalin glycerin preservative after decanting the supernatant fluid. All the samples were serially numbered, labeled with date and place of collection. Samples after examination are deposited in the Phytodiversity Research Laboratory, Department of Botany, Bharathiar University, Coimbatore. Since cultivation is usually necessary for detailed taxonomic studies of algae, the morphology of the species were therefore studied both from the field collected and identified. Semi permanent slides were deposited in the Bharathi Herbarium, Department of Botany, School of Life Sciences, Bharathiar University, Coimbatore.

Results

A total 56 algae belonging 20 families were identified from the study area. 56 algae [Chlorophyceae (41), Eugelinae (10) and Cyanophyceae (11)] were identified up to species level, rest 6 were identified only up to generic level (Table 1). The species Chlorophyaece *Chlorococcum infusionum, C. humicolo, Chaeaciosiphon rivularis, Pediastrum boryanum, P. simplex, P. tetras, Closteridium obesum, Chlorella vulgaris, Dispora cuneiformis, Botryococcus braunii, Ankistrodesmus falcatus, A. convolutus var. minutum, Actinastrum hantzschii, Selenastrum westii, Coelastrum morus, C. reticulatum, Crucigenia apiculata, C. tetrapedia, C. quadrata, Scenedesmus obliquus, S. dimorphus, S. bijgatus, S. bijgatus* var. *bicellularis, S. arcuatus, S. prismaticus, S. acutiformis, S. armatus, S. hystrix, S. tropicus, S. quadricauda* of Chlorophyceae are common. They are recorded as first hand report to the study area.

Out of 56 algae, 30 species of 11 genera belonging to Chlorophyceae. They are appears to be the most abundant. The species among the same genera also varies from place to place. Higher population of *Scenedesmus* and *Pediastrum* was observed during the survey. The species richness is seen in the pond sourround by Paddy (*Oryza sativa*) and Sugar cane fields (*Saccharum offinarum*) than in pond surround by mango (*Mangifera indica*), coconut (*Cocos nucifera*) and silky cotton (*Bombax ceba*) plantations.

Discussion

During the survey, it has been observed that the topography, altitudinal ranges and climate zone of Bodinayakanur provides a conductive environment for greater algal diversity. The diverse is influenced by geographical factor (Latitude, Altitude), environmental factor Fierer and Jackson (2006), were also observed that similar influences by geographical factor (latitude, altitude) and environmental factor.

In the present study observed that Chlorophyaece is the most species family. Similar observation were reported by several researchers and they have proposed temperature as a vital factor responsible for the growth of algae (Ramakrishnaiah and Sarhar 1982; Verma and Datta Munshi 1987; Kaushiket et al. 1991; Bohar and Kumar 1999). The growth and photosynthesis of algae are influenced by the pH and alkalinity of water (Brogueria 1987). Their studies corroborated with the observation of the present investigation of Bodinayakanur.

Pandey et al. (1995) recorded Chlorophyceae, Bacillariophyceae, Myxophyceae and Euglenophyceae in river Saura with Chlorophyceae being the most dominant group. Tiwari and Chauhan (2006) observed Chlorophyceae being the most dominant during winter, followed by Bacillariophyceae in Kitham Lake Agra. Synudeen Sahib (2011) recorded 35 species of phytoplanktons in the Parappar reservoir, Kerala.

Conclusion

The present study concludes that certain members of species in the Chlorophyceae and Cyanophyceae which are tolerant to organic pollution and resist the stress caused by pollutants. The presence of quality and quantity of the green algae indicates biologically non-polluted water resources of the study area. In the present study emphasizes the necessity of using phytoplankton as an effective and appropriate method of biomonitoring for evaluation of fresh water quality.

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Table 1: List of algal diversity from the study area

SI.No.	Family	Genus	Species
1	Chlamydomonadaceae	*Chlamydomonas*	*C. sphagnicola* (F.E.Fritsch) F.E.Fritsch & H.Takeda
2	Chlorococcacceae	*Chlorococcum*	*C. infusionum* (Schrank) Meneghini
3	Chlorococcacceae		*C. vitiosum* Printz
4	Characiosiphonaceae	*Characiosiphon*	*C. rivularis* Iyengar
5	Micractiniaceae	*Trochiscia*	*T. granulate* (Reinsch) Hansgirg
6	Hydrodictyaceae	*Pediastrum*	*P. duplex* var. *genuinum* (A.Braun) Hansgirg
7	Hydrodictyaceae		*P. duplex* var. *subgranulatum* Raciborski
8	Hydrodictyaceae		*P. tetras* (Ehr,) Ralfs
9	Hydrodictyaceae		*P. tetras* var. *tetraodon* (Corda) Hangirg
10	Hydrodictyaceae	*Sorastrum*	*S. americanum* (Bohlin) Schmidle
11	Hydrodictyaceae	*Tetraedron*	*T. minimum* (A.Braun) Hansgirg
12	Hydrodictyaceae		*T. incus* (Teiling) G.M.Smith forma *decolorata* (Defl)
13	Hydrodictyaceae	*Closteridium*	*C. obesum* (W.et G.S.West) G.M.Smith
14	Chlorellaceae	*Chlorella*	*C. vulgaris* Beijerinck
15	Chlorellaceae		*C. parasitica* (Brandt) Bejierinck
16	Dictyosphaeriaceae	*Dimorphococcus*	*D. cordatus* Wolle
17	Selenastraceae	*Ankistrodesmus*	*A. falcatus* (Corda) Ralfs var. *acicularis* (A. Braun) G.S.West
18	Selenastraceae		*A. convolutes* Corda var. *minutum* (Naeg.) Rabenhorst
19	Selenastraceae	*Selenastrum*	*S. westii* G.M. Simth

20	Selenastraceae	*Monoraphidium*	*M. pusillum* (Printz) Komárková-Legnorová
21	Coelastraceae	*Coelastrum*	*C. reticulatum* (Dangeard) Senn
22	Senedesmaceae	*Crucigenia*	*C. apiculata* (Lemn.) Schmidle
23	Senedesmaceae		*C. rectangularis* (A.Braun) Gay
24	Senedesmaceae		*C. tetrapedia* (Kirchner) W. et G.S. West
25	Senedesmaceae		*C. quadrata* Morren
26	Senedesmaceae	*Scenedesmus*	*S. muzzanensis* Huber-Pestalozzi
27	Senedesmaceae		*S. obliquus* (Turpin) Kuetzing
28	Senedesmaceae		*S. dimorphus* (Turpin) Kuetzing
29	Senedesmaceae		*S. bijugatus* (Turpin) Kuetzing
30	Senedesmaceae		*S. bijugatus* forma *irregularis* Wille
31	Senedesmaceae		*S. bijugatus* forma *parvas* (G.M.Smith) comb.
S.No.	FAMILY	GENUS	SPECIES
32	Senedesmaceae	*Scenedesmus*	*S. arcuatus* var. *capitatus* G.M.Smith
33	Senedesmaceae		*S. arcuatus* var. *capitatus* G.M.Smith
34	Senedesmaceae		*S. prismaticus* Bruhl and Biswas
35	Senedesmaceae		*S. hystrix* Lagerhein
36	Senedesmaceae		*S. spinulatus* Biswas
37	Senedesmaceae	*Scenedesmus*	*S. brasiliensis* Bohlin
38	Senedesmaceae		*S. quadricauda* (Turpin) Brebisson
39	Cheatophoraceae	*Draparnaldiopsis*	*Draparnaldiopsis sp.*
40	Zygnematophyceae	*Zygnemopsis*	*Z. indica* Randhawa
41	Zygnematophyceae		*Z. cylindrosporum* Czurda
42	Zygnematophyceae	*Spirogyra*	*S. singularis* Nordstedt
43	Zygnematophyceae		*S. submaxima* Transseau
44	Zygnematophyceae	*Sirogonium*	*S. sticticum* (Eng.Bot.) Kuetz
45	Surirellaceae	*Surirella*	*S. tenera* Greg.
46	Euglenaceae	*Euglena*	*E. viridis* (O.F.Müller) Ehr.
47	Euglenaceae	*Phacus*	*P. iyengarii* Suxena
48	Euglenaceae		*P. lismorensis* Playf.
49	Euglenaceae		*P. pleuronectes* (O.F. Müller) Dujardin emend.
50	Euglenaceae		*P. hamatus* Pochmann, Arch. Protistenk.
51	Euglenaceae		*P. orbicularis* Hübner emend.
52	Eugleniaceae	*Phacus*	*P. polytrophos* Pochmann, Arch. Protistenk.
53	Euglenaceae		*Phacus sp.*
54	Scytonemataceae	*Scytonema*	*Scytonema sp.*
55	Oscillatoriaceae	*Oscillatoria*	*Oscillatoria sp.*
56	Spirulinaceae	*Spirulina*	*Spirulina sp.*

New distributional novelties of *Selaginella* sp. Beauve. in Southern Eastern Ghats of India

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ABSTRACT: *Survey was conducted in the Yercaud Hills of Eastern Ghats in South India at bimonthly intervals during the peroid 2012 to 2014. The showery water species viz. Selaginella opaca Warb., Selaginella eurynota A. Braun., Selaginella arbuscula (Kaulf.) Spring. and Selaginella miniatospora (Dalz.) Bak. are reported as new distribution to the South India. In the present study, a detailed taxonomic description, distribution, potency of ethnomedicinal uses of these newly recorded species are provided.*

Key words: *New Distribution, Showery water, Selaginella, India.*

Introduction

Selaginella a solitary genus belonging to the family Selaginellaceae, constitutes a natural and distinctive genus of about 700 species, chiefly occurring in tropical and subtropical regions throughout the world. The only comprehensive work on the Ferns of South India by Beddome (1864) included 271 species of ferns from South India and Sri Lanka. Kunze (1851) listed five species from the Nilgiri hills of the Southern Western Ghats. Alston (1945) enumerated 58 species of *Selaginella*, of which 45 species were reported from India. Meahra and Bir (1964) and Reed (1965-66) reported the occurrence of a few additional species from India. Dixit (1984) and Manikam and Irudayaraj (1992) reported 18 species from South India. Out of 18 species, 14 species were reported only from Eastern Ghats of Andhra Pradesh and Tamil Nadu.

Recently 4 species of the genus *Selaginella* from potential showery/rocky wetland was collected during the floristic studies of the Yercaud hills of the Eastern Ghats in South India. On critical examination and careful scrutiny of relevant literatures (Alston 1945; Baishya and Rao 1982; Barua et al. 1989; Bhattcharya et al. 1995; Bhattcharya et al. 1998; Bir 1976, 1987, 1993; Bir et al. 1992, 1989; Borthakur et al. 2001; Dixit 1984, 1992; Dixit and Vohra 1984; Dutta et al. 1980; Fraser-Jenkins 2008; Handique and Konger 1986; Islam 1983; Jain 1991; Kachroo et al. 1989; Kaur and Chandra 1994; Manickam and Irudayaraj 1992; Mukhopadhyay 2001; Nath and Bhattacharya 2002; Panigrahi 1960; Panigrahi and Choudhury 1962; Panigrahi and Dixit 1967; 1967a, 1968; Singh and Panigrahi 2005; Thakur 1962; Roy and Borthakur

2013). They were identified as *Selaginella opaca* Warb., *Selaginella eurynota* A. Braun., *Selaginella arbuscula* (Kaulf.) Spring. and *Selaginella miniatospora* (Dalz.) Bak.

Materials and Methods

The present investigation was undertaken with a view of potential rocky wetland species of *Selaginella* P. Beauve. of the Yercaud hills in Eastern Ghats of South India. Several field trips were made from 2011 to 2015, covering different seasons, in order to know the phenology of the plants which are included for the present study.

Yercaud got its name from the Tamil words, Yeri and Kaadu, meaning lake and forest respectively. Yercaud is located in a part of Sanyasimalai Reserve Forest of Shevaroy Hills, North-East of Salem, Tamil Nadu between 11˚45′56″ N latitude, 78˚17′55″ E longitude and altitude of 1515 meters (4970 ft) above sea level. The Shevaroy hills occupy an area of about 150 square miles (390 Sq. km). The highest point in Yercaud Taluk is Shevaroy Temple, which is situated at a height of 5326'. The average elevation of the mountain range is about 600 m and the highest peak is Shevaroy Hills that reaches up to a height of 1700 m. Several forest vegetation types including tropical deciduous, mixed dry deciduous, dry evergreen forest occur in the region.

Exploration and plant collection

The native tribal people of Yercaud or Shevaroys are called Malayalis. "The Malayali denotes inhabitant of the hills (Malai = Hill or Mountain Alu = Person) or (Malai = Hill, Ali=Dwells or Inhabits) or Mountain Man. The Malayalis living in the Kollimalai, Sheveroys and Pachaimalai. Most of the Malayali tribes have a general knowledge of medicinal plants that are used for first aid remedies, treatment of various diseases.

During the field trips plants were collected with exhaustive information regarding their use by the tribals of Malaiyali and as well as elderly men and women in Yercaud hills in Eastern Ghats of South India. Most plant taxa were collected and made in the form of herbarium specimens (including both dry and spirit specimens), but some species were only observed and noted. Ecological data, habitats, habit and some diagnostic characters of each specimen, such as color, smell, etc. were noted and photographs were taken in the field. Ethno botanical data was collected along various lines in different manners - by enquiry, observation, interview and participation. Repeated visits also helped to get specific additional information that was not mentioned throughout the first interviews.

Results

Evergreen creeping heterophyllus lycophytes without erect or ascending stems. Stems 20-30cm, slender, palebrown on drying, creeping, undersides and upper sides, irregularly and dichotomously branched, branches copiously pinate, lax. Rhizophores, wiry, originating from basal side of stem of branching, 0.2 mm dia., axillary trophophylls present at branching. Trophophylls conspicuously dimorphic, arranged in 4 ranks (2- dorsal and 2- ventral) ascending, distant on the main stem contiguous on the branches, dark green membranous, margin entire. Ventral (lateral) trophophylls lanceolate, entire, obtuse at base, slightly oblique at apex, 0.2 × 0.1 mm, sparsely ciliate margin; dorsal (ventral) trophophylls broadly ovate, 3 × 1.25 mm, obtuse at base, acute at apex, entire, ciliate of margins. Strobili always solitary, tetragonus, 10×0.2mm, sporophylls tetragonous, dimorphic, resupinate ciliate, larger trophophyllus ovate-oblong, subacute, smaller sporophylls lanceolate, acuminate at apex. Megaspores pale yellow. Microspore orange.

Phenology

Sporophylls are formed during November on wards and sorus mature in January. *Selaginella opaca* Warb morphologically looks more or less like *Selaginella proniflora* (Lam.) Bak. of India and *Selaginella lutchuensis* Koidz of Taiwan. It superficially resembles the former in general habit and it has lateral leaves similar to those of the latter. However, it can be easily distinguished from *S. proniflora* (Lamk.) Bak. and *S. lutchuensis* Koidz by its leaf shape, glabrous leaves and solitary tetragonous strobili. Usually grows on the showery or wet rocky slopes with some wed shaded rock surface areas. It also used as a medicinal plant for curing wounds, menstrual disorders, post- child birth and to increase stamina. Malayali tribes used alternative sours of antidiabetic. It also eaten by monkeys and ants in Yercaud hills.

Selaginella eurynota terrestrial, heterophyllous throughout; stems prostrate or decumbent, branched throughout, articulate; rhizophores springing from the upper surface of the stem for V3-3/4 of its length; lateral leaves commonly spreading from the axes at 90 angles, 3-6 mm long, narrowly oblong to elliptic–lanceolate, acute or subacute, the margins entire to serrulate (sometimes ciliolate towed base and then the cilia 0.1-0.2 mm long), slightly to deeply cordate at base, the basal acroscopic lobe commonly broadly arching over and directed down along the axis; axillary leaves liner-to elliptic-oblong, the margins subentire to serrulate, the base slightly to deeply cordate with the lobes subparallel, contiguous (rarely imbricate) and usually abundantly ciliolate (cilia 0.1-0.2 mm long); median leaves 1.5-2.5 mm long, obliquely ovate–acuminate, the margins entire to serrulate, peltate, i.e., a single

rounded to subacute lobe like projection extending below the point of attachment, this ca. V3-V4 the length of the leaf; strobili 5-13 mm long, acute or acuminate, slightly to sharply carinate; megaspores yellowish or beige, with strongly pronounced, white, reticulate ridges; microspores tawny.

Sporophylls are formed during November onwards and sorus is matured on December to January. *Selaginella eurynota* A. Br. is closely allied to *S. horizontalis* (Presl) Spring. and *S. adunca* A. Br ex. Hieron but differs from the former in possing the basal lobes of axillary leaves mostly imbricate and bearing alia 0.3 to 0.5 mm long and from the latter in rhizophores springing from the upper surface of the stem for V3-3/4 of its length. Usually grows on the showery or wet rocky slopes with some wed shaded rock surface areas. The attractive plant structure and fascinating greenish colour can be recommended to grow as an ornamental plant in residents, park and also in rock gardens. Leaf paste used as curing wounds.

Selaginella arbuscula **e**vergreen creeping, medium sized, terrestrial, dark green leaves, ovate to ovate-lanceolate, long acuminate, tips acute, bipinnate, sori are arranged in many ways on the undersides of the leaf. Strobili at tips of branches. Stems erect from decumbent bases, unbranched in lower ½ and pinnately branching abundantly above (occasionally branching near base). Usually flattened in one plane, *c.*6.60 cm long, without swollen joints, blade like portion ranging from much branched and lanceolate, dark green to light green rhizophores restricted to bases of stems (rarely, along, lower ¼), often in conspicuous, fili form, less than 3cm long, *c.*0.2 mm in diameter; Sterile leaves on main stem of 2 kinds in 4 rows, slightly separated at bases becoming more imbricate in upper stems and branches, lateral leaves flat to firm, convex to ovate – oblong, *c.*2-6x1-3.5 mm, membranous, tip acute, apiculate to long–acuminate; ovate, trophophylls ovate-lanceolate to narrowly triangular, *c.*1-3x0.5-1.5 mm tips acute; fertile leaves ovate to ovate-lanceolate, keeled, long acuminute, tips acute. Strobili at tips of branches, narrower than stems, *c.*3-50 mm long, square. Sporophylls are formed to December & Sorus matured January to February. The *Selaginella arbuscula* grows intermingled with *S. umbrosa* and gives an similar appearance, probably due to this, it might have been remained unnoticed. It is closely allied to *S. umbrosa* in its overall morphology. However, the former differs from the latter in the presence of solitary strobili, branching from the base and rhizophores arising from the stems. Usually grows on the showery or wet rocky slopes with some wed shaded rock surface areas. Malayali tribes use the leaf paste mixed with cow milk to treat skin infection.

Selaginella miniatospora (Dalz.) Bak., semi-erect, plant body *ca.* 5-20 cm, terrestrial, found growing on the forest floor or on the inclined bases of hills or on the humus

deposits on the rock surface. Light green young plants become pale-green to pale-brown at maturity. Primary stem is copiously-branched and the branches are erecto-patent. Rhizophores born and remain confined at the base of the primary stem. Vegetative leaves dimorphic, light green, distantly placed on the primary stem and branches exception at terminal position, denticulate, denticules minute and occur distantly; axillary leaves *ca.* 2.5-3x1.50-2mm, ovate, cordate, obtuse, entire, prominent mid-vain extends from the base to nearly apex; lateral leaves *ca.* 2.5-3x1-1.25mm, ovate oblong, obliquely-cordate at base and obtuse, inner-half of leaves semi-ovate, imbricated at base, outer-half of leaves semi-oblong; median leaves *ca.* 1-1.25x0.41-0.5mm, very small in comparison to other vegetative leaves, ovate, denticulate, cuspidate, apex acute. Ligule thin, membranous and lanceolate. Strobilli *ca.* 5-7.5X2-2.5mm, one or two at branch apices. Sporophylls dimorphic; larger sporophylls *ca.* 1.75-2x0.40.5mm, lanceolate-rhomboid, minutely but regularly dentate, an extra membranous structure called laminar flap develop from the base of the sporophyll and extends to one third of its length; smaller sporophylls *ca.* 0.50.85x0.35-0.41mm, semi-orbicular, cuspidate and uniformly dentate from the upper half of the leaf to apex. Microspore 20-30 µm, deep-yellow, verrucoid and trilete. Megaspore 180-225 µm, deep-yellow. Phenology**:** November to February. Endemic to India (Dixit 1992). Usually Epiphyte or chasmo endolithophytic (rock crevies) high altitude fern growing along wetland or slope. Fine particles of soil and rock that fill the space among root and rock conditions make good interaction for water flow.

Conclusion

Selaginella opaca Warb. species distributes in Java, Philippines, Sumatra, Lombok, New Guinea, Ceram and Indonesia. *Selaginella eurynota* A. Braun. species report in Nicaragua, Costa Rica, Mexico, Guatemala and Panama. *Selaginella arbuscula* (Kaulf.) spring. also report in United States, Makawao Forest Reserve, Maui, Hawaii (Endemic), Kauai, Oahu, Molokai, Lanai and Sandwich Islands and *Selaginella miniatospora* (Dalz.) Bak. occur in Maharastra, Goa, South Kanara, Assam but now first time we are collected this four species from rocky wetland or slopes in Yercaud Hills of Eastern Ghats in south India. *Selaginella opaca* Warb., *Selaginella eurynota* A. Braun. and *Selaginella arbuscula* (Kaulf.) Spring. are new distributional record to India. *Selaginella miniatospora* (Dalz.) Bak. are new distributional to Tamil Nadu. In this study, a detailed taxonomic description, distribution of the newly recorded taxa are provided. The present study concluded that, this treasure of information is gradually vanishing in the near future due to lake of interest among the younger generations of tribal people as well as their tendency to migrate to

cities for luxuriant jobs. These studies assume great importance in enhancing our traditional skills and technology about the plant grows and used for native or tribal communities for their sustenance. Ethnobotanical studies documented the indigenous knowledge and also paved the way for the conservation of biological resources as well as their sustainable utilization.

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Present Status of RET gymnosperms conserved in MBGIPS

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***ABSTRACT**: Conservation of RET plant species is an important issue. The present study deals with the present status of RET gymnosperms conserved at MBGIPS. Out of 12 gymnosperms, 9 come under RET category. Among these 4 are trees, 4 are shrubs, 3 are geophilous woody herbs and one liana.*

***Key words:** RET gymnosperm, MBGIPS, Conservation*

Introduction

Gymnosperms are the most ancient seed plants whose seeds are not enclosed by a ripened ovary. Most species are woody plants or shrubby habits but some are climbers in tropical environment. Many gymnosperms have become extinct today and represented only by their fossil records. Extinction may be due to habitat loss, pollution, pathological factors, forest fires, climate change and degradation. The representatives of both fossil and living gymnosperms are Cycadales, Ginkgoales, Coniferales and Gnetales. Living gymnosperms are widely distributed in cold climates of the temperate as well as in the dry arid conditions of the tropics. In India they are mostly distributed in Himalayas in temperate/montane climate.

Gymnosperms play an important role in the preservation of the environment. Some members are resistant to pollution (Sahni 1990). They are of considerable economic importance as sources of wood and wood- derived products. Now a days, plant population becomes declined due to various reasons. List of RET plants in Red Data Book rise, and increase day by day. Therefore, the need of conservation is very high. There is less focus on the conservation of gymnosperms than angiosperm. So conservation of these plants is very important before they are lost forever. In Kerala only 3 natural species of gymnosperms occur viz., *Cycas circinalis, Gnetum edule, Nageia wallichiana.*

MBGIPS is a Kerala Govt. Institution for conservation and research in Botany. The thrust areas of conservation are: a) Aquatic Plants b) Primitive Plants such as pteridophytes, Bryophytes, gymnosperms, etc. The gymnosperms conserved in the MBGIPS are given in Table 1.

Materials and Methods

Materials were collected from various sources, including private collections of plant lovers and introduced in to Gymnosperm Section of the MBGIPS. Care was taken to provide suitable condition as much possible to these introduced exotic plants and for their regular maintenance.

Results

The threatened species are enumerated below with their botanical name, family, status, distribution and taxonomic notes.

***Araucaria heterophylla* (Salisb.) Franco** [ARAUCARIACEAE]

Status : Vulnerable

Distribution : Endemic to Australia

Taxonomic notes : Conical (young) trees. Branches whorled, horizontal or pendent; branchlets mostly distichous, horizontal or drooping. Juvenile leaves awl-shaped, curved, 4-angular, apex sharp pointed; leaves on older stems broadly lanceolate or ovate, dorsally flattened.

***Cycas circinalis* L.** [Cycadaceae]

Status : Endangered

Distribution : Indo Malaysia & Tropical East Africa

Taxonomic notes : Large shrub to small trees with terminal crown of large pinnately compound leaves; dioecious. Microsporophylls densely aggregated to form large terminal cones. Megasporophylls loosely arranged, crowded round the apex of stem; ovules 1-5 on either side of sporophyll. Seeds globose.

***Cycas revoluta* Thunb.** [CYCADACEAE]

Status : Least Concern

Distribution : Native of East Asia

Taxonomic notes : Palm like shrubs. Leaves recurved, pinnate, dark green and very stiff leaflets; dioecious. Trunk is dark brown and thick. The microsporophylls are large yellow cone that reaches lengths of up to 2

feet. Megasporophylls loosely arranged, yellow in colour. Seeds globose and covered by a dense mat of yellowish hairs.

***Cupressus macrocarpa* Hartw.*ex* Gordon** [CUPRESSACEAE]

Status : Vulnerable

Distribution : Native of North America

Taxonomic notes : Trees; branches hortizontal, obscurely whorled; bark reddish-brown, ridged; branchlets 2-pinnate, alternate, ascending, 4-angled. Leaves 3-angular, scale-like. Male catkins oblong; female catkins club-shaped, brownish, peltate, scales each with numerous ovules. Cones subglobose, brown when ripe. Seeds 16-20 per scale, narrowly winged, tubercled.

***Dioon edule* Lindl.** [ZAMIACEAE]

Status : Near Threatened

Distribution : Endemic to Mexico

Taxonomic notes : Palm like shrub. Leaves pinnate and no spines on the borders of leaflets. Spines are present on juvenile leaves. Female cones are densely tomentose.

***Encephalartos lehmannii* Lehm.** [ZAMIACEAE]

Status : Near Threatened

Distribution : Endemic to South Africa

Taxonomic notes : Palm like shrub. The leaves are up to one hundred and fifty centimetres long, blue or silver and strongly keeled. The leaflets are lanceolate, do not overlap each other and have smooth margins. The male cones are green or brown and sub – cylindrical. The female cones are barrel – shaped and green or brown in colour. The seeds are deep red.

***Gnetum edule* (Willd.) Blume** [GNETACEAE]

Status : Least Concern

Distribution : Peninsular India

Taxonomic notes : Woody lianas. Leaves opposite, elliptic-ovate or oblong, acute, base rounded or acute. Panicles axillary arising from mature wood; bracts cupular; male strobilus up to 5 cm long; stamens 1, exserted; female strobilus to 7 cm long; ovary globose. Fruit ellipsoid, yellow.

***Pinus patula* Schl. & Cham.** [PINACEAE]

Status : Least Concern

Distribution : Central and East Mexico

Taxonomic notes : Tree; bark reddish-brown. Leaves in 3s, margins serrulate, apex acute. Staminate cones oblong, to clustered. Microsporophylls obovoid-oblong. Pistillate cones 2-8-clustered, broadly ellipsoid, later ovoid-ellipsoid, pale brown. Ovuliferous scales broadly obovate becoming obovate-oblong; exposed part rhomboidal, concave with 2 shallow furrows at right angles to each other, apex mucronate; bract-scale obcordate.

***Platycladus orientalis* (L.) Franco** [CUPRESSACEAE]

Status : Near Threatened

Distribution : East Asia

Taxonomic notes : Small trees; bark reddish brown to light grayish brown, thin, flaking in long strips; crown ovoid-pyramidal when young. Leaf apex bluntly pointed; facial leaves rhomboid, with a conspicuous, linear, glandular groove at center abaxially; lateral leaves overlapping facial ones, boat-shaped, ridged, apex slightly incurved. Male cones yellowish green, ovoid. Seed cones when immature bluish green, subglobose; when ripe reddish brown, subovoid. Seeds grayish brown or purplish brown, ovoid or subellipsoid.

***Stangeria eriopus* (Kunze) Bail.** [STANGERIACEAE]

Status : Vulnerable

Distribution : Endemic to South Africa and Southern Mozambique

Taxonomic notes : Geophilous woody herbs with a subterreanean, carrot-shaped stem. Mature leaves pinnate, fern-like, erect or arching, dark green, glabrous; leaflets linear-lanceolate to lanceolate. Male cones narrowly cylindrical, solitary on each growing point, covered with short silver hairs when young,

yellowish brown when mature; Female cones ovoid to ellipsoid, covered with silvery hairs when young, dark green and less hairy at maturity. Seeds ovoid to obovoid.

***Zamia fischeri* Miq.** [ZAMIACEAE]

Status : Endangered

Distribution : Endemic to Mexico

Taxonomic notes : Geophilous woody herbs. Stem sub globose. The leaves are about smaller about 15 to 30 centimeters long; the petioles are 5 to 10 centimeters long, and the rachis has 5 to 9 pairs of leaflets. The leaflets are papyraceous, tapering toward the base, and are acute apically with margins having several serrations in the outermost half. The cones are greenish-gray to gray, cylindrical to ovoid-cylindrical in shape. Seed is red coloured about 1.3 to 1.8 centimeters long and 0.5 to 0.8 centimeters in diameter.

***Zamia furfuracea* L.f.** [ZAMIACEAE]

Status : Endangered

Distribution : Endemic to Mexico

Taxonomic notes : Geophilous woody herb. The plant with subterranean trunk. The leaves radiate from the center of the trunk; the leaflets are toothed toward the tips. The circular crowns of leaves resemble fern or palm fronds. Female cone is rusty-brown and egg shaped. Male cones are smaller and clustered.

Discussion

India has one of the richest biodiversity centres in the world. But recently this biodiversity faces Becomes challenges due to anthropogenic activities and climatic changes. As a result, our plant population becomes depleted. These factors also have affected the gymnosperm population throughout the world.

9 RET gymnosperms are conserved in MBGIPS. Among these *Cycas circinalis*, *Zamia fischeri*, *Zamia furfuracea* are endangered. According to Vandana Krishnamoorthy et al. (2013) land clearing and illegal collection of young leaves and seeds leads to the destruction of original habitat of *Cycas circinalis.* Habitat destruction and over collection of plants for ornamental purpose is the main threats of *Zamia fischeri* & *Z. furfuracea. Araucaria heterophylla, Cupressus macrosperma, Stangeria eriopus* are

vulnerable due to logging, land clearance, over grazing and illegal collection of plant parts for medicinal purposes. *Dioon edule, Platycladus orientalis, Encephalartos lehmannii* are near threatened. According to Vovoides and Iglesisa (1994) the wild population of *Dioon edule* was declined due to habitat destruction and illegal collection of buds. Exploitation for timber in natural habitat is the main threat of *Platycladus orientalis* and illegal exploitation of *Encephalartos lehmannii*. *Cycas revoluta, Gnetum edule* and *Pinus patula* of the category Least Concern are also conserved at MBGIPS, their current population is stable.

Gymnosperms are among the economically most important plant species especially because of their timber and other forest produces. Compared to other groups of plants, very little research has been done on gymnosperms. Rare and threatened gymnosperms of some regions have been studied by some workers like Loren (2004) from Mexico, Vovides and Iglesias (1994) from Mexico, Chamberlam (1935) from Chicago, Uniyal and Aswathi (2000) from Uttar Pradesh, Reddy et al. (2007), Singh et al. (2008), Tripathi (2008) from Nainital, Tewari et al. (2010) from Uttarakhand, Ranjay (2010), Singh et al. (2011), Prasad Rao et al. (2014) and Rita Singh et al. (2015) from Andhra Pradesh. Majority of the gymnosperms are included in IUCN red list category. So conservation of these RET plants has become the need of the time. The best ways of conservation is the *in situ* and *ex situ* conservation. MBGIPS is committed to the conservation of lower plants, gymnosperms and angiosperms. This study reveals that there are many gymnosperms are presumably in danger of extinction. As a part of a conservation programme these RET gymnosperms are conserved in *ex situ* and recover, multiply and reintroduce these plants by tissue cultures. Also inducing the awareness among the peoples can promote the knowledge about importance of the diversity and existence of these RET plants.

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Table 1: Gymnosperm Conserved at MBGIPS

Sl. No.	*Botanical Name*	Status
1.	*Araucaria heterophylla* (Salisb.) Franco	Vulnerable
2.	*Cycas circinalis* L.	Endangered
3.	*Cycas revoluta* Thunb.	Least Concern
4.	*Cupressus macrocarpa* Hartw.*ex* Gordon	Vulnerable
5.	*Dioon edule* Lindl.	Near Threatened
6.	*Encephalartos lehmannii* Lehm.	Near Threatened
7.	*Gnetum edule* (Willd.) Blume	Least Concern
8.	*Pinus patula* Schl. & Cham.	Least Concern
9.	*Platycladus orientalis* (L.) Franco	Near Threatened
10.	*Stangeria eriopus* (Kunze) Bail.	Vulnerable
11.	*Zamia fischeri* Miq.	Endangered
12.	*Zamia furfuracea* L.f.	Endangered

Conservation of Bryophytes in Malabar Botanical Garden and Institute for Plant Sciences, Kerala, India

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***ABSTRACT**: Malabar Botanical garden and Institute for Plant Sciences, Kozhikode-14 conserves aquatic as well as lower group plants (Bryophytes and Pteridophytes) especially the plants of Western Ghats of South India. At present 65 species of bryophytes are conserved here. Of these 20 belong to Liverworts (Marchantiopsida) 2 belong to Hornworts (Anthoceratopsida) and 43 belong to Mosses (Bryopsida). All bryophytes are provided with optimum moisture using foggers.*

***Key words**: MBGIPS, Conservation, Bryophytes*

Introduction

Bryophytes are ubiquitous in the wet terrestrial environment and are considered to be the pioneer land plants. They are the second largest group of land plants next to flowering plants and are considered to be 'Amphibians of Plant Kingdom' due to their preference to aquatic or wet habitats. This group includes three distinct lineages viz., Liverworts, Hornworts and Mosses. They inhabit in a variety of microhabitats such as soil (terrestrial), bark (epiphytic), leaves (epiphyllous), rocks (lithophytes) and water (aquatic) and play an important role in ecosystem functions such as sequestering nutrients, retaining water, regulating the soil microenvironment and acting as carbon sinks (Vitt 2000). Like other plant groups, bryophytes are threatened by habitat loss, pollution, over exploitation, invasive species and other factors. Several species of bryophytes (*Orthotrichum truncato-dentatum* and *Dactylolejeunea acanthifolia*) have been found extinct in the wild for many years and because of the depletion of their original habitat. Even though there has been less focus on bryophytes than on seed plants by botanical gardens and other conservation organization, perhaps because they are often less charismatic or less well known than seed bearing taxa, and their identification requires specialised expertise. Malabar Botanical Garden and Institute for Plant Sciences have an important role in the conservation and related research work of lower plants.

Materials and Methods

Collections are made systematically in different seasons made from all major National parks, Bio reserve and other protected areas of Kerala State. Collections were done with all necessary required items in the field. Photographs were taken from the field using SLR Sony α 100 camera and altitude was calculated using Garmin 680. Epiphytic species were collected by climbing up trees as high as possible. High canopy species were collected from fallen branches. Corticolous and lithophytic species were collected along with the substratum. Two or more species usually grow together and hence were collected by giving the same field number. Field data including date, locality, habitat, altitude, etc. were noted down in the field book. The specimens kept in paper packets usually remain fresh and alive for few days and so that can be conserved them alive. Identification of specimens was done after careful examination using literature. All the collected specimens are introduced to the Bryophyte-Pteridophyte conservatory (Apushpi) of MBGIPS. The lithophytic species are kept on rocks and epiphytic species kept in the husk of coconut and terrestrial species are kept in round bottomed pot keeping vertically. In the conservatory maintaining suitable microhabitat for each species and provide microclimate with the help of automated fogger system.

Results

The collected species are planted in the conservatory and many species are well established. At present a total 66 plants are maintained in the conservatory including 20 Liverworts, 2 Hornworts and 44 Mosses. They are well labelled with species name and family. The species name, family and habit of each conserved species are mentioned in Table 1.

Discussion

The present work reveals that a total 66 plants were established in conservatory. Among these, some species are rare eg: *Calycularia crispula* Mitt. is a new distributional record to Western Ghats of Peninsular India. The study also showed that many high altitudinal species are established in the conservatory shows their ecological adaptability under controlled conditions. The well-established species are: *Asterella khasiana* (Griff.) Grolle, *Dumortiera hirsuta* (Sw.) Nees, *Cyathodium cavernarum* Kunze, *Pallavicinia lyellii* (Hook.) Gray, *Hyophila involuta* (Hook.) *A. jaeger*, *Philonotis hastata* (Duby) Wijk & Margod. etc. The International Union for the Conservation of Nature (IUCN) Red List (Bold et al. 1980) listed 92 species of bryophytes as threatened worldwide. In situ conservation should be the primary

focus for conserving these threatened species (Hallingback and Tan 1996; Wagner 1995), ex situ growth and germplasm storage is also equally important complementary aspects of plant conservation (Given 1987; Laliberte 1997). Species preserved ex situ could serve as important resources for research on various aspects such as reproduction and growth, physiology, medicinal properties, etc. for conservation programs. It will be advantageous to the students and teachers of biology and nature lovers to observe the live specimens of bryophytes conserved at MBGIPS.

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Table 1: List of Bryophytes conserved in MBGIPS

Species	Family	Habitat
	LIVERWORTS	
Asterella khasiana (Griff.) Grolle	Aytoniaceae	Terrestrial, Lithophytic
Calycularia crispula Mitt.	Calyculariaceae	Lithophytic
Cheilolejeunea intertexta (Lindenb.) Steph.	Lejeuneaceae	Epiphytic
Cyathodium cavernarum Kunze.	Targioniaceae	Terrestrial
Dumortiera hirsuta (Sw.) Nees	Marchantiaceae	Terrestrial, Lithophytic
Fossombronia cristula Austin	Fossombroniaceae	Terrestrial
Heteroscyphus argutus (Nees) Schiffner	Geocalycaceae	Epiphytic, Lithophytic
Heteroscyphus hyalinus (Stephani) A. Srivast. & S. C. Srivast	Geocalycaceae	Epiphytic,Lithophytic
Marchantia linearis Lehm. & Lindenb.	Marchantiaceae	Terrestrial, Lithophytic
Marchantia polymorpha L.	Marchantiaceae	Terrestrial, Lithophytic
Pallavicinia indica Schiffn.	Pallaviciniaceae	Terrestrial, Lithophytic
Pallavicinia lyellii (Hook.) Gray	Pallaviciniaceae	Terrestrial, Lithophytic
Plagiochila chinensis Steph.	Plagiochilaceae	Terrestrial, Lithophytic
Plagiochila arbuscula Lindenb.	Plagiochilaceae	Terrestrial, Lithophytic
Radula obscura Mitt.	Radulaceae	Coticolous
Riccardia multifida (L.) Gray	Aneuraceae	Terrestrial, Lithophytic
Riccardia tenuicostata Schiffn.	Aneuraceae	Terrestrial, Lithophytic
Riccia discolour Lehm. & Lindenb.	Ricciaceae	Terrestrial
Riccia frostii Austin	Ricciaceae	Terrestrial
Targionia hypophylla L.	Targioniaceae	Terrestrial
	HORNWORTS	
Anthoceros angustus Steph.	Anthocerotaceae	Lithophytic
Anthoceros crispulus (Montin) Douin.	Anthocerotaceae	Terrestrial
	MOSSES	
Atrichum undulatum (Hedw.) P.Beauv.	Polytrichaceae	Terrestrial
Aerobryum speciosum Dozzy & Molk.	Meteoriaceae	Lithophytic
Anomobryum auratum (Mitt.) A. Jaeger	Bryaceae	Lithophytic
Bryum capillare Hedw.	Bryaceae	Terrestrial, Lithophytic
Bryum cellulare Hook.	Bryaceae	Lithophytic
Bryum coronatum Schwaegr.	Bryaceae	Terrestrial, Lithophytic

Bryum pseudotriquetrum (Hedw.) G. Gaertn., B. Mey. & Scherb.	Bryaceae	Terrestrial, Lithophytic
Bryum wightii Mitt.	Bryaceae	Lithophytic
Bryum billardierii Schwagr.	Bryaceae	Terrestrial
Calymperes afzelii Sw.	Calymperaceae	Epiphytic ,Corticolous
Calymperes burmense Hamp.	Calymperaceae	Epiphytic ,Corticolous
Calymperes tenerum Muell. Hal.	Calymperaceae	Epiphytic ,Corticolous
Campylopus flexuosus (Hedw.) Brid.	Dicranaceae	Terrestrial, Lithophytic
Fissidens anomalous Mont.	Fissidentaceae	Terrestrial
Fissidens ceylonensis Dozzy & Molk.	Fissidentaceae	Terrestrial
Fissidens crispulus Brid.	Fissidentaceae	Terrestrial, Lithophytic
Fissidens zippelianus Dozy &Molk.	Fissidentaceae	Terrestrial
Floribundaria chrysonema (Mull.Hal.) Broth.	Meteoriaceae	Lithophytic,Epiphytic
Floribundaria floribunda(Dozzy & Molk.) M. Fleisch.	Meteoriaceae	Lithophytic,Epiphytic
Funaria hygrometrica Hedw.	Funariaceae	Terrestrial
Himantocladium plumula (Nees) M. Fleisch.	Neckeraceae	Epiphytic
Homaliodendro nexiguum (Bosch. & Sande-Lac.) M. Fleisch.	Neckeraceae	Epiphytic
Homaliodendron flabellatum (Sm.) Fleisch.	Neckeraceae	Epiphytic
Hyophila involuta (Hook.) A. Jaeger	Pottiaceae	Terrestrial, Lithophytic
Hypnum macrogynum Besch.	Hypnaceae	
Leucobryum juniperoideum (Brid.) Mull. Hal.	Leucobryaceae	Corticolous
Leucoloma amoene- virens Mitt.	Dicranaceae	Epiphytic, corticolous
Leucolomat aylorii (Schwagr.) Mitt.	Dicranaceae	Epiphytic, corticolous
Macromitrium sulactum (Hook.) Brid.	Orthotrichaceae	Epiphytic, corticolous
Macromitrium turgidum Dix	Orthotrichaceae	Epiphytic, corticolous
Macrothamnium macrocarpum (Reinw. & Hornsch.) M. Fleisch.	Hylocomiaceae	Epiphytic, corticolous
Meteoriopsis squarrosa (Hook. ex Harv.) M. Fleisch	Meteoriaceae	Epiphytic, corticolous
Octoblepharum albidum Hedw.	Leucobryaceae	Epiphytic, Corticolous
Philonotis hastata (Duby) Wijk & Margod.	Bartramiaceae	Terrestrial, Lithophytic
Philonotis mollis (Dozy & Molk.)	Bartramiaceae	Terrestrial, Lithophytic

Mitt.		
Pogonatum microstomum (R. Br. ex Schwagr.) Brid.	Polytrichaceae	Terrestrial
Philionotis thwaitesii Mitt.	Bartramiaceae	Terrestrial
Pterobryopsis orientalis (Mull.Hal.) M. Fleisch.	Pterobryaceae	Corticolous
Sematophyllum subhumile (Mull. Hal.) M. Fleisch.	Sematophyllaceae	Epiphytic
Sematophyllum subpinnatum (Brid.) E. Britton.	Sematophyllaceae	Epiphytic
Thuidium pristocalyx (Mull.Hal.) A. Jaeger	Thuidiaceae	Lithophytic
Thuidium delicatulum (Hedw.) Schimp.	Thuidiaceae	Lithophytic
Vesicularia vesicularis (Schwagr.) Broth.	Hypnaceae	Lithophytic

Effect of alterations in the photosynthetic pigments and carbohydrate content of *Hydnocarpus alpina* wight due to gall formation by an eriophyid mite species (acari: eriophyidae)

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ABSTRACT: *The pouch like galls on the adaxial surface of the leaves of medicinal plant, Hydnocarpus alpina wight (Flacourtiaceae) induced by an unidentified eriophyid mite are new to science. The inducer may probably be a new species. In the present study, the effect of gall induction on the photosynthetic pigments and carbohydrate content of leaves of H. alpina through quantitative analysis of chlorophyll and carotenoid pigments and total carbohydrate content of the gall tissue of different age classes of leaves like the young (10 days) and mature (35 days) was determined. Results of the study revealed highly significant differences in chlorophyll a and b and carotenoid content in different tissue types, namely uninfested (C), galled leaf (GL) and gall tissue (G) in both young and mature leaf. Gall tissue had significantly higher concentration of total carbohydrate when compared with other tissue types and the difference was highly significant. It is inferred that gall formation induces stress in plants by lowering the energy transduction efficiency of photosystem II, thus adversely affecting the smooth functioning of the organelles concerned with photosynthesis and storage organs, hampering the normal physiological process of the plant. This would substantially lead to a reduction in the growth rate, leaf area, biomass etc. of the host plant, H. alpina.*

Key words: *Hydnocarpus alpina, Eriophyid mite, Chlorophyll, Total carbohydrates.*

Introduction

Among phytophagous mites, the eriophyid mites are the most diverse group and highly host specific causes different forms of plant abnormalities such as galls, erinea, leaf blisters leaf curling, rusts, silvering, fruit russetting, and deformed buds, etc. on their respective host plants Castagoli (1996). For initiation and maintenance of galls on plant organs (leaves, stems, fruits and buds) have been lead to alteration in host plant traits, such as plant architecture (Larson and Whitham 1997), shoot growth (Vuorisalo et al. 1990), and nutrient allocation (McCrea et al. 1985), in addition to impacting whole-plant growth and survival (Hakkarainen et al. 2005). Photosynthesis is the basis of energy gain in any ecosystem ineffective (Zelitch 1975), or any decrease in this process due to infestation generally proves injurious to plant growth and productivity. Phytophagous mites are capable of causing

damage to plants, resulting inhibition of their growth, destroying their photosynthetic structures and storage organs (Schmidt 2014). This resulted in altered primary metabolism of infested plants, exclusively changing the metabolism of amino acids and carbohydrates.

The unidentified gall forming eriophyid mite while sucking up the leaf sap of *H. alpina*, it stimulates the formation of innumerable number of pouched galls of varying dimensions. On severe infestation, individual galls become fused to form complex structures, covering the entire leaf lamina. This results severe distortion, reduction in leaf area and subsequent drying up of leaves. Considering the medicinal and other economical importance of *H. alpina*, and the severity of this newly reported mite galls on the plant, the present work was taken up with an objective to find out the effect of alterations in the concentration of photosynthetic pigments (chlorophyll a & b) and in the total carbohydrate content of the plant.

Materials and Methods

Samples of leaves/leaf galls of *H. alpina* were collected from the University campus, Malappuram district of Kerala for a one year period from April 2013 to April 2014. The samples of both young (Y) and mature (M) galls, approximately of the age of 10 days and 35 days respectively collected from the host plant randomly were transported to the laboratory for subsequent microscopic observation under a Zeiss Stemi DV4 Stereo Zoom Microscope. The galls were cut off using a slicer, and all life stages of the mite residing in the galls were carefully removed with the help of a Camel hair brush. For rating the degree of damage, 6 categories of leaf tissue were considered as follows: (A) 10 days old uninfested leaf (C) (control for young) (B) 35 days old uninfested (C) (control for mature) (C) 10 days old heavily infested galled leaf (GL). (D) 35 days old heavily infested galled leaf (GL) (E) 10 days old intact gall tissue (G) and (F) 35 days old intact gall tissue (G). For biochemical analysis, 9 replicates were prepared for each set to be used for control and experiment.

Total carbohydrates was estimated by Anthrone method (Hedge and Hofreiter 1962). Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were measured spectrophotometrically and calculated based on the formula by Arnon 1949. The amount of the various pigments was expressed in µg/g of fresh mass basis. Data analysis were performed one-way ANOVA to determine the significant difference between different tissue types in both age classes followed by Tukey's post hoc test for multiple comparisons (SPSS version 16.0).

Results and Discussion

Photosynthetic pigments

Mite infested leaves of *H. alpina* bearing pouch like galls developed on the adaxial surfaces (Fig. 1) is reported here for the first time as per the data listed in Catalogue of the eriophyoidea of the world (Amrine and Stasny 1994; Mani 2000). Data obtained on quantitative analysis performed during the present study revealed remarkable decreases in the mean content of Chlorophyll 'a', Chlorophyll 'b', total chlorophyll and total carotenoid contents in G in both age classes and the differences was highly significant between different tissue types (Fig. 2) ($f=2752.59$, $p=0.000$) and ($f=194.70$, $p=0.000$.) for Chlorophyll 'a' in Y and M samples respectively, ($f=1556.15$, $p=0.000$) and ($f=1727.45$, $p=.0.000$) for Chlorophyll 'b' in Y and M samples respectively, ($f=864.07$, $p=0.000$) and ($f=525.73$, $p=0.000$) for total Chlorophyll in Y and M samples respectively, ($f=18383.05$, $p=0.000$) and ($f=26.4$, $p=0.000$) for total caroteniod in Y and M samples. Statistical analysis based on Tukey's post hoc test for multiple comparisons to determine the difference between the concentration of photosynthetic pigment present in the different tissue types in both age classes showed a marked significant difference in the concentration of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents, between C and GL ($p=0.000$ for both Y and M samples), between C and G ($p=0.000$ for both Y and M samples). Significant differences could be observed in the concentrations of chlorophyll a, chlorophyll b and total chlorophyll contents between GL and G ($p=0.000$ for both Y and M samples), however in the case of concentration of total carotenoid content, the difference was found significant in Y samples ($p=0.000$) and no such difference was observed in M samples ($p=0.134$).

Total carbohydrate

The mean values for total carbohydrate content were found highest in Y as well as M gall tissue (G) followed by the GL, whereas it was lowest in C in both age classes and the differences were found highly significant statistically ($f= 47.49$, $p=0.00$ and $f= 24.58$, $p=0.00$ for Y and M samples respectively) (Fig. 3). In both age classes, multiple comparisons were made to assess the differences in content of carbohydrates between different tissue types. The results showed significant differences between C and GL ($p=0.00$ for young and mature sample), C and G ($p=0.00$ for young and mature sample). Also a significant difference was found between young GL and G ($p=0.003$) but no such difference was found between mature GL and G ($p=0.34$).

Discussion

In concurrence with present observations Patnaker et al. (2013) observed that mite galling causes a considerable reduction in physiological activity and associated protective leaf pigments (chlorophyll and carotenoid) in leaves of *Salix pulchra* and *S. glauca*. Interestingly, several authors (Huang et al. 2014; Castro et al. 2012) suggest a galls on *Machilus thunbergii* (Lauraceae) leaves showed a great decrease in carotenoids and chlorophyll related compounds with great deficient in the pigment-protein complexes of PSI and PSII throughout the whole period of gall formation which adversely affect energy transduction efficiency. Consequently galled leaves are incapable to repair photosystem II damage during the periods of high light and low temperatures because the energy and resources needed for the repair has to be spent on defence against mites in galls (Patnaker et al. 2013). This very clearly indicates that the gall mite drastically affects the photosynthetic activity of the plant. Thus the present study proved that damage inflicted by eriophyid mite galling on the leaf of *H. alpina* appears to affect the photosynthetic apparatus of the host plant leaf thus normal growth and physiology. Furthermore, gall formation by *Dryocosmus kuriphilus* reduced the leaf area, biomass ratio of leaf per shoot, resulted in the retardation of development of photosynthetic organ and biomass reduction of host trees in the following year (Kato and Hijii 1997) and early leaf abscission of galled leaf (Yukawa and Tsuda 1986).

The results of the present study supports earlier findings (Motta et al. 2005) on the lepidoperan gall on leaves of *Tibouchina pulchra* which showed higher levels of total carbohydrates than either surrounding leaf tissues or neighbouring gall-free leaves. Similarly, Hartley (1998) found increases in total soluble carbohydrates in gall tissues of four eriophyid mite species. The increases in total carbohydrates in the gall may be due to fact that plants will translocate sugars to locations were infestation has occurred particularly for the synthesis of defence related components such as ethylene (Samsone et al. 2012). This increased carbohydrate content in galls by galling herbivore also indicates the high metabolic rate, creating a sink of photoassimilates to the gall site (Hartley 1998). Castro et al. (2012) established that gall on *Copaifera langsdorffii* has not only low chlorophyll content, but also acts as a sink of nutrients. As a result, primary metabolism of infested plants is disturbed. The significant impact of mite infestation on *H. alpina* which influences the photosynthetic processes is likely to affect the other physiological phenomenon needs further investigation.

Conclusion

Results of quantitative analysis performed during the present study enabled to establish the damage potential of the unidentified eriophyid gall mite, which induces pouch like galls on the plant, *H. alpina.* The infestation decreases the photosynthetic pigments and increases the carbohydrate contents in the gall tissue, indicates that translocation of carbohydrates from leaves to galls. High concentrations of carbohydrates in gall tissues, together with the lower energy transduction efficiency of PSII, further confirmed that galls are sinks for host leaf photoassimilates. This is a clear indication that galled leaves suffer heavy chlorophyll loss. During severe infestation, individual galls become fused to form complex structures, covering the entire leaf lamina leading to complete distortion of the leaf. This in turn would lead to reduction in leaf area, the subsequent drying, defoliation and loss of biomass.

Acknowledgment

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Figure 1: Pouch like galls on the leaf of *H. alpina* due to infestation by unidentified eriophyid mite.

Figure 2: Mean± SEM (n=9) Concentration of chlorophyll (a, b & total) and carotenoid contents in C (Uninfested), GL (Galled leaf), G (Gall tissue) in both young (Y) and mature (M) leaf due to infestation by an unidentified eriophyid mite on *H.alpina*.

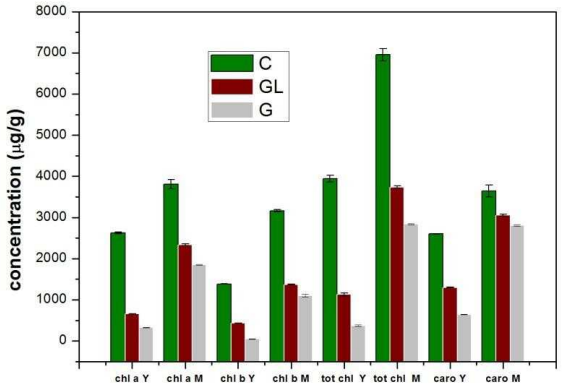
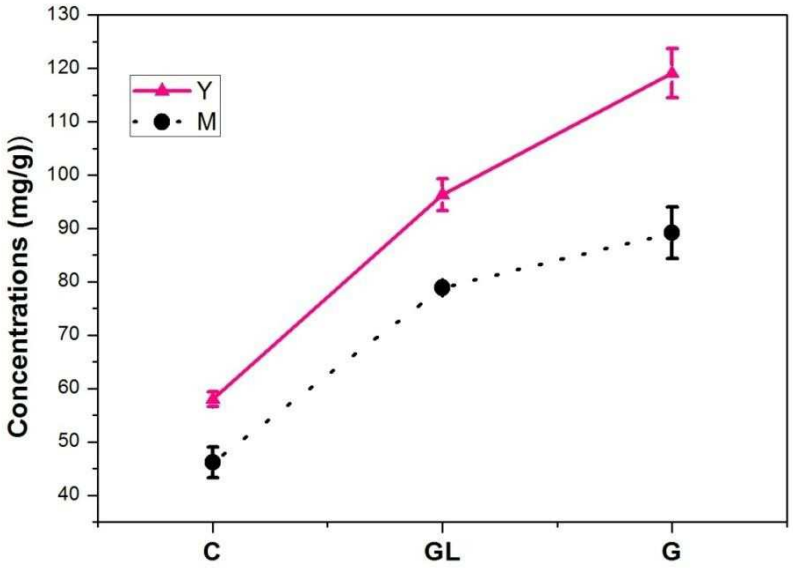


Figure 3: Mean± SEM (n=9) Concentration of total carbohydrate contents in C (Uninfested), GL (Galled leaf), G (Gall tissue) in both young (Y) and mature (M) leaf due to infestation by an unidentified eriophyid mite on *H.alpina.*



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Biodiversity in Bittergourd and Ashgourd

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ABSTRACT: *Bittergourd and Ashgourd are important warm-season cucurbit vegetables. A wide genetic diversity in vegetative and fruit characters as well as quality traits is found in bittergourd and ashgourd. The extent of genetic variability indicates the amenability of a given character for its improvement through breeding. The rich variability among bittergourd and ashgourd genotypes have many value-added characters and will bring fruits in its improvement. If they are considered while breeding, no doubt bittergourd and ashgourd will be promising vegetables.*

Key words: *Bittergourd, Ashgourd, Genetic variability*

Introduction

Bitter gourd or balsam pear (Momordica charantia L.) cultivated for its immature fruits is having considerable nutritional, economic and medicinal importance. India is endowed with the wealth of bittergourd germplasm consisting of both wild and cultivated forms. The diverse visual characters (i.e., sex expression, growth habit, maturity, fruit shape, size, color and surface texture) of bitter gourd provide for relatively broad phenotypic species variation in India. Being a monoecious and highly cross-pollinated crop, large variation is observed in fruit and vegetative characters.

Ashgourd is a monotypic genus with only one cultivated species *Benincasa hispida* (Thunb.) Cogn. and is grown for its succulent hairy fruits, used in confectionary and in ayurvedic medicinal preparations. Ashgourd is known by several names: waxgourd, winter melon, hairy melon, ash pumpkin, white pumpkin, whitegourd and Chinese preserving melon. The name waxgourd refers to the thick, waxy cuticle that typically develops on mature fruits. The specific epithet '*hispida*' refers to the hirsute pubescence on the foliage and immature fruits. In India, a wide range of variability in vegetative and fruit characters is available in ashgourd. Surprisingly, this crop has not been much exploited on commercial basis in the past. Although ashgourd is becoming a crop of industrial importance, relatively less attention has been paid towards the varietal improvement of existing strains available in different parts of the country.

Genetic diversity

Two types of cultivars have been grown in northern India and nine types in southern India. Today, Indians cultivate primarily two varieties of M. charantia (i) var. charantia, which produces large fusiform fruits and (ii) var. muricata (wild) with small and round fruits. Three cultivar types are present in waxgourd group on the basis of fruit characters namely, fruits nearly round and essentially hairless, fruits nearly round and hairy, fruits oblong and hairy are found. Four major categories as cultivar groups have been recognised in Benincasa hispida viz., unridged winter melon group, ridged winter melon group, fuzzy winter melon group and waxgourd group.

Chemical constituents

In bittergourd, Small fruited variety is richer in nutrients than long fruited variety. The small fruits contain 2.0% protein, 1.0% fat, 2.8% carbohydrates, 9.8 mg iron, 210 IU of vitamin A and 88 mg vitamin C per 100 g edible portion. Big and long fruit contains 1.6% protein, 0.2% fat, 4.2% carbohydrates, 2.2 mg iron, 210 IU of vitamin A and 88-92 mg vitamin C per 100 g edible portion. White-fruited Indian varieties are, in fact, relatively high in polypeptides, phenolics and polyphenolic compounds, which are natural antioxidants; thus are alternatives to replace synthetic antioxidants to enhance food quality. Variation is also observed in respect of extent of bitterness. White coloured varieties are less bitter in taste and preferred in South India.

In ashgourd, the fruit is a rich source of vitamins A, B, C, proteins and minerals. The fruit has high moisture content and is low in calories and carbohydrates. In addition, the fruit also contains Ca, P, Na, Mg, Fe, K,S and starch in minute quantities. Major compounds in ashgourd include E-2 hexanol, n-hexanal and n-hexyl formate. The plant contain 2,5-dimethyl pyrazine, 2,6-dimethyl pyrazine, 2-methyl pyrazine, 2- ethyl 5-methyl pyrazine, 2,3,5-trimethyl pyrazine; flower contains arginine, lysinr, aspartic and glutamic acids, fruits contain isomultiflorenil acetate (fruit wax), α-amino butyric acid, serine, proteinase, glucose, mannitol, rhamnose, n-triacontanol, lupeol, α-sitosterol and seeds contain arachidic, linoleic, linolenic, oleic, palmeic and stearic acids.

Materials and Methods

The basic material for the study included 33 genotypes of bittergourd and 25 landraces of ashgourd collected from different agroclimatic regions of the country.

They were evaluated at the vegetable plot in the Department of Olericulture, College of Agriculture, Thiruvananthapuram. The crops were grown in a randomized block design with two replications and they received timely management practices as per package of practices recommendations of Kerala Agricultural University (KAU, 2011). The descriptor developed by IBPGR (1983) for cucurbits was used for cataloguing.

Results and Discussion

Among the bittergourd collection wide biodiversity in morphological and qualitative characters of fruits was observed e.g. vine length, 103.75 - 620.00 cm; internodal length, 1.25 to 5.58 cm; fruit length, 4.91 - 38.83 cm; fruit girth, 6.96 - 25.53 cm; fruit number, 8.75 - 34.25; average fruit weight, 4.26 - 578.75 g; β-carotene, 52.58 - 138.96 µg/100 g; Vitamin C, 62.54 - 124.29 mg/100 g; iron content, 2.38 - 6.88 mg/100 g; chlorophyll a, 0.00110 - 0.54365 mg/g; chlorophyll b, 0.00045 - 0.03075 mg/g; total chlorophyll 0.003 - 0.12 mg/g and bitterness value, 1227.50 - 10400 units/g.

Of the germplasm, most of the genotypes fall in moderate to high viny growth habit and medium to long internodal length. Tendrils are present in all the thirty three genotypes. Leaf size varied from small to large with ovate, pedate or reniform shape (Figure 1). Most of the genotypes had shallowly lobed leaves but exceptions of deeply lobed cases were also found. All the genotypes had soft hairy leaf dorsal surface pubescence and moderate density of foliage hairs per branch.

Variability was more pronounced for flower and fruit characters (Fig. 2 and 3). There are reports on high variability for morphological characters in snakegourd (Ashok 2000) and bittergourd (Dey et al. 2006). Flower and fruit size ranged from small to very large. Fruit form was either round, oval, globular, cylindrical, elliptical, elongate or dumbell. Skin texture was either smooth, rough or with spines. Wide variation was noticed in skin colour ranging from white to dark green, majority having green skin coloured fruits. Fruit shape at stem end and blossom end ranged from deep round to taper point. Peduncle length ranged from short to long. Peduncle detachment from fruit was easy in most of the genotypes.

The collection has wide biodiversity in morphological and qualitative characters of fruits e.g. vine length, 283.00 - 875.00 cm; internodal length, 7.20 - 17.55 cm; fruit length, 13.65 - 56.00 cm; fruit girth, 22.20 - 78.05cm; fruit number, 1.87 - 9.12; average fruit weight, 0.27 - 9.50 kg; yield per plant 1.62 - 21.20 kg. Most of the landraces fall in moderate to high viny growth habit and short to long internodal length. Density of foliage hairs per branch ranged from few to dense. Leaf size

varied from small to large with ovate, pedate or reniform shape. Most of the landraces had shallowly lobed leaves but exceptions of deeply lobed and lobeless cases were also found. Leaf dorsal surface pubescence was either soft hairy or bristle like.

Variability was more pronounced for flower and fruit characters. Flower and fruit size ranged from small to very large. Fruit form was either round, oval, globular or elongate bottle like (Fig. 4). Skin colour was green in smooth textured fruits and white in waxy textured fruits. Fruit shape at stem end and blossom end ranged from deep round to taper point. Peduncle length ranged from short to long. Peduncle detachment from fruit was difficult in most of the landraces. Most of the landraces possess medium earliness of harvest. Fruit storage ability was more than six weeks in most of the landraces.

Seed quantity per fruit ranged from very few to many with small to large seed size. Most of the landraces had yellow seed colour, but exceptions of brown and whitish yellow colour was also found. Glossy to dull seed surface lushe was noticed. Seed separation from placenta was easy in most of the landraces.

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Figure 1: Variability in fruit charcters of *B. hispida*

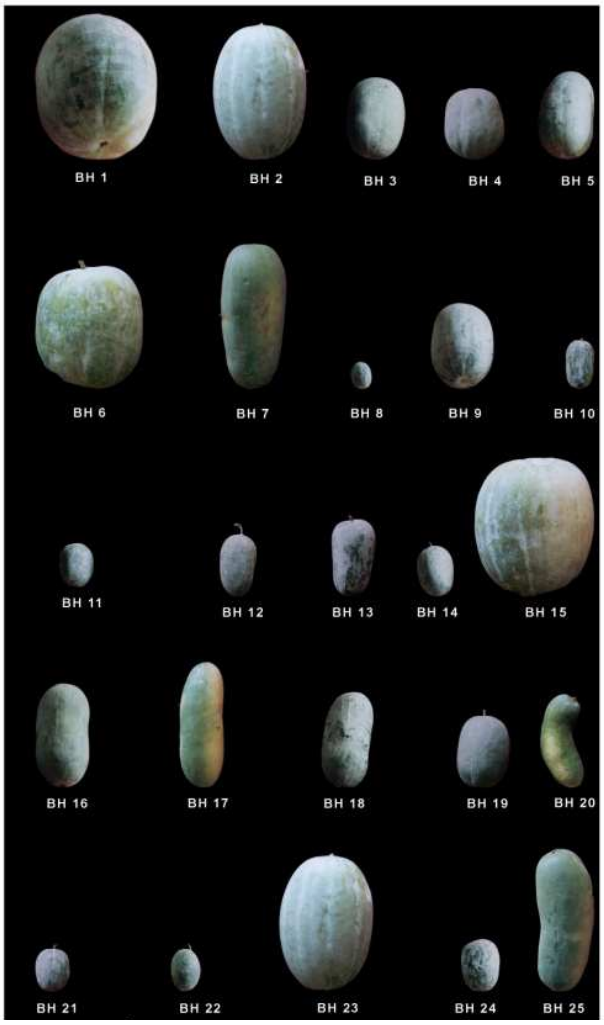


Figure 2: Varibility in flower characters in *M. charantia*

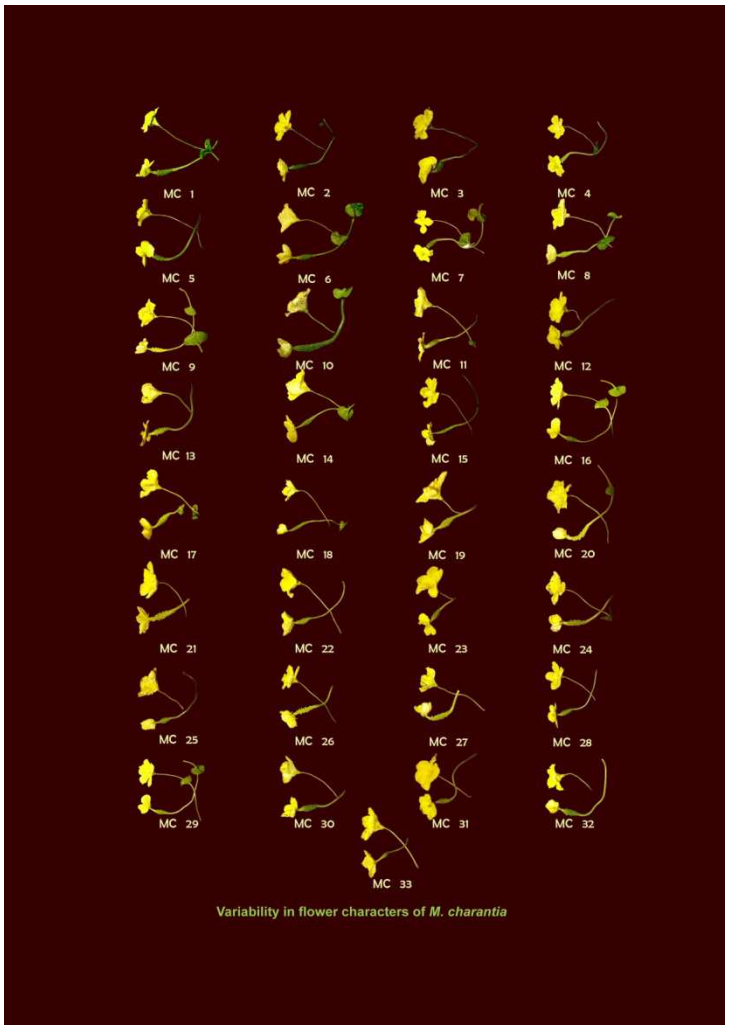


Figure 3: Variability in fruit characters in *M. charantia*

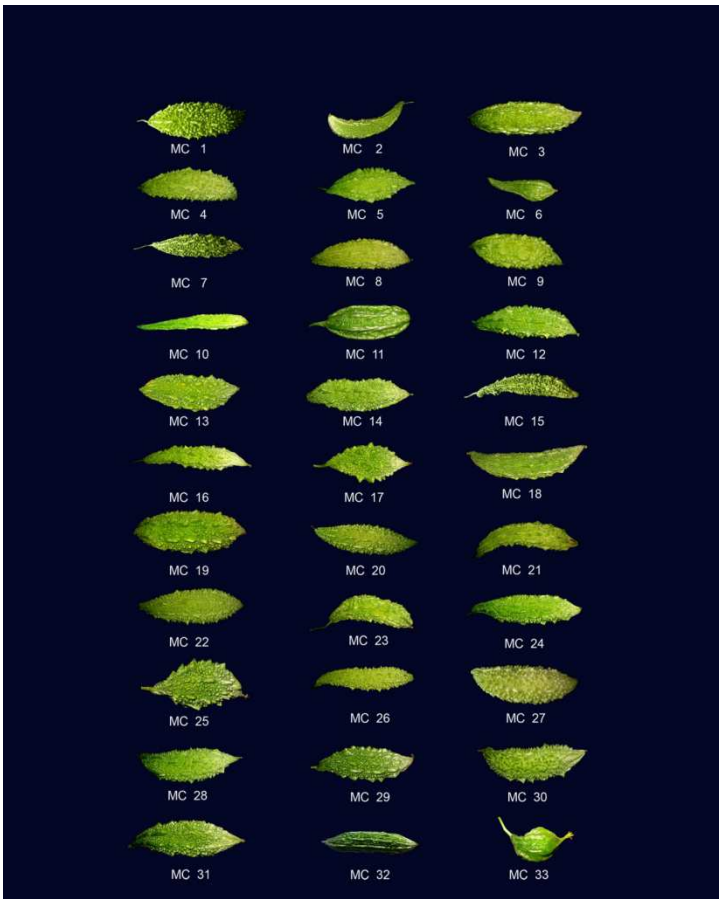
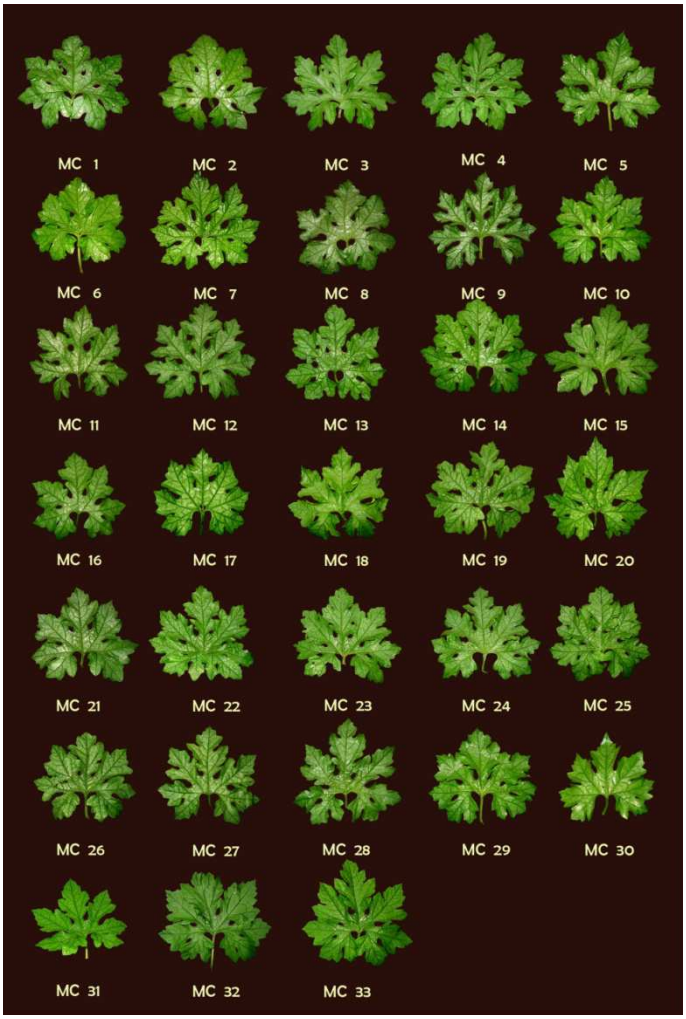


Figure 4: Variability in leaf characters of *M. charantia*



Bio-Monitoring of the Thiruvananthapuram museum lake using phytoplankton

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ABSTRACT: *The pollution status of the Museum Lake in the Thiruvananthapuram Botanical Garden and Museum using phytoplankton as indicators was carried out. The study of different community structures of the planktonic taxa revealed that the water body is organically polluted which is due to the impact of organic litter from the riparian vegetation. A total of 21 pollution tolerant genera were identified from the water body of different classes- Chlorophyceae, Bacillariophyceae, Cyanophyceae and Euglenophyceae. Scenedesmus, Pinnularia, Euglena, Ankistrodesmus falcatus, Closterium sp, Crucigenia, Kirchneriella, Merismopedia aeruginosa, Oscillatoria subbervis were some of the phytoplankton pollution indicators noted. Excessive nutrient loading from the surrounding was degrading quality of this urban lake ecosystem. Detailed information of the status of pollution of any water body is of much importance because it ultimately helps in the proper management of the water body.*

Key words: *phytoplankton, pollution, museum lake*

Introduction

Increased monitoring activities that focus on all major biological compartments are needed to quantify and evaluate the present condition of the aquatic resources. Biological monitoring is a valuable method used in the conservation studies that uses the bio indicators for the determination of pollution status. Biological indicators are species used to monitor or assess the health and environmental integrity of any ecosystem. Planktonic microalgae satisfy conditions to qualify as suitable indicators in that they are simple, capable of quantifying changes in water quality, applicable over large geographic areas and can also furnish data on background conditions and natural variability (Onyema 2007). Phytoplankton are an ecologically important group in most aquatic ecosystems but are often ignored as indicators of aquatic ecosystem change (Sharma and Bhardwaj 2011). Because of their nutritional needs and their position at the base of aquatic food web, phytoplankton indicators provide relatively unique information concerning ecosystem condition compared with commonly used animal indicators. They respond rapidly and predictably to a wide range of pollutants and, thus provide potentially useful early warning signals of deteriorating conditions and the possible causes and their assemblages provide one of the few benchmarks for establishing

historical water quality conditions and for characterizing the minimally impacted biological condition of many disturbed ecosystems. Preliminary comparisons suggest that phytoplankton indicators are a cost-effective monitoring tool as well (McCormick and Cairns 1994). Physical and chemical measurements provide quantitative data on the presence and levels of aquatic pollution and degradation, but these parameters do not reflect the extent of environmental stress reaching the living organisms or the subsequent effects of this stress (Omar 2010). Phytoplanktons are of great importance in bio monitoring of pollution (Shekhar et al. 2008; Davies et al. 2009). The use of phytoplankton in bio monitoring was started in early 19th century. Kolkwitz and Marsson (1908) were among the first to use presence and absence of phytoplankton species as an indicator of specific water quality studies. Studies on the phytoplankton and physico chemical factors of its habitat are urgently needed for the management of water resources and to predict the evaluation of the aquatic ecosystem (Gaballa 2014). Palmer (1969) developed an index based on the tolerance of phytoplankton to the pollution levels. The lake inside an *ex citu* conserved area should be more aware on its quality standards. The authorities should be aware of the quality standards as it is a major tourist spot too. Regular monitoring and recording of water quality should be performed and water quality should be maintained to appropriate levels. The present study deals with the bio monitoring of the lake which is inside Govt. Botanical Garden and Museum using phytoplankton.

Materials and Methods

Study area

Govt. Botanical Garden and Museum is one of the oldest in the country which is located at the heart of the temple city of Thiruvananthapuram (08˚30' N, 076˚57'E). Swathi Thirunal (1813-1847), illustrious king and music composer who ruled Southern Kerala (Travancore) during 1830-1847 is the visionary behind establishment of the Thiruvananthapuram Museum and Zoo (Nair 2003). The Botanical Garden and Museum is of unique in its nature which includes Zoological Garden, Natural History Museum and a Botanical Garden. The lake in the present study is inside the Zoo. The lake is a big, oval shaped, perennial water body extended over 1.90 acres, often named as the 'The lungs of the city'. There is an immense number of rare and exotic floras associated with the lake and are kept as part of conservation and beautification in mind which never failed to attract these winged beauties as well as tourists. About 60 species of resident water birds like Pond Herons, Oriental Darter and Cormorant are some of them associated with the water body. The photograph of the study area is given as Fig. 1.

Phytoplankton Identification

Phytoplankton samples were collected at monthly intervals from the selected sites of the water body from February 2013 to January 2014. Quantitative enumeration of phytoplankton was carried out by passing a known (1 litres) volume of sample water through the plankton net No. 25 (mesh size 64µm). The filtered samples were preserved by adding a few drops of Lugol's solution. The samples were then reduced to a known volume of 15, 30 ml in a centrifuge. Enumeration of phytoplankton was done by taking 1 ml of sub-sample in a Sedgwick Rafter cell and counting its entire contents up to the statistical accuracy. The works of Desikachary (1959) and Prescott (1978) and online websites like *Phycokey, Algae base* and *Desmids* were considered for the identification of phytoplankton. The micro-photographs of each representative sample were taken using a camera attached microscope Leica DM 500.

Species diversity of phytoplankton community was calculated from the mathematical expressions suggested by Shannon and Weiner 1949. Shannon and Weiner's Diversity Index is an expression of correlation with pollution status of the ecosystem (Wilhm and Dorris 1966; Washington 1984) which is based on Shannon's information theory (Shannon and Weiner 1949).

The Pollution Index (Palmer 1969)

Scientists developed a method to determine the level of organic pollution by studying the algae present in a sample of water. A pollution index factor of 1 through 5 has been assigned to each of the 20 types of algae that are most tolerant to organic pollution. Types of algae most tolerant of organic pollution were assigned a factor of 5. Less tolerant types were assigned a lower number. If there are 5 or more cells of a particular kind of algae on a slide, the alga must be identified and recorded. The index numbers of the algae are then added. Any algae that are not listed have a pollution factor of zero.

Results and Discussion

A total of 88 phytoplankton species were observed from the study area of the different classes - Chlorophyceae, Bacillariophyceae, Cyanophyceae and Euglenophyceae. The abundance of phytoplankton recorded was represented Fig. 2. Throughout the study period the Chlorophycean members showed abundance. From them 21 of the pollution tolerant species were considered. The concept of diversity indices indicates pollution stress. The species richness of a community

which relates the number of species has been regarded as an important index of diversity. The application of phytoplankton diversity index to the biological monitoring of water quality is based on the premise that communities under stress undergo a reduction in diversity (Trivedi et al. 2008).

Shannon - Weiner's Diversity Index of four Classes of Phytoplankton Observed in the lake Table 1. Wilhm and Dorris (1960) have suggested a relationship between species diversity and pollution status of aquatic ecosystems and classified as follows: >3 = clean water; 1-3 = moderately polluted; < 1 = heavily polluted. Staub et al. (1970) proposed a different scale of pollution in terms of species diversity index which is a modified one and states a negative correlation between Shannon Weiner's Diversity index and pollution. 3.0-4.5 = slight; 2.0-3.0 = light; 1.0-2.0 = moderate; 0.0-1.0 = heavy. Thus, from both the limnological expressions of diversity index it can be stated that the Thiruvananthapuram Zoo lake is heavily polluted.

Palmer's Algal Index

The numbers scored by each genus as proposed by Palmer (1969) are totalled to get the value of algal genus index. A score of 20 or more for a sample is indication of high organic pollution. While a score of 15-19 denotes the presence or moderate rate of organic pollution. Palmer has shown that genus like *Euglena, Oscillatoria, Scenedesmus, Nitzschia, Navicula* are generally found in organically polluted water and the same has also been endorsed by Goel et al. (1986). In the present study, the phytoplankton was dominated by typical organic pollution indicating species like *Oscillatoria, Euglena, Lepocinclis, Nitschzia, Synedra, Ankistrodesmus.* Similar observations were mentioned by Basavaraja et al. (2012), Kumari et al. (2008) and Vutukuru et al. (2012) and also supports the view to categorize the lake as eutrophic in nature as also reported by Nandan and Aher (2005) and Shekhar et al. (2008). It is reported that excessive growth of certain plankton genera, viz., *Scenedesmus, Anabaena, Oscillatoria* and *Melosira* indicate nutrient enrichment of aquatic bodies (Zargar and Ghosh 2006). The microphotographs of the pollution indicator phytoplankton are shown in Fig. 3. Even though the lake is inside an ex citu conserved area, anthropogenic disturbances are observed in minor forms. Plastic bottles thrown by the visitors of Museum and Zoo carelessly into the lake can deteriorate the quality of the water (Ajayan and Kumar 2014). Also the water body is affected by autochthonic sources of pollution i.e. growth, death and degradation of macrophytes and litter from the riparian vegetation. The result for Palmer's Algal Pollution Index is shown in the Table 2.

Phytoplankton diversity and abundance is a very important tool in the monitoring of the deteriorating condition of the Lake. This could be a viable tool both for long term and community based monitoring putting into consideration of its inexpensive nature and ease to collect data. Palmer (1969) is of the view that only *Euglena* is more important than *Oscillatoria* as a genus of algae indicative of organic pollution. *Euglena* was a common genus found throughout the study period.

Conclusion

From the study it is concluded that the lake is organically polluted and it needs more care in quality parameters to check pollution for a healthy environment and to enjoy the tourism. The study confirms the role of phytoplankton as an excellent tool for bio monitoring. Regular clearing of macrophytes to remove pollutants from lake is the best way for the restoration. Therefore, the lake has to be preserved for its intended use, a sustainable and aesthetic management planning is necessary for the conservation of this water body

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Table1: Shannon – Weiner's Diversity Index of four Classes of Phytoplankton observed in the lake

Month	Chlorophyceae	Cyanophyceae	Bacillariophyceae	Euglenophyceae
February	3.90	1.10	1.43	1.10
March	1.44	1.29	1.32	0.68
April	1.37	0.34	1.28	0.69
May	1.42	1.05	0.69	0.85
June	1.69	0.69	0.85	1.16
July	1.42	0.69	0.69	0.69
August	1.83	0.85	1.10	0.85
September	1.40	0.26	1.40	0.26
October	1.44	0.22	0.89	0.36
November	1.36	0.34	0.75	0.42
December	1.56	0.36	0.63	0.75
January	2.00	0.77	0.62	0.86
Average	**1.73**	**0.66**	**0.97**	**0.72**

Table 2: Palmer's Algal Pollution Index for Museum Lake Thiruvananthapuram

Pollution Tolerant Genus	Score	Abundance and Occurrence
Chlorophyceae		
Ankistrodesmus	3	+
Chlorella	*3*	+++
Closterium	0	++
Dictyosphaerium	1	++
Kirchneriella	0	++
Micractinium	*1*	+
Pandorina	3	++
Scenedesmus	4	++
Cyanophyceae		

Anabaena	1	+
Oscillatoria	4	++
Merismopedia	0	++
Microcystis	*1*	+
Bacillariophyceae		
Cyclotella	2	+
Diatomella	1	++
Fragillaria	1	+
Gomphonema	1	+
Melosira	*1*	+
Navicula	3	++
Nitzschia	3	++
Pinnularia	1	++
Synedra	3	++
Euglenophyceae		
Euglena	1	++
Lepocinclis	1	++
Phacus	0	++
Trachelomonas	0	+
Total Score	**33**	

(+ = Rare; ++ = Common, +++ = Abundant)

Figure 1: Study Area

Figure 2: Abundance of phytoplankton recorded

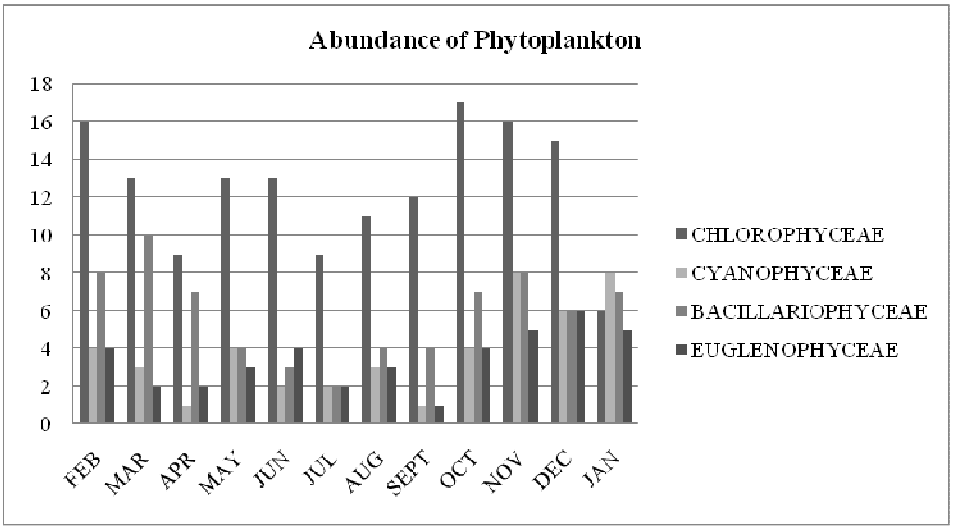
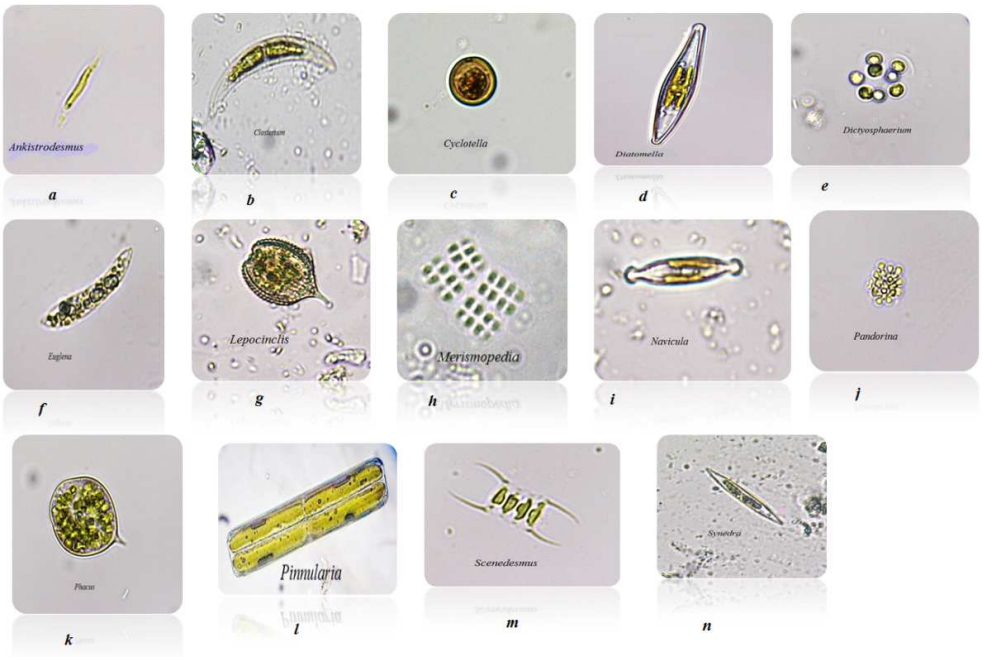


Figure 3: Pollution indicator phytoplankton bbserved. (a). *Ankistrodesmus,* (b). *Closterium,* (c). *Cyclotella,* (d). *Diatomella,* (e). *Dictyosphaerium,* (f). *Euglena* (g). *Lepocinclis,* (h). *Merismopedia,* (i). *Navicula,* (j). *Pandorina,* (k). *Phacus,* (l). *Pinnularia,* (m). *Scenedesmus* and (n). *Synedra.*



A study of the estimation of the above ground biomass and Carbon sequestration of Sacred groves of Thiruvananthapuram district, Kerala.

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ABSTRACT: *Sacred groves are the micro repositories of genetic and ecosystem diversity and are deteriorating at an alarming rate. They are ecosystems which help to conserve valuable biodiversity and help in regulating several cycles. A field survey was conducted in five selected sacred groves (Attingal, Pachalloor, Chirayinkeezhu, Vattappara and Nedumangad) for analysing the aboveground biomass and the total carbon sequestered in the groves during the months from January to June 2015. The diameter at breast height (DBH) and height of the tree was measured using non-destructive method. Our observation found that the total above ground biomass was minimum in Attingal Sacred grove (68,916.33 tonnes) and maximum at Nedumangad Sacred grove (5,95,251.67 tonnes). The Sacred grove at Nedumangad reported maximum species density. Regarding carbon sequestration, the lowest carbon storage value was reported from Attingal Sacred grove (33,079.84 tonnes) and the highest was reported in Nedumangad Sacred grove (2,85,720.80 tonnes). Our findings showed that there is a relationship between species density, total aboveground biomass and the total carbon sequestered.*

Key words: *DBH, diversity, biomass, carbon sequestration.*

Introduction

Biomass refers to the total amount of energy that is being stored in the trees for the next tropic level. The first step to know atmospheric carbon harvest is the Biomass estimation. It is an important indicator of ecological process and is directly related to the amount of carbon that is trapped in different parts of the tree. The increasing carbon emission is of major concerns for entire world as well addressed in Kyoto protocol is explained (Ravindranath et al. (1997); Chavan and Rasal 2012). There is a disturbingly increasing trend in the atmospheric concentration of carbon dioxide in the last five decades. This increase in carbon dioxide concentration in the atmosphere is mainly by human developmental activities and has led to remarkable changes in the natural global carbon cycle. The nature has its own mechanism of sequestering the carbon from the atmosphere and storing it in its reservoirs like the oceans, forests and soils. If not, the planet would have been overheated by now. And this has helped in achieving balance of carbon dioxide levels in atmosphere and maintaining the global carbon cycle. The terrestrial ecosystem and the oceans act as natural carbon "sinks" or "sponges".

The amount of biomass in a tree can be used to determine the potential biomass production for bioenergy and also be used to build carbon budgets and to evaluate the environmental sustainability of different ecosystems. The living biomass of trees constitutes the main carbon pool but it is mainly affected by deforestation and land degradation. In order to assess the magnitude of carbon exchange between various ecosystems and the atmosphere, estimating the carbon stocks is very important. An estimate of the amount of carbon emitted into the atmosphere can be done by the assessment of amount of carbon sequestered by an ecosystem. It helps us to understand the current status of carbon sequestered and the changes in this in the near future. The present study helps us to analyse the amount of carbon sequestered by the trees in the selected Sacred groves and the need to conserve the Sacred groves and the natural vegetation.

Materials and Methods

A study was conducted in five sacred groves spread across coastal plains, mid and high lands, in Thiruvananthapuram district, Kerala. These are spread over Thiruvananthapuram, Nedumangad and Chirayinkeezhu taluk. Survey was carried out in Kavil Shree Maheswarashramam from Nedumangad, Indiliyappan kavu from Vattappara, Erumakkavu from Chirayinkeezhu, Pulikottukonam Nagarkavu associated with Shree Bhadra Devi Temple Trust from Attingal and Sacred grove associated with Pachalloor Sree Bhadrakali Devi Temple, Thiruvananthapuram. Plant materials were identified with the help of local flora and experts.

Tree Height and Diameter at Breast Height (DBH)

Non-destructive method was selected to estimate biomass of different trees. The biomass of each tree was estimated on the basis of DBH and tree height. DBH was determined by measuring the tree Girth at Breast Height (GBH), approximately 1.3 meter above ground surface. The GBH of all perennial trees having diameter greater than 30 cm were measured directly by using a measuring tape. The tree height was measured using Smart measure application on mobile phone. And the same indicator parameters (e.g. tree DBH and height of the tree) were selected for calculating the biomass and for estimating the carbon sequestration.

Above ground biomass (AGB) of trees

The above ground biomass of trees were calculated by taking into consideration, the whole shoot, branches, leaves, flowers and fruits. It was calculated using the following formula

AGB kg = Volume of tree (m^3) x wood density ($Kg/m^{3)}$ (Hangarge et al. 2012).

$V = \pi r^2 H$…… (1)

Radius of the tree is calculated from GBH of the tree. Tree diameter (D) was measured (3.14) to the actual marked girth of species i.e. GBH/3.14 (Bohre et al. 2012). The standard mean density of wood for Asia is 0.57 (Sandra Brown 1997).

Carbon sequestration

All plant tissue falls between 45-50% carbons, so this estimate is reasonable for all dead, live and non- wood samples. The final step in estimating carbon sequestration is multiplying AGB by 0.48. Total Carbon sequestered= Above Ground Biomass x 0.48 (Condit 2008).

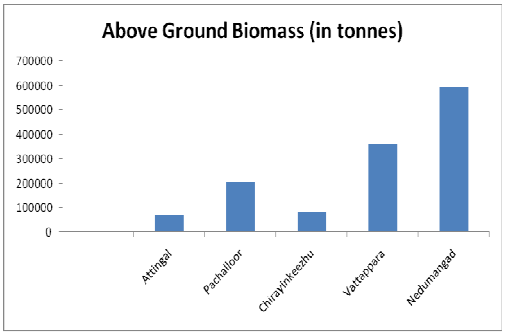
Results and Discussion

Above ground biomass (AGB) of trees

The main aim of the study was to estimate the biomass and total carbon sequestered in five selected sacred groves from geographically different places in the Thiruvananthapuram district. There were 34 trees from 16 species in Attingal Sacred grove. In Pachalloor Sacred grove, 13 species and a total of 38 trees were present. The number of trees in Chirayinkeezhu Sacred grove is 37 which belong to 12 species whereas in Vattappara Sacred grove 30 species and a total of 76 trees were present. Nedumangad Sacred grove reported the maximum number of trees. A total of 145 trees belonging to 47 species were present in this grove.

The total aboveground biomass varied from 68,916.33 tonnes in Attingal Sacred grove, 2,07,860.48 tonnes in Pachalloor Sacred grove, 84,764.06 tonnes in Chirayinkeezhu Sacred grove, 3,62,155.51 tonnes in Vattappara Sacred grove and 5,95,251.67 tonnes in Nedumangad Sacred grove (Fig. 1). Hence from the analysis it is clear that the Nedumangad Sacred grove reported the maximum above ground biomass compared to other Sacred groves. The very importance of estimating the biomass is that it helps in knowing the state of the ecosystem and number of organisms it support. The ecological status of these groves can be determined from the biomass calculated. It also helps to locate the amount of energy stored that explains about the primary productivity of the ecosystem and the role of different species and the hydrologic properties of the sites.

Figure 1: Above ground biomass estimation of five sacred groves in Thiruvananthapuram district



Carbon Sequestration

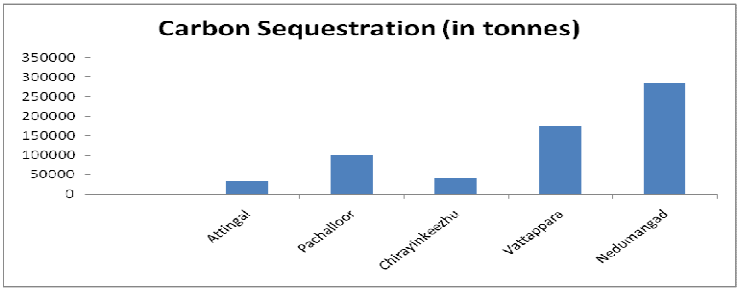
The lowest carbon storage value was estimated at 33,079.84 tonnes in Attingal Sacred grove and maximum carbon storage was 2,85,720.80 tonnes in Nedumangad Sacred grove (Fig. 2). As the diameter of trees increased, its biomass and carbon storage capacity also increased. Hence sequestered more carbon and removes more carbon dioxide from the atmosphere. Trees to a great extent will hold CO_2 gases emitted from automobiles with consequent reduction in health and environmental related issues.

The largest carbon pool is the above ground biomass of the trees. It is directly affected by deforestation and human intrusion. The changes in the ecosystem and the biomass influence the transfer of carbon between the terrestrial ecosystems and atmosphere. Therefore estimating the total carbon sequestered is very important for assessing the magnitude of the carbon exchange that takes place. By estimating the amount of carbon sequestered by the trees in these sacred groves, help to estimate the amount of carbon emitted into atmosphere when this ecosystem is degraded. From the calculations it is observed that the Sacred grove with thick vegetation, Nedumangad, had high carbon sequestration potential compared to other sacred groves. So this grove contributes more towards reducing the level of carbon dioxide in the atmosphere. Large fluctuations in plant biomass are associated with low diversities and more constant biomass and greater productivity accompanied high diversities.

The efforts that have put up have helped to obtain more accurate data which is crucial for climate change research and mitigation activities in these ecosystems. But from the analysis it is very clear that the sacred groves are shrinking due to

anthropogenic activities and other human needs. So legal actions must be taken to protect and preserve these areas as sacred patches.

Figure 2: Total Carbon sequestration of the five sacred groves in Thiruvananthapuram district



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