2nd National Training Workshop on Electronic Herbarium and Digital Database Preparation

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Edited by
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Content

PREFACE

Herbarium is a part of academic and research curriculum at various levels of teaching and in persuasion of research activities in the subject, botany. This is an important and scientific method of preservation of dried plant specimens. However, there are several disadvantages, which we feel in the processes of preparation, preservation and handling of herbarium sheets in addition to the problems associated with herbarium such as fading of original colours, bio-degradation and pathogenic attacks etc. Now a days, traditional herbarium preparation is discouraged in many universities and colleges for conservation of natural resources. No doubt, the traditional method has occupied its own importance besides its problems. However, a new eco-friendly Electronic herbarium has emerged out to overcome certainly many of the problems associated with traditional method and added many new facilities and it became much more attractive, friendly as well as informative.

India is one of the countries in the world with the richest biodiversity of plants and hotspots. But we are lagging behind many countries due to lack of digital database of plants facility. Naturally, we do not know exact number of species available in India and we have no chance to see them all at once. The existing traditional method of herbarium is confined to four walls of that centre, have no way to access them from far and wide.

Under these circumstances, the department of Botany is proud to announce that we have already prepared Electronic herbarium and digital database of Mumbai. We organized First National Training Workshop on "Electronic herbarium and digital database preparation" in 2003, when we initiated it. We are holding the second one now with an intension to train the people from various walks of life to make uniform method of database preparation for the benefit of people and the entire country in the present global scenario. I welcome you to the department of Botany, Institute of Science, Mumbai for two days National Training Workshop. I thank you for very good participation and keeping confidence on the technique and persons involved in this endeavor. Finally, I wish you all, to participate actively and implement the technique at your end and make every citizen say proudly that "WE BUILT UP PLANT DATABASE FOR OUR COUNTRY".

T. Srinivasu
Organizing Secretary,
NATWED.

INSTITUTE OF SCIENCE

The Institute of Science, Mumbai formerly known as The Royal Institute of Science (RIS), was established in 1920. This institute is managed and organized by the Government of Maharashtra and affiliated to the University of Mumbai. It is a full-fledged premier post-graduate centre for teaching and research in science. Over the years, the Institute has come to occupy a place of repute in academic and scientific world. As an academic institution, the Institute has not only succeeded in setting and maintaining high standards of research and teaching, but has also provided an atmosphere congenial for the pursuit of science.

Education is a learning process and research is a process of expansion of ideas, which exist. This is supported in the tenets of the motto of the Institute. “It’s good to seek the causes of things’, which has been followed over the years at the Institute.

During the visit of His Majesty the Emperor in 1912, the word “Royal” was associated with the Institute and it was designated as the Royal Institute of Science (RIS).

In 1920, the building was made available to function as Royal Institute of Science. The formal opening of the Institute was made on 27th march 1924 by then Governor of Bombay, Sir Leslie Wilson.

In her prime years, the Institute was fortunate to have the association of several britishers like Dr. J. J. Fox, who was the first Principal, Dr. A. Meldrum and Dr. T. S. Wheelar, who were all renown chemists, besides host of reputed Indian Scientists like Prof. G. R. Paranjape, Prof. D.D. Kanga, Prof. P.R. Awati, Prof. S. L. Ajrekar, Prof. K. R. Gunjikar etc. who set very high academic standards for the Institute.

In the more recent years, the Institute was lucky to have great scientists like Dr. F. R. Bharucha, Dr. Mrs. Kamala Sohonie, Prof. B. C. Haldar and others as its Directors. Prof. B. C. Haldar name requires a special mention, as it was

during his tenure of good vision that the Institute scaled to new heights by introducing interdisciplinary subjects and research projects undertook were international standards. He started courses in nuclear and radiochemistry and research work in the field of environmental pollution, acquired separate building for housing nuclear and radiochemistry laboratories etc.

Renaming and Expansion:

After independence, the word, “Royal” was dropped from the name of the Institute and it was re-designated as the “Institute of science”.

The Institute today conducts M.Sc. (by papers & by research) courses, and Ph.D. programmes in several disciplines namely Biochemistry, Botany, Chemistry (Analytical, Inorganic, Organic, & Physical), Mathematics, Microbiology, Physics & Zoology. Recently, the Institute has also started multidisciplinary M.Sc. course in Environmental Science for the first time in the University of Mumbai. With an intake capacity of about 480 students for M.Sc. by papers and about 200 students for M.Sc. by research and Ph.D., the Institute contributes a lion’s share in teaching and research in University of post-graduate level in these subjects.

Department of Botany

The Department of Botany is one of the oldest departments of the Institute. It was established in the year 1923 and has a spacious area of 3662 sq. meters. Department has the honour of having Botanist of International fame on its past staff list. They are Prof. L.A. Ajrekar, Prof. R. H. Dastur, Prof. F. R. Bharucha, Prof. Mrs. E. Gonzalves, Prof. V. R. Dnyansagar, Prof. S. B. Chaphekar to name a few.

The department offers M.Sc. by papers and research as well as Ph.D. programmes. The intake capacity of the department is 20 each for M.Sc. I & II. The specializations offered at M.Sc. II are Cytogenetics & Molecular Biology, Environmental Botany, and Plant Physiology & Biochemistry.

The teaching staff of the department includes Dr. Mrs. Shahana Khan (HOD), Dr. T. Srinivasu, Dr. V. D. Mendhulkar, Dr. Mrs. S.V. Pradhan, Dr. Mrs. A. N. Hodavadekar, Dr. Archana Rangare, Mr. R.W. Raut, and Mrs. R. N. Pande.

All the staff members are actively engaged in teaching and research as well as co-curricula. They are also involved in teaching of environmental science & Biotechnology. Major thrust areas of the department include Biodiversity, mutation breeding, tissue culture, phyto-chemical analysis, Bio-remediation, Bio-fertilizers and Pollution control.

Some of the facilities available with the department includes Traditional and Electronic herbarium, Botanical garden with some of the rare and endangered species, equipments like Gas Chromatography, micro photographic equipment, UV-VISIBLE spectrophotometers, Cold Centrifuge, Plant growth chambers, trinocular, binocular, monolocular, dissecting, phase contrast, projection microscopes, computers, digital cameras, CCD cameras and other basic equipments.

The Botanic garden, which has spread 0.2 hectares in the prime area of South Mumbai, is one of the well-maintained gardens (NAAC, 2002) and is included in the list of Botanic gardens of the world and also in the directory of botanic gardens in India (BSI). The oldest collection in the herbarium (*Ribes nigrum)* is which dates back to the year 1884, which was presented by Dr. J. R. Drummond to the Head of the department, Prof. R. H. Dastur.

The department of Botany is constantly receiving a number of research project grants from various funding agencies since 1960's. And in recent time, UGC sanctioned Rs. 7 lakh grant for "Electronic herbarium" project for the first time in India. At the same time DAE-BRNS sanctioned Rs. 9.87 lakh for the project, Development of High yielding genotypes with improved oil quality in Niger (*Guizotia abyssinica* Cass.).

With these achievements, very recently, Department of Botany, Biotechnology, microbiology and Zoology combined received grants of Rs. 31

lakh from Science & Technology, New Delhi under FIST programme for the development of molecular biology & biotechnology laboratory set-up with networking facilities.

The department of Botany received huge funds from various funding agencies for organizing several conferences, symposia, workshops and refresher courses for the benefit of the botanists all over India in the past. As evidenced, this department organized UGC & DAE-BRNS sponsored '*First National Training Workshop on Electronic Herbarium and Digital Database Preparation'* in February 2003. This is the second one in line. It has also planned to organize one more conference in this year i.e. "*12th All India Congress of Cytology and Genetics*" in collaboration with AICCG society, Calcutta from 28-31st October 2005.

This department has tradition of publishing many research papers in several reputed journals in addition to books such as "**Plant Geography** " by Prof. F.R. Bharucha and recent book "**Wonderful World of Wild Flowers of Mumbai**" by Dr. T. Srinivasu, Dr. S. N. Pathan and S. N. Pardeshi.

COMPUTER VISION TECHNOLOGY FOR PLANT SCIENCES

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Abstract

Computer Vision, which can be defined as the process of recognizing objects of interest from images, includes both Image Processing and Pattern Recognition. Computer vision technology, which exploits the developments in imaging science, is a new and efficient tool to quantitatively evaluate image information. Several novels applications of digital imaging in various disciplines of plant sciences like plant physiology and development, plant biochemistry, plant breeding, agronomy, cytogenetics etc are described.

Introduction

What we see we believe. Photographs and pictures have unique place in research in biology. Previously it was very difficult to quantitatively evaluate photographic data. The advances in personal computer based digital imaging technology have resulted in evolution of the versatile imaging systems for research in life science, which can be called as computer vision systems. Essential features of digital image processing and its applications in life sciences have been described by Sainis *et al* (1). A typical imaging system has a digital imaging device such as CCD camera, or scanner to input the image data. These devices discretize the image data spatially into pixels as well as digitize the intensity of the image as gray value for each pixel. The color imaging devices digitize the intensity data in Red, green and blue channels. Thus both monochrome and multicolor images are made amenable for computing. The digital data can be stored or processed before reproduction. It also can be analyzed to obtain quantitative information about several features present in the image. The images captured by these imaging devices can be stored and analyzed using general or specific software packages operating on personal computers. The availability of portable digital imaging camera has extended

applications to areas such as imaging samples under field conditions where quantitative imaging was not possible. Some interesting applications of imaging technology are described in the following.

Plant physiology and development

Several parameters of plant growth and development are now amenable for measurements using digital image analysis systems. Earlier it was difficult to measure the growth and development of different parts of plants such as roots, shoots, leaves flowers *in situ*. A non-destructive measurement of biomass, leaf area and other morphological parameters can now be undertaken using digital image analysis systems.

Image analysis was used to measure leaf area index and plant size of young hazelnut plants by Bignami and Rossini (2). Knowledge of leaf area index of woody plants is crucial for research on physiological responses of trees to various environmental changes. The measurements of leaf area dynamics of these plants were crucial but not practical because of destructive sampling procedures. In the above case a correlation was established between total leaf area and area canopy projection on the ground. Plant size parameters were also correctly predicted using image analysis. Once calibrated, the image analysis method was a simple, rapid and effective tool for measurement of crown leaf area index and plant growth repeated in time and space.

Roots control the uptake of water and nutrients by plants, and hence the dry matter production. The greatest constraints in understanding the root dynamics have been the laborious tedium associated with accurate measurements of various root parameters. The image processing and analysis techniques made this impossible task look very easy. An easily accessible method for root length measurement using an image analysis system was described by Tanaka et al (3) which involved taking pictures of root system against a dark back ground. The method was more accurate and reliable compared to normal root scanner.

The effect of gravitropism on surface extension in maize roots was studied by Ishikawa *et al* (4) using image analysis. It was generally agreed that root gravitropism occurred by establishment of asymmetric growth across the elongation zone with slower growth on lower side as compared to that on upper

side. Understanding the growth-rate pattern during garvitropism was important for the assessment of models pertaining to cellular basis of curvature. In order to facilitate measurement of localized extension rates, glass beads (0.25 mm) were stained with India ink and placed on root surface. One bead was placed at the root tip and ten others were placed evenly along the opposite sides at 0.7-mm intervals. The TV camera fitted with 16 mm lens and high resolution CRT monitor connected to time lapse recorder, video digitizer and to IBM PC computer was the hardware used for recording the images of the roots at various intervals. Since white roots were placed on white background, the software was written to track the black objects on white background using special software. This software measured the angle of curvature of beads with respect to each other and the respective distance from the bead at the root tip. A special algorithm was used to locate the center of each bead. Using this system Ishikawa *et al* (4) observed that the curvature began 25±7 min after gravity stimulation and resulted from enhanced growth along the upper surface and reduced growth along the lower surface. The roots curved at the rate of 1.4 ± 0.5^{o} min^{-1}. The final angle of 90^{o} was reached in 110±35 min. The surprising feature of this study was that there was a rapid translocation of gravity stimulus from root cap to the elongation zone or that gravitropism can be perceived directly by the elongation zone. Thus it seems unlikely that gravity-induced changes in pattern of hormone distribution alone can account for gravistimulation.

Bassetti and Westgate (5) used image analysis system for quantifying floral asynchrony and kernel set in maize. A delay in the onset of silk emergence relative to pollen shed often decreases kernel set in maize (*Zea mays*). Using a computer-aided image analysis system, lack of pollen, failure of silks to emerge, and loss of silk receptivity were measured daily to monitor the progress of silk emergence and the intensity of pollen shed in the field. The finding suggests that selection for silk emergence prior to pollen shed (protogony) may improve kernel set in maize under conditions known to delay silk emergence. Image analysis system was also used to predict growth in lettuce plants grown in plant factory by Shibata *et al* (6).

Genty & Meyer (7) have developed a method for routine, non-invasive monitoring of the topography of leaf photochemistry. The method uses video images of leaf chlorophyll fluorescence, taken during steady-state photosynthesis

and during a transitory saturation of photochemistry, to construct, pixel by pixel, an image of the photochemical yield of photosystem II (PSII). The effectiveness of the method was shown by mapping the heterogeneous distribution of photosynthetic activity after treatment with either a herbicide (DCMU), abscisic acid, or during the course of the induction of photosynthesis. Leaf CO_2 assimilation was simultaneously monitored under non-photorespiratory conditions to estimate the average quantum yield of linear electron transport. This new ability to quantitatively visualize leaf photochemistry provides a powerful tool to probe the spatial distribution of leaf photosynthesis.

Peterson & Aylor (8) also have described an application of video imaging of fluorescence to the study of light utilization in photosystem II of attached leaves of *Phaseolus vulgaris* infected with the obligate biotrophic fungus. The video-based detection system produced a spatially resolved, quantifiable signal that was highly specific for chlorophyll fluorescence.

It is now possible to study quantitatively translocation and metabolism of labeled phosphate. Barry *et al* (9) have described the detailed distribution of the tracer from the auto-radiographs of the corresponding photographs using image analysis. Horner *et al* (10) were able to quantitate the sieve tube elements in foliar terminal veins. Similarly other elements of vascular system can also be quantitatively estimated using image processing and their relation to the phenomenon of translocation can be measured using radio labeled compounds. Olofsdotter (11,12) have established the dose-response curves for variety of herbicides on various crop plants in cell culture assays and in whole plant assays using image. The technique was very sensitive to small differences in responses than manual counts of cell colonies.

Plant biochemistry

In a new approach to study enzyme activity *in situ,* a relatively low-cost computer-assisted image analysis system has been developed by Crevecoeur *et al* (13). Special software was written for the continuous monitoring of absorbance readings on cryostat sections of plant tissues incubated in media to reveal activity of glucose-6-phosphate dehydrogenase in cryostat sections from shoot apices of spinach plants. The reaction rate of the dehydrogenase activity was monitored at two incubation temperatures, 20^{o}C and 30^{o}C. Control incubations

were performed in media lacking substrate. The specific test minus control reaction at 30°C was twice that at 20°C. Variation of the substrate concentration at 30°C yielded a Km value of 0.37 mM. These results show that image analysis system can be used for kinetic measurements of dehydrogenase activity in frozen tissue sections. It is a new approach to study enzyme activity *in situ*.

Agronomy

Root and root-soil contact of plants in relation to soil macroporosity can be determined using image analysis system. The effects of soil salinity on crop growth and yield were studied using digital systems by Wiegand *et al* (14). Influence of heavy soil compaction on the morphometric parameters of soil structure was investigated by Domzal *et al* (15). In this study, a digital system was used for the morphometric analysis of soil kneaded with tractor wheels. It was found that even a single passage of a light tractor over a loose soil of 60% field water capacity caused a significant deterioration of physical properties of the soil.

Plant breeding

Digital images are important in generation of databases of germplasms. Mincione *et al* (16) have constructed an image-based germplasm database for Phaseolus. The database has the capability of simultaneous display of images and data fields. Quantitative Evaluation of Soybean leaflet shape has been reported by Furuta *et al* (17) Leaflet shape of thirty-nine soybean varieties was quantitatively evaluated by principal components scores based on the elliptic Fourier descriptor of contours. The results were analyzed to understand the genetics of leaf morphology. Digital image analysis was also used to study polymorphism in oat kernel size by Pietrzak & Fulcher (18). Genetic aspects of starch granule traits in barley were studied by Oliveira *et al* (19) using image analysis system. Starch granules in barley kernels were classified as large, Type A, and small, Type B. Fourteen genetically diverse barley genotypes were evaluated in four environments, and four populations were evaluated in a parent-offspring study. Significant differences were found among the 14 barley genotypes the granule traits.

Plant pathology

Effects of common root rot on discoloration and growth of the spring wheat root system was studied by Kokko *et al* (20). Image analysis procedures were used to quantify the effect of common root rot on the growth and discoloration of the subcrown internode (SCI), crown roots, and seminal roots of spring wheat. The linkage of discoloration of the SCI to a reduction in root growth was most evident for the crown roots. Image analysis procedures developed in this study can be used to objectively and precisely measure damage caused by root diseases in other host species. This procedure validates the most commonly used field research method of rating plants for common root rot.

Rust resistance of maize was quantitated using image scanner by Miyaga *et al* (21). Seedlings of 5 to 6 leaf stage were inoculated by dusting urediospores mixing with talcum powder. Urediospores mass-produced on the leaf surface were stripped off by adhesive tape and these tapes were stuck on a white paper. The paper was read with an image scanner. Black dots, image components of urediospore mass, were counted by a personal computer and total urediospore mass area per unit leaf area was calculated using the area-measuring program. Resistance to southern rust of 5 to 6 leaf stage plants was clearer than 2 to 3 leaf stage. Difference of resistance within varieties measured by this method was equal to the difference examined in the field. Color image analysis was also used for quantitative assessment of powdery mildew in cucumber by Kampmann & Hansen (22).

Image analysis has been used to estimate the dynamics of chlorophyll content of leaves under field conditions. The green color of leaves indicates the amount and proportion of chlorophyll in the leaves and indicates nutrient status of plants. Though several methods were used previously to measure reflectance of light from green leaves, leaf color diagnosis with color camera appears to be an effective, easy and low cost method to estimate intensity of green color of leaves of individual plants in the field. Kawashima and Natakani (23) correlated the chlorophyll content of leaves with red (R) green (G) and blue (B) wavelengths with accuracy of 0.1 g/m $^{-2}$ using digital values in RGB. The normalized function (R-B)/(R+B) was found to be correlated better with chlorophyll. Chlorophyll content of the leaves can also quantified using color scanners.

Morphometry of seeds

Morphological features of grains are important and grain appearance is widely used as the basis for establishing the class and identity of given sample of grain. Features such as kernel size, shape, color, texture are heritable and can be easily used for visual grain inspection. Grade assignment to the grains is normally based on visual inspection of grains by experts, which is completely subjective process, susceptible to human error of judgement. Besides it is also tedious and imprecise. Correct visual identification results from comparing a pre-existing mental image of a variety and mental image created from the new sample, which puts unusual demands on expertise. Biochemical and molecular methods are also being developed for variety identification but are expensive and time consuming. An objective, quantitative, and routine methods for measurement of grain characters that lend themselves to visual identification, or, more correctly, to discrimination of varieties of wheat and other cereal grains, would be highly desirable. The advances in digital image technology have offered a scope to bring objectivity and quantitation in estimation of morphological features of grains. This process uses the digital images to measure size of individual grains and mathematically extract features and shape related information from the images. Several attempts were made previously to use image analysis for grain characterization of oats, rye, barley and wheat and deal with use and quantitation of variety of morphological features of grains

Sakai *et al* (24) analyzed the shapes of brown and polished rice of Japonica, Indica and Javanica types composed of four rice varieties with three polishing methods using image processing. Area, perimeter, maximum length, maximum width, compactness and elongation were measured. The maximum length, maximum width and elongation of a rice particle defined here were different from the traditional dimensions such as length and width. Further, separating the rice varieties using the shape difference of a rice particle was also examined. The results suggest that separating the rice varieties was possible at a probability level of 95.4% with combined dimensions and shape factors or with single ones.

We have been interested in applying the digital image analysis technology to variety of problems (1). With the advancements in computing, imaging and microelectronics, it was felt necessary to develop an imaging system for quantitative measurement of morphometric features of grains. The COMPREHENSIVE IMAGE PROCESSING SOFTWARE (CIPS) PACKAGE developed in-house, is currently capable of measuring several geometric parameters from the grain images as well as carry out moment analysis on these images in addition to performing image enhancement operations (Shouche *et al,* 2001). The gray images of wheat grains, captured in crease down position using digital scanner in transparency mode are used for analysis. Geometric features such as area, perimeter, compactness, major and minor axis length and their ratios, slenderness, spread and several shape factors are computed on the binary image of each grains generated using a suitable threshold. Moment analysis is also carried out on the gray images of wheat grains after rotation normalization for extended analysis of shapes of grains.

Cytogenetics

The field of cytogenetics is benefiting immensely by introduction of PC based imaging technology. Image analysis was used for human chromosomes karyotyping much before the advent of personal computers. These image analysis systems were dedicated, extremely costly and difficult to adapt for karyotyping other chromosomes. With the advances in digital technology it is now possible to use image analysis systems for variety of chromosomes including those of plants. Plants have a wide variety of chromosomes numbers and with different morphological features. The existence of polyploidy and aneuploidy makes identification of chromosomes very difficult. A more flexible architecture in terms of hardware and software is required for analysis of plant chromosomes. Fukui developed a special image analysis system CHAIS for plant chromosomes (26, 27). This system allows for gross structural examination of plant chromosomes. Automatic karyotyping involves image formation of chromosomes picked up directly through TV camera mounted on a microscope. The image is first digitized and frozen in image frame memory. In a typical program 16 gray images are averaged, editing, shading correction is done on average image and this image is binarized. The extracted image is checked, midribs are drawn and

centromere positions are determined. This is done for all the chromosomes and length of each chromosome is measured. An identification number is automatically assigned to each chromosome and ideogram is generated by computer graphics based on numerical data. This system can process 20 chromosome images in 10-20 min. including interactive modifications of image.

Fukui & Iijima (27) and Iijima *et al* (28) have used image analysis to classify rice chromosomes. Rice chromosomes are very small (1-2 μm) at mitotic metaphase and identical in morphology. There was no objective identification method to recognize these tiny chromosomes. Image analysis was, therefore, used to characterize the condensation pattern reflecting the compactness of the chromatin fibers along the chromatid. By combing image data with numerical parameters of length and arm ratio nearly 118 specific features were obtained to classify 12 rice chromosomes (28) In cytogenetics the banding analysis is also important to characterize the chromosomes and their aberrations and abnormalities. Normally banding pattern is studied visually under a microscope by examining large number of metaphase cells. Image analysis was used to study banding pattern in barley and maize by Kakeda *et al* (29,30). In an interesting computer aided innovation Fukui & Kamisugi (31) simulated 10 different chromosomal microscopic images produced by 5 different microscopic optical techniques and staining procedures using one black and white image from a desktop imaging system. These experiments have demonstrated the proficiency of digital image processing to simulate the optical effects produced by microscopic devices and staining dyes. Thus computer simulation in future may be able to replace the optical systems such as phase contrast, negative staining, Normarsky differential interference contrast system and dark field system.

Gene bank activities

Preservation of genetic variability of different crop plants is one of the important activities in Gene bank. As a result of national and international efforts, national and regional gene banks have been established in many countries. Initially gene banks concentrated on collecting and saving ex situ threatened material of land-races of the most important crops. This is now gradually complemented with conservation of wild forms of cultivated plants and their relatives as well as biodiversity in general. Considering the utilization of the gene

bank material in breeding programs it is important to have a data bank of all the morphological and as many biochemical characters as possible for each genetic stock. Digital imaging systems can now be adapted to keep quantitative records all the visual parameters of a given genetic stock. Digital images can also used in maintaining the taxonomic records the flora and fauna.

Novel applications

In an interesting study the densitometric analysis was used to measure DNA content in lentil roots grown in space using image-processing system by Driss-Ecole *et al* (32). The seedlings were grown for 28 hours on board in space lab and the roots were frozen for further analysis. The roots were fixed and feulgen stained nuclei of cortical cells were analyzed using integrated optical density. In micro gravity there was decrease in DNA synthesis and promotion of arrest in G2 phase of cell cycle.

Pyke and Leech (32) used computer assisted automatic image analysis systems linked to light microscope to probe into mechanism of control of chloroplast division. It has been observed that 90% of chloroplasts in mature leaf are the products of division of young green chloroplasts in expanding leaves. Ideal material to understand the regulation of chloroplast division is to get mutants where the tight correlation between the number of chloroplasts per mesophyll cell is disrupted. Identification of such mutants requires a rapid screening procedure capable of identifying small changes in microscopic cell phenotypes. The image analysis system was used to measure cell area and counting the number of chloroplasts per cell by thresholding the images after iodine staining. Mutants line with 80% reduction or 50 % increase in chloroplast number per mesophyll cell was obtained from M2 population of ethyl methane sulfonate-mutagenized seeds of *Arabidopsis thaliana*.

Biologists have been traditionally measuring the intensity of color using mainly the spectrophotometers. In these instruments the intensity of light transmitted through a solution is measured in comparison to the reference solution. It is rather difficult to handle very large number of samples in spectrophotometric analysis because numbers of samples that can be measured at a time are limited. Besides, normally the sample has to be present in the solution form only. In contrast the digital technology allows for quantitation of light intensity of millions of spots simultaneously depending on the imaging device

used. Fast computers are used to analyze the digital output. This has lead to a new frontier of research in digital technology called Vision Science. How are the patterns recognized, colors seen and distinguished, images subtracted by the eye-brain machines of the animal world, are some of the problems that are being addressed in vision science today. This is a multidisciplinary science where several applied and basic disciplines are merging together. The recent introduction of spectrally resolved imaging has added another dimension to this filed. A new instrument has been developed to measure light intensity between 400-1000nm using 12-bit mono color cooled CCD camera and interferometer. The technology has found applications in multicolor chromosome classification. This will now facilitate the study of many biochemical reactions *in situ.*

Image analyses are a task that requires steps of pattern recognition and attention orientation functions of brain, which are not yet fully understood, and are subject of intense research (34). Computer vision techniques will improve further when we understand more about image processing and analysis as the human brain carries it out. This will allow us to combine the precision and reliability of the computer vision and pattern recognition ability of human visual perception

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ELECTRONIC HERBARIUM AND DIGITAL FLORA OF MUMBAI

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Key Words: Digital database, electronic herbarium, species

ABSTRACT

Electronic herbarium may be defined as “virtual images of plant specimens in digital format”. This could be done by taking digital photos of plants in their natural habit with the help of digital camera and are processed in the computer. The digital database of the plants was developed by selecting two hundred variable characters by using software package ‘DELTA’. The digital photos are attached to the database after feeding characters. Nearly 975 species belonging to 142 families of dicots and monocots are incorporated in the digital flora till now. This includes some rare, endangered, endemic plants and new records to Mumbai.

This eco-friendly electronic herbarium has some edge over traditional one as the plant original colors are retained forever, no pathogenic attack, no bio-degradation problems, no space problem, maintenance cost is nil etc. In addition to these advantages, this database of plants can be used for Cladistic and Phenetic classification analysis, information retrieval, generation of conventional key and interactive key, and to get full description, brief description and diagnostic features of plants, current status of the plants, socio-economic values of plants etc.

INTRODUCTION

One of the dynamic branches of botany is plant taxonomy because it forms pillar to other branches and at the same time, it borrows information from other branches of botany in deciding the exact systematic position of species in modern taxonomy. For identification and classification of species, taxonomists take help of floras for description and herbaria to match the living specimen with previously identified preserved specimen.

In general, herbarium is a store house of plants specimens collected from far and wide, dried on blotting paper for a fortnight or so, treated with anti-fungal, anti-insect and antibacterial agents and mounted as well as labeled on standard size sheets, arranged according to some known system of classification and kept in pigeon holes of steel or wooden cupboards (Jain and Rao, 1977). The steps involved in the preparation of herbarium are 1) collection of different plant specimens by various methods; 2) pressing and drying of specimens; 3) preservation of specimens by poisons, fumigants etc.; 4) mounting of specimens by gluing or pasting or strapping or stitching methods; 5) labeling sheets on right lower corner, giving some details of the plant; 6) filling in pigeon steel cupboards and 7) preparation of accession (Jain and Rao, 1977).

Herbarium serves as vital link for various disciplines of biology not only to provide information about plants from the preserved specimens but also to give insight, the changes occurred in the existing plant biodiversity with past ones from time to time. However, there are several disadvantages, which we feel while dealing with preserved specimens in identification. Those are common problems such as pests and insects attack as well as bio-degradation of specimen, physical damage due to improper handling of specimen, fading of colors, space problem, missing of main identification key characters in specimens, high maintenance cost, availability of plant information pertaining to herbarium specimen restricted to that centre (not freely accessible from anywhere). In addition to them, it needs regular replacement of damaged specimens which is a load on nature (wastage of living material, in case of rare and endangered specimens), needs fumigation which leads to pollution and health problems, relay on field note books for information such as habit, habitat etc., problems related to preservation of large plant parts such as fruits (in cucurbits), culms (in bamboos), etc. With the advent of computers, digital cameras, these problems can be overcome easily and can make herbarium more eco-friendly, attractive and colorful as well as much more informative.

Electronic herbarium may be defined as “virtual images of plant specimens in digital format”(Srinivasu, 2003). This can be done by taking digital photos of plants in their natural habit and are processed in the computer. The digital database of the plants was developed by selecting a number of (more than two hundred) variable characters by using software package ‘DELTA’.

'The Flora of Presidency of Bombay' was studied a century ago by Cooke (1903); and later on 'The Bombay flora, Bombay' by Dalzell and Gibson (1961). In recent times, the entire Mumbai flora was not studied in detail after industrialization and mega-metropolitan city formation. Further, there is a lot of demand for database of plants all over the world especially from bio-diversity rich countries like India, China, Brazil as there are a number of economically and medicinally important plants available, which are untapped till now. Hence, it was thought to undertake the present (plant biodiversity) study and to digitalize the existing plants to fulfill the needs of people of various walks of life.

MATERIAL AND METHODS

Plants growing all over Mumbai (Sanjay Gandhi National Park, Maharashtra Nature Park, railway track sides, Malabar hills, Institute of science, Jijamata gardens, open vacant spaces in the city, sea-coasts and creeks etc.) are covered by taking digital photographs of plants in their natural habitats with special attention to some important features for identification and database preparation by using digital camera, computer and software DELTA (Descriptive Language for Taxonomy - worldwide accepted package). The digital database is indigenously prepared with more than 200 variable characters.

RESULTS & DISCUSSION

The electronic herbarium and digital database of plants of Mumbai (±1% area of Maharashtra) which now contains 871 dicots and 104 monocots (3025 species are recorded for entire Maharashtra) belonging to 142 families (187 families reported for Maharashtra State) with plant description in detail, family, botanical names (ICBN) and their synonyms, common and vernacular names, distribution in Mumbai, present status of the plants in nature, socio-economic (including medicinal) value of these plants and digital photographs. Addition of more number of plants to the existing database is in progress. This database includes more than 250 medicinal plants and some rare, endangered (*Crotalaria filipes* Bth., *Alysicarpus belgaumensi* Wight. var. *racemosus* Baker; *Curcuma inodora* Blatt.; *Achyranthes coynei* Sant; *Commelina hasskarlii* Clarke; *C. paleata* Hassk.; *Cynospermum asperrimu* (Nees) Vollesen; *Haplanthodes tentaculata* (L.) R. B. Majumdar; *H. verticillatus* (Roxb.)Nees; *Neanotis carnosa* Dalz.;

Typhonium bulbiferum Dalz.*; Gloriosa superba* L. *),* endemic plants *(Canscora diffusa* (Vahl.) R. Br. ex R. & S. *var. diffusa; Diospyros peregrina (Gaertn.); Ficus Talboti King; Lindernia parviflora (Roxb.) Haines {*Endemic to Asia*}; Lindenbergia muraria (Roxb. ex D. Don) Bruehl; Lindernia crustacea (Linn.) Mueller.* (Endemic to Asia*); Lindernia antipoda (Linn.)* (Endemic *to Asia); Limnophila dubia (L.); Pavetta indica L.; Neolamarkia cadamba (Roxb.) Bosser; Morinda pubescence Sn.; Mitragyna parvifolia (Roxb.) Korth.; Haldina cordifolia (Roxb.) Ridsd.; Alysicarpus vaginalis DC. var. stocksii; Jasminum auriculatum Vahl.; Nymphoides hydrophylla (Lour.) O. Kuntze.; Hibiscus Talbotti (Rakshit.) (*Endemic to Maharashtra*); Xenostegia tridentata (Linn.) Austin and Staples; Merremia vitifolia (Burm. f.) Hall.; Merremia gangetica (L.) Cufod.; Ipomoea marginata (Desr.) H. Mantz.; Argyreia nervosa (Burm. f.); Tricholepis glaberrima DC.; Phyllocephalum scabridum (DC.); Gymnema sylvestre (Retz.) R. Br. ex Schult.; Wrightia tinctoria R. Br. ssp. tinctoria; Wrightia arborea (Dennst.) Mabb. (*Endemic to Asia*);Rauwolfia serpentina (L.) Bth. ex Kurz. (*Endemic to Asia*); Holarrhena pubescens (Buch-Ham.) Wall. ex Don (*Endemic to Asia*); Cerbera manghas L.;(*Endemic to India & Lakshwadweep*); Achyranthes coynei Sant. (*Endemic to Maharashtra*); Haplanthodes verticillatus (Roxb.)*, mangroves and new records to Mumbai *(Barleria* lawii T. And.*; Haplanthodes verticillatus* (Roxb.)Nees*; Achyranthes coynei Sant.; Amaranthus caturus* Heyne ex Hook. f.*; Spilanthes clava* DC. *; Xenostegia filiformis* (Thumb.) Almeida (Comb. Nov.); *Euphorbia coccinea* Roth. *. Urena labota . var. lappago* (Sm.) Almeida *; Neptunia triquetra* (Vahl.) Bth.*; Alysicarpus vaginalis. var. stocksii* Baker*; Crotalaria nana* Burm. f. *; Mucuna minima* Haines*; Lindenbergia muraria* (Roxb.) Bruhl. *; Physalis longifolia* Nutt. *;Oxalis dehradunensis* Raiz*; Cistanche tubulosa* (Schrenk) Wight*).*

Mumbai occupies less than 1% area of Maharashtra state, but has nearly ⅓ of plant species of the entire state, indicates its rich plant biodiversity.

The applications of this database are many in addition to merits over traditional methods of herbarium and some of them are as follows:

1. Retrieval of individual plant information, 2. Generation of differences between two or more species, which can be used to decide the systematic position of plants and their families 3. Generation of key and intkey (interactive key) from the digital data which can be used for Cladistic and Phenetic analysis, 4. To know the

distribution pattern of plants in Mumbai and their present status in general, 5. Socio-economic values of plants etc.

Further, this technique can be adopted not only to angiosperms but also for various plant and animal groups. This electronic herbarium and digital flora of Mumbai could certainly provide recent information on new records and species of this region instantly. This database can be updated easily at regular intervals of time. Finally, it can be stated that this database may not be alternative to the existing herbarium but certainly complimentary to the existing herbarium and shares functional dynamism (load). It forms an important centre of data storage system for faster dispersal of information from one place to anywhere in the world i.e. bio-informatics of plant species in information technology era (web site form).

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A NUTSHELL OF PROTOCOL FOR ELECTRONIC HERBARIUM AND DIGITAL DATABASE PREPARATION

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This paper deals with various steps involved in the preparation of Electronic herbarium and digital database of plants in a nutshell. This is done by selecting various morphological characters with a number of possible variable states. It is also possible to take other than morphological characters such as anatomical, cytological, embryological, palynological, geographical, phytochemistry, molecular characters etc. in modern taxonomy. However, in this case, morphological characters of plants were taken as a model.

The following chief steps involved in the preparation of Digital database and electronic herbarium of plants are

I. Preparation of database, feeding data & retrieval of the data:

1. Double click on the drive (C/D drive wherever delta package is loaded)
2. Double click on DELTA (Descriptive Language for Taxonomy)
3. Click on FILE, sub-menu list is displayed
4. Click on New Dataset – Document 1 opens
5. Click on view – sub-menu list is displayed
6. Click on Character editor
7. Select Character No. 1 and type character on right upper box and select character type (as unordered multistate /ordered multistate/integral/ real/ text)

   For example: 'Family' is a character and select character type as 'unordered multistate'.
8. Type character variable in the lower right box and press enter

Add one by one variable in the same way and press done

9. Select next character (2), type 'synonyms' in the upper right box, select character type (UM/OM/R/I/**T**).

   For example: Synonyms was typed on the right upper box and selected character type as TEXT and press 'done'. Here fixed variables are not available, no need to type variables.

11. Select next character (3), type 'longevity of plant' in the upper right box, select character type (**UM**/OM/R/I/T), type character variables one by one in the lower right box and press enter every time.

   For example: annual <plant that completes its life cycle in a season/year>

   Press enter

   biennial < plant which completes its vegetative growth in the first year and reproductive stages in the next year> press enter.

   perennial < plant lives more than two years> press enter.

   (**Note**: If you want to write meaning/comment of the term (variable state), put it in the bracket "<>" to understand the meaning of the term while preparing database. The meaning will not be quoted but the term will only be displayed at the time of generation of retrieval of information).

10. Select another character (4), type 'stem' in the upper right box and select character type (UM/**OM**/R/I/T) and type variables such as absent & present and press done.

   *For example: '*Absent' press enter

   'Present' press enter

   tick mark 'implicit' and press done.

   (**Note:** Tick mark implicit check box, it allows the specification of the currently selected state as the implicit state).

11. Select another character (5) 'stem height' in the upper right box and select character type (UM/OM/**R**/I/T) and type units of measurements in the lower box as 'cm. high', and press done.
12. Select another character (6) 'stipule number' in the upper right box and select character type (UM/OM/R/**I**/T) and press done.
13. Select another character (7) 'flowering and fruiting period' in the upper right box and select character type (UM/**OM**/R/I/T) and type variables one by one by pressing enter and finally press done.

    For example: 'January' press enter

    'February' press enter

    'March' press enter

    etc. and press done.

14. *Tick mark 'mandatory' in the check box for a character, if you want that character must be recorded (compulsory) in every item.*
15. *Tick mark 'exclusively' in the check box for variable state in the multistate characters i.e. only a single state of the character can be associated with any given item).*
16. Click on 'view' menu, sub-menu is displayed. Select taxon editor, 'item edit' Opens.
17. Type genus and species names separately with suffix 'author name' in the edit taxon name box.
18. Select genus and species name and click button '*i*' in the formatting tool bar and press done.
19. Genus and species name appears in the upper left box and characters in the upper right box.

Feeding datasheet:

20. Click on the first character 'family', variable states appear in the lower right box. Now click on the suitable variable state.

For example: Barleria cuspidata – Tick on the family variable state 'Acanthaceae'.

21. Select next character - Synonyms. Here it is 'Text character', hence type the matter on the lower left box.
22. Similarly select Real/integral number type of character. In this case, type the measurements in the lower left box.
23. While feeding data, if you want to edit specific character, right click mouse sub-menu is displayed. Click on **edit selected character** in the sub-menu for editing.
24. Similarly, click on **append to add new character at the end of the list** (Last character number).
25. Like-wise click on **insert new character at this position** for addition of new character above the selected character.
26. If you want to remove unwanted character, click on **delete this character** in the sub-menu displayed.
27. If you want to see all the variable states of any one character in the upper right box, click on **expanded all**—all the variable state are visible at a glance.
28. If you want to see default (normal) position, Click on **collapse all –**now all the variable states of character in the upper box disappear and appear in the lower box.
29. For altering the sizes of these 4 boxes (resizing), place cursor on the divider of the boxes (the cursor appears like ↔ it), then move the mouse according to your requirement.

30. Once the feeding of the data of species is over, click on 'file' menu and save dataset (**Note:** *Try to save dataset at regular intervals for safety of data).*

II. To transfer digital photographs from camera to the computer:

1. Plant digital photos may be taken in the natural habit by using digital camera with good resolution.
2. Connect the frame grabber to digital camera and USB port of the PC.
3. Switch on digital camera in play mode.
4. Double click on Fine pix viewer icon on desktop (shortcut) or open the same through start, program menu, Fine pix viewer, and *Fine pix viewer* icon.
5. Double click on DCIM.
6. Double click on 100 Fuji (camera folder). It will display the images currently in the camera chip.
7. For transferring images for the first time click on 'settings' menu on the menu bar, click on 'Automatic save settings' in the sub-menu (set file type in JPEG/BMP, then fix destination folder by browsing). Next time or subsequent transfers, go to file and select automatic save or click on Automatic save icon on the menu bar, the digital photos in the frames are saved automatically in the fixed TMP (temporary) folder in the specified drive.

OR

8. Double click on **removable disk drive** on my Computer. Double click on DCIM. Copy whole folder of 100 Fuji (camera folder) and paste it in the destination folder.
9. Delete the digital images from camera chip by selecting all (Control & A Key simultaneously) and right click mouse to display sub-menu and then select delete.
10. Switch off Camera (**Note**: Don't close Fine-pix before switching off the camera).

11. Close the Fine-pix viewer window now.
12. Remove the frame grabber from the USB port.
13. Double click 'My computer' icon on desktop and open specified drive where photographs are stored in TMP file folder for editing.
14. Open TMP file folder, where digital photos are saved with their number (year & image number). The number should be replaced with plant (ICBN) name by right clicking mouse on photo, a sub-menu appears, then select 'Rename' option and then type plant name & family name.
15. Select the digital photo after renaming, cut and paste it in the "image" folder inside the DELTA folder. The images are ready to attach to the corresponding species.

III. Attachment of digital photographs for display in the DELTA:

1. Open DELTA and dicot folder.
2. Click on 'View' menu in the menu bar and select 'Taxon editor' or double click on (any specific) genus and species name, dialog box will be displayed.
3. Click 'Add' button, another dialog box 'Select Image To Add' will appear. Click 'Look in Files'. Select 'image' folder and see the list of the plant images names available in the image folder
4. Select specific species image name (for e.g. *Annona squamosa* Linn., Annonaceae) and click on 'use' button for attachment and save dataset. Click on the 'display' button in the image files dialog box, the image will be displayed.
5. Now click on 'scaled' in the window menu, the same full image is displayed in the condensed form.
6. Right click mouse on the image, select insert overlays and then select item (taxon) name in the sub-menu – a dialog box appears.

7. Click on '*i*' button and then tick integral height and finally click 'ok' button, taxon name is displayed within digital photo. The taxon name can be placed anywhere within the frame by dragging the cursor.
8. Similarly attachment 1or 2 images one after another in the same way.
9. To display digital images one after other, select subject in the menu, a sub-menu with all the names are displayed. Select any one image, that image is displayed.
10. Close the window and then press done to comeback to database.
11. Save dataset.

IV. EXPORT AND IMPORT DIRECTIVES TO RETRIEVE INFORMATION AND TO GET INTKEY FACILITIES:

1. Click 'File' in the menu bar
2. Click on export directives
3. Click 'OK' button, the process is finished. Press done.
4. Open the same file in the menu bar and click save dataset-the whole process is saved.
5. Click on import directives, a dialog box appears. Press Ok.
6. If there are errors anywhere, the error messages appear, please correct them before pressing done or correct them and run import directives again for proper import of files and press done.
7. Close down the window.
8. Click 'View' on the menu bar and select 'Action Set'.
9. Click on each action from the 'confor' list and run. Click Ok (If any file write failed, message is shown, correct mistake) and run again same steps.
10. Do Actions for all the files in 'confor' list and press done.
11. Click on intkey and run, Query export dialog box appears, click on **yes.**
12. **Select DELTA files export** dialog box appears, click Ok.

13. **Export status** dialog box appears (If there are mistakes, please correct them as per message) and press done - **INTKEY** appears.

V. INTKEY (Interactive key) – FACILITIES:

1. Select any one taxon and click '*i*' (information about taxon) button, taxon information dialog box appears.
2. Select full description or brief description or diagnostic description or three together or illustrations to get full description or brief description or diagnostic description or all or illustrations.
3. To get differences between two or more taxa: Select any two or more species, click on differences between taxa- differences details and total number of differences at the end will be displayed.
4. **Query & search facility:** *To identify an unknown taxon*.

   Select one by one character from the left upper box and feed requirement /select one variable state and OK.

   For example: Ask rose coloured flowers list by selecting 'select state - rose' click OK. Eliminated taxa can be seen on right lower box and remaining taxa on the right upper box.

   Select another one and repeat the same steps and ultimately you reach the target taxon remaining in the right upper box. Once you know the name of taxon, you can get information and illustrations.

   (Note: Text characters cannot be used in query & search facility).

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