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An Insite into the Vanda Genera: Vanda`S Natures Wonder

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Abstract

Orchids are the most beautiful and unique group of plants in the angiosperm. They are ubiquitous in distribution and have a universal appeal. Taxonomically they represent the highly evolved family among monocotyledons with 600-800 genera and 25,000-35,000 species are highly variable in habitat and form. In India, Orchidaceae form 9% of our flora and are the largest botanical family of angiosperm. About 1300 orchid species had been reported from India scattered all over North Eastern Himalayas (600 species), North Western Himalayas (300 species), Maharashtra(130 species), Andaman and Nicobar Islands (70 species) and Western Ghats (200 species). Many species of Dendrobium, Phalaenopsis and Vanda are renowned for their very showy and attractive flowers and their ornamental value. South-East Asia has developed into a major supplier of orchid hybrids.

Keywords: Taxonomically; Angiosperm; Dendrobium;

Introduction

The vast majority of orchid species are epiphytic, but 4,000 known species are primarily terrestrial. Orchids exhibit two distinct growth habits: sympodial and monopodial. Monopodial orchids have indeterminate stems that grow continually each season and bear lateral inflorescences. Sympodial orchids have stems that grow for a determined period of time and cease to grow further, with new growth coming from lateral meristems and can have lateral or terminal inflorescences.

Vanda (van-da) comes from the Sanskrit word describing the plant today known as Vanda tessellate from Bengal (India). It was discovered in first time. The genus Vanda consists of about 80 species, mainly distributed across East Asia, Southeast Asia, Bumeo, Indonesia, Australia and New Guinea, with a few species extending into Queensland and some of the islands of the western Pacific. These mostly epiphytic, but sometimes lithophytes or terrestrial orchids, are distributed in India, Himalaya, Southeast Asia, Indonesia, the Philippines, New Guinea, southern China, and northern Australia. Vanda's are found in tropical areas, requiring lots of bright light, plenty of water, warm temperature and high humidity [1]. Some of the important species are Vanda tessellate, Vanda thwaitesii, Vanda wightii, Vanda testacea, Vanda pumila, Vanda motesiana (found in India - Arunachal Pradesh to Assam), Vanda griffithii (Eastern Himalaya), Vanda jainii (Assam), Vanda lilacina (China-Yunnan to Indo-China), Vanda liouvillei (Assam to Indo-China), Vanda stangeana (India-Arunachal Pradesh to Assam), Vanda testacea (Indian subcontinent to China).

Vanda is one of the best known and attractive Asian Orchid genera, and it can mostly be found in tropical lowlands and foothills. This genus is highly prized in horticulture for its showy, large, beautiful, fragrant, long-lasting, and intensely colourful flowers. This genus is one of the five most horticulturally important orchid genera, because it has some of the most magnificent flowers to be found in the orchid family. This has contributed much to the work of hybridists producing flowers for the cut flower and pot plants market. Vanda coerulea is one of the few botanical orchids which can produce varieties with blue flowers, a property much appreciated for producing interspecific and intergeneric hybrids. Vanda dearei is one of the chief sources of yellow colour in Vanda hybrids.

(a) Vanda tricolor with strap-like distichous, stem-clasping leaves, keeled, green above and light beneath, the apex erose.

(b) Vanda teres with terete leaves above a thick as a pencil, the base of these leaves encircles the stems. The inflorescence is axillary, erect, and racemes with few too many flowers.

A common physiological trait in epiphytic orchids like vanda's is the utilization of the Crassulacean Acid Metabolism (CAM) photosynthetic pathway. CAM has been linked to the evolution of epiphytism in orchids and is likely due to the need for increased water efficiency.

Some important Vanda species distributed in Indian sub-continent are listed below:

Vanda coerulea commonly known as blue Vanda or autumn lady's tresses is a species of orchid found in Assam and neighbouring Khasi hills with its range extending to China. In the wild, Vanda coerulea grows on exposed deciduous trees (primarily oak) at elevations of 900-1500 m. It has been recorded from India (Assam, Arunachal Pradesh, Meghalaya, and Nagaland), Nepal, Burma, northern Thailand, and is also likely to occur in Bhutan, Laos and Vietnam. It is a striking species with large, flat, vivid blue, long-lasting flowers. It is greatly prized by growers who have used it extensively in breeding to produce deep blue and purple hybrids. The plant bears up to 20 to 30 spikes. Vanda coerulea is a spectacular ornamental and widely used by orchid breeders to produce a range of hybrids [2]. Recent laboratory research indicates that extracts from V. coerulea may have potential use in anti-ageing skin treatments.

Vanda wightii is from southern India and Sri Lanka. For some time it was thought to be extinct after being described in 1849. The species was however rediscovered and it is now known from India. It is closely related to Vanda thwaitesii. Primarily it is found at the altitude of 60-80 m but has been found as high up as 700 m.

Vanda bensonii Bateman found in Assam India, Myanmar and Thailand in deciduous forests at low to moderate elevations as a small to medium-sized, hot to warm growing, monopodial epiphyte with an erect, leafy stem carrying lorate, coriaceous, alternate leaves that blooms on an axillary, erect, racemose, several inflorescence with fragrant flowers occurring in the spring.

Vanda cristata is a medium sized, monopodial epiphytic species from the montane forests of Bangladesh, India, Nepal, Bhutan and Tibet China on moss covered trees at altitudes of 600 to 2300 meters and is a small sized, warm to cold grower that can take some full sun with a stout stem carrying 2 to 6 flowered inflorescence having fragrant floral scented, waxy, long-lived flowers with horns on the apex of the lip which is the determining factor between this species. Flowers during May-June.

Vanda bicolor is mainly found in the eastern Himalayas, Assam, Bhutan and Myanmar in open forests and on riverbanks at elevations 700 to 2000 meters as a small sized, warm to cool growing epiphyte with a leafy stem enveloped almost completely by leaf sheaths and each carrying oblong, curved, bilobed, each lobe tridentate leaves that blooms in the winter and spring (March-May) on an axillary, glabrous, 3 to 6 flowered inflorescence with triangular floral bracts.

Vanda alpina also known as the montane Vanda, mainly found in western Himalayas, Assam, eastern Himalayas', Nepal, Bhutan, Sikkim, Yunnan China and Northern Vietnam at elevations of 900 to 2000 mts. This species is often mislabelled and often Vanda alpina plants actually turn out to be Vanda cristata. The difference between the two is that Vanda cristata has horns on the apex of the lip and Vanda alpina does not. It blooms during June, July.

Vanda coerulescens is a monopodial species comes from Assam India, eastern Himalayas, Yunnan China, Myanmar and Thailand as a small-sized, hot to cool growing epiphyte at elevations of 300 to 1200. This Shy Blue Vanda will flower during the later winter, and spring flowers have a pleasing scent of grape and are borne on an axillary erect or drooping inflorescence, longer than the leaves.

Vanda thwaitesii is an endangered orchid species endemic to southwesternn Ghats of India and Srilanka. Vanda epiphyte, originally reported from Sri Lanka where it is now believed to be extinct. In India, it is found in the Western Ghats in Kerala and Karnataka at elevations of about 800-1000 MSL.

Vanda motesiana Choltco is found in Manicure, a region in eastern India along the border with Burma, and in Assume (Meghalaya on current maps), which is just west of Manipur and includes the orchid-rich region of the Khasi Hills. Plants are found mostly at 3950-4900 ft. (1200-1500 m). It flowers during the month of March - May.

Vanda ampullacea (ROXB.) is native to the Himalayas, covering areas of Nepal, Sikkim, Bhutan, Khasia Hills in North-East India, Myanmar, Thailand, Laos, and Yunnan Province in southwestern China. In India, these plants grow in diffused light, often on leafy trees, at heights of 100-1000 m [3]. Vanda ampullaceum orchid, also called as Vein-like Ascocentrum, Aerides ampullaceum, Gastrochilus ampullaceus, Oeceoclades ampullacea, Saccolabium ampullaceum, Vanda ampullaceal, is a species of the genus Ascocentrum earlier. This species was described.

Vanda pumila is an endangered monopodial orchid with beautiful flowers that are native to Thailand but now found across South Asia. This orchid species is also found in North East India, Nepal, Myanmar, China, Laos, Thailand and Vietnam at the elevation of 1200-2300 m. Vanda pumila is important for breeding cultivars, and most of the species have great medicinal value.

Vanda hindsii. mainly distributed in Queensland Australia, Papua and New Guinea in areas with a distinct dry season in winter and spring as a large sized, hot growing, monopodial epiphyte found at the altitude of 300-600 mts. Flowers during the of November to March.

Vanda ampullacea Lindley is a perennial epiphytic orchid, summer bloomer on an axillary, [8 to 20 cm] long, shorter than the leaves, compact inflorescence that have many small flowers, found in central China, western Himalayas, Assam India, Bangladesh, eastern Himalayas, Nepal, Bhutan, Sikkim, Andaman Islands, Myanmar, Thailand, Laos and Vietnam at elevations of 300 to 1500 meters in subtropical forests near rivers often on deciduous trees..

It has had various classifications, initially called Aerides ampullacea by William Roxburgh when it was first described in 1814. It was most recently reclassified in 2012 during a taxonomic revision of Vanda.

Vanda tessellata also known as The Lattice-Like Patterned Flower Vanda, Aerides tessellatum, Cymbidium allagnata, Cymbidium tessellatum, Cymbidium tesselloides, Epidendrum tesselatum, Epidendrum tesseloides, Vanda roxburghii, Vanda roxburghii var. wrightiana, Vanda tesselloides, is a species of the genus Vanda. This species was described by William Jackson Hooker ex George Don in 1850.

Vanda tessellata is native to Bangladesh, India, Myanmar, Nepal, Sri Lanka. They are growing on trees in dry and intermediate zones at elevations around 1500 meters. At Bodimettu on the Kerala-Tamil Nadu border in Southern India, this species grows as a lithophyte in the open sun. It is a medium to large-sized, cool growing epiphyte, which reach up to 1m in height, with a 30-60 cm long, climbing stem carrying linear, narrow, tridentate apically, 15-20 cm long leaves.

The Lattice-Like Patterned Flower Vanda blooms on a sub-erect, 15 to 50 cm long inflorescence carrying 5 to 12, fragrant, long-lived flowers occurring in the summer, fall and early winter. The flowers are 5 cm in diameter.

Their colour varies from light to dark brown with darker veins and pink, blue or purple lip. The sepals are yellow, tessellated with brown lines and with white margins. The petals are yellow with brown lines and white margins, shorter than the sepals.

Vanda spathulata (L.) Spreng. found in southern India and Sri Lanka in the low brush at elevations of sea level to 300 meters as a large-sized, hot growing, vine-like, scrambling epiphyte. At present this species is extremely rare and this has been listed in the IUCN red list (species status: vulnerable).

In India it is mainly found in Karnataka, Kerala, Tamil Nadu, commonly called as a spoon- leaf Vanda, spathulata Vanda, Ponnampu-maravara in Malayalam and flowers from September to January (Figures 1-3).

Figure 1: *Vanda spathulata (L.) Spreng, V. alpine, V. ampullaceal, V. bensonii, V. bicolor, V. coerulescens.*

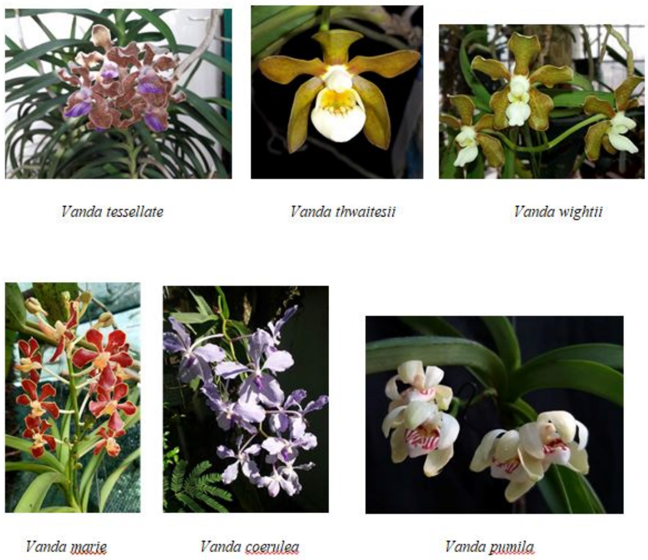

Figure 2: *Vanda tessellate, V. thwaitesii, V. wightii, V. marie, V. coerulea, V. pumila.*

Figure 3: *V. motesiana, V. denisoniana, V. pumila, V. testacea, V. dearie, V. tessllata.*

Photos credits: Mr. Waribam Amarjeet from Guwahati an orchid grower, Mr. Sriram Kumar from Bangalore an Vanda orchids enthusiast, Mr. Nageshwar Mahadev from Bangalore an orchids enthusiast.

Medicinal property

The potential of orchids to be used as medicinal plants are reported mainly in Chinese, Indian, Sumerian, and Egyptian systems of medicine. These plants are relatively unexplored for their chemical constituents and pharmacognostic properties.

Vanda species are being used in the folk medicine in various part of Asia, mainly in India, Nepal, China and Bangladesh. However, due to extensive use of Vanda species for various applications, the natural plant populations are being decreased, and some of them are now categorized as threatened species. As such, six species are placed under IUCN Red List including Vanda hindsii (least concern), Vanda javierae (endangered), Vanda thwaitesii (endangered), Vanda scanders (endangered), Vanda spathulata (vulnerable) and Vanda tessellata (least concern).

The literature survey revealed diverse traditional uses of different species of Vanda, mainly against indigestion, wounds, hepatitis, dyspepsia, bronchitis, piles, rheumatism, and bone fracture. However, only a few of them are investigated scientifically for their chemical constituents and pharmacological activities. Bioactive compounds including eucomic acid derivatives, phenanthrene derivatives and other phenolic compounds are reported from Vanda species. Different extracts were so far evaluated for the number of pharmacological activities including neuroprotective, anti-ageing, antimicrobial, anti-inflammatory, antioxidant, anti-cancerous, membrane stabilizing, wound healing and hepato-protective activities.

Traditional uses

Vanda tessellata, Vanda coerulea and Vanda testacea are also used as herbal drugs in the treatment of inflammation, cancer, nervous disorders and rheumatism according to Yunani medicines and traditional Indian medicine. Few Vanda species are studied for their pharmacological activities using in vitro and in vivo models.

Vanda tessellata roots are alexiteric and antipyretic useful in dyspepsia, bronchitis, inflammations, piles and hiccup. Externally the root is used in rheumatism and allied disorders and diseases of the nervous system [4]. It is also employed as a remedy for secondary syphilis and scorpion stings. The juice of the leaves is used topically in otitis, and a paste of them finds use as a febrifuge. In the Yunani system, the root is used as a tonic for the liver and brain; effective against bronchitis, piles, lumbago, toothache, and boils of the scalp; it also is said to lessen inflammation and heal fractures.

Vanda thwaitesii Hook.f. is an endangered epiphyte endemic to southwesternn Ghats of India and Srilanka. According to the ethnobotanical evidence Vanda thwaitesii possess medicinal properties like anti-microbial, anti-inflammatory and anti-cancerous activity.

The juice of Vanda coerulea flower is used as eye drops against glaucoma, cataract and blindness. Active ingredients of Vanda coerulea may fight against the visible signs of ageing skin.

Wound-healing activity- The aqueous extract of Vanda roxburghii was evaluated for wound healing activity in rats. Topical administration of the extract at a dose of 150 mg/kg/day showed potent activity, which was due to either increase in collagen deposition or improved alignment and maturation.

Hepatoprotective activity reported that the petroleum ether extract of leaves of Vanda tessellata showed dose-dependent hepato-protective activity as determined by serum markers like cholesterol, triglycerides, and alanine amino transferase in rats.

Anti-inflammatory activity evaluated the potential anti-inflammatory activity of hydro-alcoholic extracts of the stem of Vanda coerulea on HaCaT irradiated keratinocytes. These extracts were further analyzed for the isolation of various active biomarkers, which exhibited significant antioxidant and anti-inflammatory activity.

Antimicrobial activity evaluated the ethyl acetate extracts of Vanda tessellata stem, for antimicrobial activity by using the agar well diffusion method. The extracts showed broad-spectrum antimicrobial activity against Escherichia coli, Candida albicans and Aspergillus niger.

The antimicrobial activity of different extracts of the whole plant of Vanda tessellata. Among the extracts, the chloroform extract showed inhibitory activity against tested pathogenic bacteria (Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, and Vibrio cholerae) and fungal species (Aspergillus, Rhizopus spp. and Penicillium spp.) followed by hexane extract. In another study, reported that 3-ethoxy- 10, 17 dimethyltetradecahydro-1H-cyclopenta[a] phenanthren-17(2H)-one, a new compound isolated from Vanda tessellata, showed antibacterial activity against B. subtilis, E. coli and Proteus mirabilis.

Anticonvulsant activity-Pathan and reported the potent anticonvulsant activity of ethanolic extract of roots of Vanda roxburghii against picrotoxin, pentylenetetrazole and maximal electroshock-induced convulsions.

Anti-cholinesterase activity reported the anti-acetylcholinesterase and anti-butyrylcholinesterase activities of the chloroform extract of Vanda roxburghii. Similarly, extracts of the flowers of Vanda spathulata were found to be containing potent nootropic agents and their effects on the cholinergic system of mice brains was studied.

Antioxidant activity- Petroleum ether extract of leaves of Vanda tessellata showed potent 1,1-diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide (NO) radicals scavenging activities.

Anti-aging activity isolated and investigated three new derivatives of glucopyranosyloxy benzyleucomate i.e. vandateroside I-III along with eucomic acid from Vanda teres stem for their anti-aging effects in immortalized keratinocyte cell line of human origin (HaCaT). The results indicated that eucomic acid and vandateroside II increased the activity of cytochrome c oxidase, in this way activating cellular respiratory function, and as a result reducing the signs of epidermal ageing.

Investigated plant extracts of Vanda coerulea for antiaging effects in cosmetics. Their results proved that the extracts of this plant could be used as an active constituent in cosmetics to diminish the ageing signs of the skin. In this context, Vanda coerulea has been proved as a potential agent to preserve the skin and tissue firmness and to counteract skin pigmentation disorders.

Antidepressant activity examined the methanolic extract of the flowers of Vanda spathulata for antidepressant activity in mice using the forced swim test and the tail suspension test, and the results were significant in both models. The study further indicated that the antidepressant activity of methanolic extracts might be due to the inhibition of monoamine oxidase (MAO-A and MAO-B) in mice brain.

The extracts of the aerial parts of Vanda roxburghii showed significant antinociceptive activity. Investigated the antiulcer activity of petroleum ether extracts of leaves of Vanda tessellata in Wistar rats induced by aspirin. They reported that the rats pretreated with petroleum ether extracts exhibited considerable protection against gastric ulcer as compared to the untreated group without any detectable toxicity. Alcoholic extracts of flowers of Vanda tessellata showed aphrodisiac activity in male mice by stimulating their sexual behavior.

Flower biology

Vanda Orchid flowers are typically bilaterally symmetrical and trimerous with inferior ovaries. Orchid flowers are highly variable. The most characteristic features of the Orchidaceae are related to their flowers. The stamens are arranged on the abaxial side of the flower, the pistil and stamen are either partially or fully fused into a structure known as the column, and a highly-modified petal (labellum) acts as a landing pad for pollinators. Orchid flowers usually display ` Resupination´ twisting or bending of the pedicel, during development. Because of this, the labellum, which is adaxial in the flower bud, comes to rest in an abaxial position. Another defining characteristic of orchids is that their stamens are all located on one side of the flower rather than being radially dispersed like those of other angiosperm orders. In addition, the style and filaments of orchid flowers are fused to form a column or gynostemium. The column is marked by the presence of the rostellum, a modification to the column that separates the stigmatic surface from the anthers which are located at the end of the column. While primitive orchids still exhibit powdery pollen, the pollen of more derived orchids is contained in discrete, hard packets or pollinia that are attached to a sticky disc termed the viscidium. The viscidium attaches to potential pollinators, resulting in the removal of pollinia and subsequent pollination.

Darwin wrote extensively about the mechanisms by which orchid pollination occurs. While he studied and presented examples of specific pollinators to illustrate his theories about co-evolution, these may be the exceptions rather than the rules in the orchidaceae. Most orchids appear to be adapted for insect-mediated pollination and often exhibit "deceptive" pollinator attraction strategies.

As many as 400 species of orchids are sexually deceptive, offering some resemblance to the sexual partners of pollinator species, whereas one-third of all orchids are food deceptive.

Deception takes many other forms in the Orchidaceae including simulation of prey, simulation of substrata and "resemblance to antagonists". Attraction without reward (i.e. deception) is believed to be evolutionarily primitive in orchids, as it is a prevalent characteristic and maybe a long-term adaptive strategy that limits inbreeding depression and excessive fruit set.

Following pollination, the ovary develops into a dehiscent fruit known as a capsule. Upon maturity, capsules split along their three carpels, releasing many dust-like seeds.

Orchid seeds are primarily adapted for wind dispersal, facilitated by their reduced morphology. Seed dispersal is often aided by spring hairs that also develop within the capsule. Seed capsules may contain as many as 4,000,000 seeds.

Morphology and floral biology

The Vanda's has a monopodial growth habit with highly variable leaves according to habitat. Some have flat, typically broad, ovoid leaves (strap-leaves), while others have cylindrical (terete), fleshy leaves and are adapted to dry periods.

The stems of these orchids vary considerably in size; some are miniature plants, and some have a length of several meters. The plants can become quite massive in habitat and in cultivation, and epiphytic species possess very large, rambling aerial root systems.

The few too many flattened flowers grow on a lateral inflorescence. Most show a yellow-brown colour with brown markings, but they also appear in white, green, orange, red, and burgundy shades. The lip has a small spur. Vanda species usually bloom every few months, and the flowers last for two to three weeks.

Flowers are bilaterally symmetrical (i.e. flower can be divided by a single plane into two equal parts) so it is Zygomorphic flower.

Androecium contains two waxy pollinia (bipollinated) these are connected to two elongated stripes which in turn are attached to a sticky viscidium a disc-shaped structure that sticks to a visiting insect. Pollinia are covered in anther cap.

The gynoecium consists of a single compound pistil that together with the androecium comprises the column.

The stigma is just proximal to the single terminal stamen, and stigma is two-lobed, the ovary is inferior contains several million of numerous tiny ovules. The fruit is a capsular type (Figure 4).

Figure 4: Showing Vanda flower structure: Intact flower, exploded flower, Ventral view of gynostemium (column), tepals (sepal+petal) anther cap, Pollinarium, flower stalk.

Pollination brings about several changes in flowers of Vanda orchids. Ovule formation, along with other post-pollination syndromes is initiated by pollen-born auxin and ethylene, the production of which is initiated. Auxin alone triggers perianth death, stigmatic closure, and ovule enlargement and maturation. Ethylene alone causes petal senescence, pollen germination, and pollen tube growth. Ethylene in the presence of auxin enhances ovule development and differentiation. This phenomenon explains the lag between pollination and fertilization, which ranges from 4 months to 8 months. The typical matured capsule of Vanda is brown in colour and splits longitudinally. On the capsules, it has the prominent straight ribs. Capsules develop within the 4-8 months, turn brown and dehisce. Seeds are very minute and brownish in colour. This creates empty air space in the seed, hence seed become balloons which can float in the air and travel long distances. Such floats can explain the appearance of babies of Vanda on trees in nature.

Pollinators of Vanda orchid -There is mounting evidence that the high level of diversity amongst orchids is due to their low reproductive success, leading to high levels of pollinator specificity. The majority of orchids are pollinated by bees, butterflies, moths, flies, beetles, and birds. Despite the number of total orchid pollinators, about 60% of orchids are pollinated by a single species.

Vanda seeds are extremely small and light the seeds consist of an undifferentiated globular embryo contained within a thin testa. There is substantial airspace within the seed relative to the embryo. This allows seeds to float through the air for extended periods of time, which provides a primary means of dispersal. The considerable airspace, along with the hydrophobic lipoid layer covering the testae results in seeds with a high buoyancy, which allows them to be transported by rivers and storm run-off. Seeds can also be distributed by attaching to the feathers and fur of animals.

Vanda seeds possess morphological dormancy due to their lack of a radicle or cotyledon. Physiological dormancy has been identified in the seed of numerous Vanda species. Cold stratification has improved germination rates in seeds of Vanda species. The addition of 6-benzyl amino purine (BA), Zeatin and kinetin to germination media promoted the germination of immature seeds. Abscisic acid was identified as the germination-inhibiting compound in mature seeds of Vanda (Figure 5).

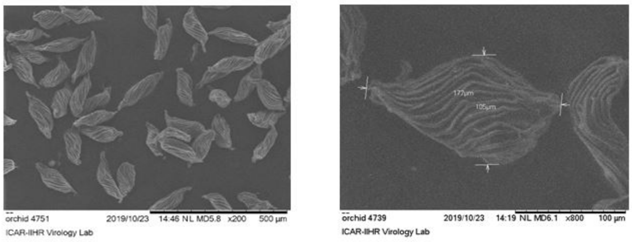

Figure 5: Showing the tiny Vanda thwaitesii Hook.f. Orchid seeds in the scanning electron microscope non-endospermic tiny seeds of Vanda, germinating seeds generally known as protocorm is the first organ that will develop into the true roots and leaves with the symbiotic association of endophytic fungus (mycorrhizal association).

Symbiotic Association

Vanda Orchid seeds generally lack endosperm and have an undifferentiated embryo. Morphological dormancy (absence of a radicle, or usually of a cotyledon) and physiological dormancy is seen in some Vanda Orchids. The orchid generally requires fungal association for the seed germination until the plant establishment because these seeds lack endosperm (non-endospermic seeds- Double fertilization does occur in most cases, but triple fusion either fails or when it occurs the endosperm nucleus degenerates shortly after). This phenomenon explains why endosperm is virtually non-existent in orchid seed which is the nutritive tissue severe as the food during the seed germination process. Seed germination and seedling growth promoted by a specific fungus clone in Vanda by providing moisture and nutrients for seeds in-turn it receives the carbohydrates the from developing plantlets. The presence of appropriate fungal mycobiont for seedling growth and plant nutritional support is essential for the long-term survival of orchids.

Orchid seeds imbibe water, but without infection by mycorrhizal fungi, further, development is impossible in nature. Water uptake in seeds also appears to be affected by mycorrhizal fungi. Fungal hyphae penetrate the testa or widen seed micropores during infection, allowing for faster water imbibition. Vanda seeds are slow to imbibe water and typically float on the surface for extended periods prior to imbibition.

Vanda seeds are unable to germinate by their own until infected with a compatible mycorrhizal fungus. Mycorrhizal fungi generally enter the seed through the suspensor cells. Although orchids depend on mycorrhizal associations primarily as a source of carbon, they can also receive nitrogen, phosphorus, and water from their host fungi.

Seed propagation

In the 1920s, Knudson demonstrated that orchid seed could be germinated without mycorrhizal fungi by growing seed in vitro on medium containing mineral nutrients and sugar. Prior to this, Noel Bernard and Hans Burgeff had presented evidence which supported the conclusion that orchid seeds could only be germinated symbiotically.

That seeds of Vanda dearei cultured on Knudson C (KC) basal medium germinated after 25 days with 63 per cent germination rate followed by half-strength Murashige & Skoog (MS) (45.4%). The results revealed that addition of 0.5 per cent (w/v) yeast extract significantly enhanced seed germination (85.9%) and shortened germination time to 23 days. For growth and development of protocorms, KC basal media recorded the highest percentage of protocorm (37.0%).

That *in vitro* seed germination of endangered orchid species endemic to South-Western Ghats, India and Sri Lanka using appropriate fungal mycobiont (Ceratobasidium clone). The results show that Vanda thwaitesii seeds showed higher germination rate (81%) when inoculated with VT3 clone of Ceratobasidium fungus majority of the control seeds failed to germinate (Figure 6).

Figure 6: showing the embryonic developmental stages of Vanda orchid, protocorm developmental stages, the emergence of the first leaf, complete plantlet development with the roots.

Micro propagation

While seed culture can be an effective means for producing plants in mass, it does not allow for the cloning of unique or highly prized specimens. In 1960, Morel published a procedure for cloning via tissue culture technique. Orchids have been successfully propagated using roots, leaves, stems, flower buds and inflorescences as explant materials. Successful micro propagation of Vanda species has been attempted using matured leaves, axenic seedling leaves, undifferentiated inflorescence buds, differentiated flower buds, and inflorescence nodes.

Shoot tip as explants

The effects of thidiazuron (TDZ) on PLB production from shoot tips of Vanda coerulea cultured on VW basal medium supplemented with 7 g L-1 agar, 2.0 g L-1 casein hydrolysate, 0.5 g L-1 L-glutamine, 1.0g L-1 meso-Inositol, 30 g L-1 sucrose, and 250 mg L-1 peptone. It was reported that the addition of 11.35 µM TDZ resulted in optimum PLB production (96% of 30 cultures responded).

Vanda spathulata reported on the effects of different concentrations of BA and IAA alone and in combination on initiation of direct shoots. They reported that after three months culture, explants cultured on medium supplemented with 4.4–22.2 µM BA produced one shoot. Higher BA concentrations (44.4–88.8 µM) resulted in the production of fewer than three shoots per explant. Explants cultured on medium containing 44.4 µM BA with 17.1 µM IAA and 66.6 µM BA with 28.5 µM IAA produced 12.6 and 12.1 shoots/explant, respectively [5].

Inoculated shoot tip explants in Murashige and Skoog (MS) media to regenerate Vanda tessellata L. Nearly 90 per cent of explants inoculated were found to respond to basal media and the results showed that combination of 1.5 mgl-1 NAA and 1.0 mgl-1 BAP was proved to be the best medium formulation for multiple shoot formation (13.19 numbers) as well as maximum shoot elongation (5.9cm) in Vanda tessellata L.

Conservation

There are two approaches to conservation: in situ, which is conservation within the natural area, and ex-situ, which is any off-site form of conservation. In situ, conservation is the highest priority since it allows for the continuation of ecological functions, but ex situ methods can be a valuable supplement in the field. Estimate that the cost of ex-situ seed conservation can be as low as 1% of in situ conservation.

Some of the Vanda species are listed in the International Union of Conservation of Nature and Natural Resources Red Data Book, and the entire genera is classified as Appendix-I or Appendix-II restriction under the Convention on International Trade in Endangered Species of Wild Fauna and Flora.

The threats facing wild orchids are numerous, including over-collection from the wild, pollinator and mycorrhizal specificity, inbreeding depression, and anthropogenic modification of the landscape, resulting in fire suppression, altered hydrology, and loss or fragmentation of land due to conversion to residential areas and agriculture. Recently thousands of hectares of natural and semi-natural land were converted for urban use and agricultural use. Global climate change will have an unknown effect on overall orchid populations. However, there is the risk of orchid flowering times and pollinator availability falling out of synchronization, and the effect of climate change is virtually unknown in regards to mycorrhizal fungi.

In vitro approaches for species, conservation is a valuable alternative in ex situ conservation. The most valuable tools offered are in vitro collections of genetically diverse species, as a living collection, reintroduction projects, and long-term gene banking using cryopreservation. These tools offered through in vitro conservation are considered priorities outlined for ex-situ conservation of orchids.

Low-temperature storage

Seed banking is the most productive and efficient means of ex situ conservation. Maintaining a seed bank can cost as little as 1% of in situ seed conservation, making it a more economically feasible option. Tissue culture, gene, and seed banks are used for many types of agricultural plants and wildflowers. However, these procedures have not been well developed for orchids. Orchid seeds are ideal for seed banking due to their minute seed size and high seed number per capsule. The genebank Standards recommend low-temperature storage of orthodox seed at -18°C as an ideal storage temperature for Vanda species.

Cryopreservation of orchid seeds

Although low-temperature storage can extend seed viability for years in some cases, immersion in liquid nitrogen (-196°C) can theoretically halt biological time, extending viability indefinitely. The direct method has been employed for the majority of Vanda orchid seed cryopreservation studies.

A study using immature and mature seeds of Vanda tricolor. In this case, immature seeds (45% WC) germinated at 10% after Liquid Nitrogen immersion compared to 26% in the unfrozen control, both of which were higher than mature seeds (40% WC) that germinated at 1% after Liquid Nitrogen immersion and 11% in control. The author suggests dormancy developed in the mature seeds, which may explain the difference in germination rates amongst controls.

The cryopreservation of orchid seeds of different species through rapid and step- freezing method. The study shows, there is a significant difference in the results when comparing the data between the species and between the treatments. The efficient treatment was step freezing with the use of DMSO as a cryopreservant and the species Epidendrum quitensium, and Epidendrum anderssonii showed better results.

Orchid seed cryopreservation by vitrification

Vitrification is another common method used for orchid seed cryopreservation ideal for seeds with high water content or intermediate storage characteristics. Seeds that were unable to survive direct cryopreservation and those with water content as high as 57% have been successfully conserved using the vitrification method.

Protocorm cryopreservation

There are fewer reports of protocorm and PLB cryopreservation than of seed, but more techniques have been employed. Encapsulation-dehydration resulted in a survival rate of 40% in Vanda coerulea protocorms.

Conclusion

Most of the orchids depend on a single species of bird, bee or other insects for pollination, if that species is eliminated; the particular orchid that depends on that species becomes threatened by extinction. Thus, habitat fragmentation and rainforest destruction may eventually prove devastating to the vast number of orchid species by the absence of symbiotic endophytic fungal associates. They are also an essential component of any forest ecosystem with a highly complex mutual relationship with other biotas. Their presence, along with other epiphytes, is an indication of a healthy ecosystem. Orchids are sensitive to even slightest disturbances to their habitats and with the tremendous pressure on natural resources, particularly forest resources, orchids and their habitat are continuously under threat. As few of the Vanda species are listed under IUCN Red List as endangered species, efforts should also be made in determining the cause of lowering the natural populations and educating local people about their conservation and sustainable utilization. Also, further research needs to be conducted on developing mass propagation and cultivation techniques for sustainable commercial uses.

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