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Malaxis acuminata D. Don : A Review

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ABSTRACT

Malaxis acuminata is a terrestrial orchid that grows in shady areas of semi-evergreen to shrubby forests. It is highly valued for its medicinal properties as its dried pseudobulbs are important ingredient of Ashtavarga drugs used in the preparation of Ayurvedic tonic 'Chyavanprash'. The tonic is energizing, cures tuberculosis, and enhances sperm formation. Low rate of natural multiplication, large scale habitat destruction and excessive collection for commercial purposes has severely endangered the natural populations of this species. This paper attempts to review the available literature on the species about its phytoconstituents, medicinal importance and in vitro propagation methods for its conservation.

INTRODUCTION

The orchids account for nearly 10% of the total flowering plant species and constitute one of the largest, diverse, and highly evolved families of flowering plants - the Orchidaceae [1]. Their numerical strength in terms of species has been variously assessed between 17,000 and 35,000 [2], but an estimation of 19,128 species [3] seems to be more realistic. They stand distinct from other plants in having intricately fabricated and colourful flowers; microscopic seeds with highly reduced embryos and suppressed development of endosperm; and dependence on a fungal infection for germination and growth in nature [4]. These plants are cosmopolitan in distribution, except for in a few isolated islands, aquatic and marine ecosystems, and in the frozen continent of Antarctica. They are, however, mainly concentrated in the tropical and subtropical climates due to the prevalence of thick vegetation and high humidity, both of which factors are congenial for their growth and development.
The orchids are rich in phytochemicals and have been extensively used in local medicines [5]. Their additional utility as aphrodisiac and restorative drugs, and as a source of food, gums, glues, and narcotics is also well documented [6]. The most important commercial produce of orchids is the essence 'Vanilla'- an odorous alkaloid obtained by sweating the cured beans of spice orchid- *Vanilla planifolia*.

The orchids are primarily sexual in nature and produce numerous, microscopic seeds which are poorly developed at maturity. Usually, the mature seed consists of the small embryo suspended within a membranous seed coat. The seeds also lack endosperm and require a suitable fungal partner for germination in nature; the fungus is believed to augment the carbohydrate, auxin, and vitamin transport in orchids [7]. A non-symbiotic (asymbiotic) culture method was developed after Knudson [8] demonstrated that fungal requirement of *Cattleya* seeds can be successfully bypassed during germination using a relatively simple culture medium containing sucrose. Subsequently, the ability of orchid seeds to germinate prior to reaching maturity led to the development of the technique of "embryo/ovule/green-pod" culture [9]. The technique involves an easy procedure of sterilization, ensures better frequency of germination, reduces the time lapse between pollination and sowing of seeds, and helps in i) propagation of rare and endangered species, ii) rescuing hybrid embryos from desired matings, iii) exploiting the polyembryonate potential of orchid seeds, and iv) cloning of apomictic (obligate) genotypes [10].

MALAXIS SOLAND EX SW.

The genus *Malaxis* Soland ex Sw. comprises nearly 300 deciduous species of terrestrial/lithophytic or rarely epiphytic orchids distributed in tropical and temperate regions, primarily in Asia and Oceania. The genus derives its name from the Greek word '*malaxis*' for soft/tender, which apparently refers to the thin texture of leaves. A majority of the constituent species have pseudobulbous stems; fibrous roots; membranaceous, often plicate leaves; and few to many, small, greenish/whitish non-resupinated flowers in terminal, erect racemes. Incidentally, it is the non-resupinate nature of the flowers in *Malaxis* which helps to distinguish it from the allied genus *Liparis* [11, 12].

The genus is well known for its leaf-bulbil producing *Malaxis paludosa* (L.) Sw. [13] and several therapeutically important species, which are used in local medicines for their aphrodisiac (*M. acuminata* D. Don), diuretic (*M. ophioglossoides* Muhl. ex Willd.), anti-inflammatory (*M. rheedi* Sw.), diaphoretic (*M. versicolor* Sant. and Kapadia), and rejuvenating [*M. acuminata*, *M. cylindrostachya* (Lindl.) O. Ktze., *M. muscifera* (Lindl.) O. Ktze.] properties [14, 15].

In India, the genus is represented by 18 species most of which are Himalayan in distribution; some are met within the peninsular and Andaman and Nicobar regions [16]. Incidentally, all the Indian species were earlier treated under the genus *Microstylis* Nutt.

For their proper growth and development, malaxes prefer warm, humid, and semi-shaded conditions; liberal supply of water; and perfectly drained and organically rich compost.

Malaxis acuminata D. Don (= *Microstylis wallichii* Lindl.)

Distribution: *Malaxis acuminata* is an Asiatic species distributed widely in Thailand, China, Burma, Bhutan, Nepal, and India. In India, it dwells in the Himalayan, Khasia and Jaintia, and peninsular (Western Ghats, Nilgiris) hills on the mainland and Andaman hills offshore. Along the Himalayas, it extends from Shimla eastwards to Sikkim within an altitudinal range of 1500-2300 m, and occupies humus-rich and moist substratum in shaded situations.

Morphology: *M. acuminata* is a small, medium-sized terrestrial orchid, up to 30 cm in length, with pseudobulbous stem covered at base by old leafy scales. Leaves 3-4, ovate-lanceolate, membranous and measuring 10-15 × 5-6.5 cm showing acute apex and undulate margins. Flower pale-green tinged purple, shortly stalked, 1-1.2 cm across, present on many flowered, 8-10 cm long spikes; bracts linear, minute. Sepals oblong; lateral broad and short with recurved margins. Petals are linear, longer than sepals. Lip shield like, broadly ovate, somewhat convex, tip notched, auricles at base straight or overlapping. Leaves have sheathing leaf base and new plants grow near the base of the decaying mother plant. Flowers, in terminal racemes, are small, pale yellowish-green in color, but with a purple tinge.

Flowering: July-September

Cytological status: The species exists as three morphologically indistinct cytotypes with 2n=30, 36, 42 **[17]**.

BIOLOGICAL/COMMERCIAL STATUS:

The species is of great medicinal significance. Its dried pseudobulbs form an ingredient of 'Ashtavarga' [a group of eight drugs, namely *Jivak, Rishbhak, Mahameda, Meda, Kakoli, Khirkakoli, Ridhi* and *Bridhi* [18]] used in the preparation of Ayurvedic tonic 'Chyavanprash'. The tonic is energizing, cures tuberculosis, and enhances sperm formation [19].

Extensive pressure of commercial collection and habitat destruction have detrimentally affected the size and frequency of its natural populations which even otherwise stand impaired because of poor fruit set and slow vegetative propagation.

PHYTOCONSTITUENTS:

Literature survey reveals that studies on the phytoconstituents of this species are limited. Bhatnagar et al. [20] reported one sterol namely β-sitosterol and an alcohol identified as cetyl alcohol, two sugars namely glucose and rhamnose and five basic compounds one of them being cholin from this species. Thin layer chromatographic studies by Gupta et al. [21] revealed the presence of constituents like Limonene, eugenol, citronellal, 1, 8-cineole, piperitone and p-cymene. Sharma et al. [22] reported that the quality of the wild crafted drug varied with the place of collection. Lohani et al. [23] analysed the metal content and volatile constituents of this species by Atomic Absorption Spectrophotometer and GC and GC-MS respectively. Chemical analysis revealed that wild plants of the species contained 6.48 ppm Cu, 43.00 ppm Zn, 35.00 ppm Mn, 331.00 ppm Fe, 21600.00 ppm K, 9000.00 ppm Ca, 2800.00 ppm Mg, 198.00 ppm Al, 26.70 ppm Ba, 55.60 ppm B, 0.30 ppm Mo, 156.00 ppm Cl. Fatty acids analysis revealed the presence of the following constituents-linoleic acid (18:2ω6) 61.20% (w/w), α-linolenic acid 18.10% (w/w), oleic acid 12.00 % (w/w), palmitic acid (16:0) 6.00% (w/w), stearic acid (18:0) 2.10% (w/w), γ-linolenic acid (18:3ω6) 2.20% (w/w), eicosanoic acid (20:0) 0.81 % (w/w), eicosenoic acid (20:1) 0.42% (w/w) and eicosadienoic acid (20:2) 0.04% (w/w). On the other hand cultivated plants of this species contained 7.18 ppm Cu, 49.50 ppm Zn, 37.00 ppm Mn, 352.45 ppm Fe, 23000.00 ppm K, 13000.00 ppm Ca, 5300.00 ppm Mg, 217.50 ppm Al, 37.50 ppm Ba, 59.70 ppm B, 0.27 ppm Mo, 148.00 ppm Cl. Analysis of fatty acids revealed linoleic acid (18:2ω6) 65.23% (w/w), α-linolenic acid 15.50% (w/w), oleic acid 14.87 % (w/w), palmitic acid (16:0) 5.90% (w/w), stearic acid (18:0) 2.50% (w/w), γ-linolenic acid (18:3ω6) 1.87% (w/w), eicosanoic acid (20:0) 0.69 % (w/w), eicosenoic acid (20:1) 0.52% (w/w) and eicosadienoic acid (20:2) 0.07% (w/w). Other chemical constituents which were isolated from wild and cultivated *M. wallichii* were vitamins α–tocopherol and γ-tocopherol 12.00-9.80 ppm and 695.00-786.7 ppm respectively while terpenoids 18.00-20.50%. It has an acid value of 1.20-1.39 and saponification value of 103.00-110.50 [23].

Garg et al. [24] tested the antioxidant activity of the butanol extract of *M. acuminata* using various available methods including 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, reduction capability by $Fe3^{+}$-Fe^{2+} transformation method and hydrogen peroxide scavenging method. They concluded that the species has a good antioxidant activity.

VEGETATIVE PROPAGATION:

Tamta et al. [25] attempted conservation of this species in its natural habitat using seeds and pseudobulbs. The pseudobulbs of sizes 3 to 4 cm in length (0.8 -1.0 cm diameter) were selected and four type of propagules were used, i.e., whole pseudobulbs, only tip part, only middle part and only bottom part of pseudobulbs. The experimental bulbs after proper shoot development were planted in selected plots in Deodar and Oak Forests at Chakrata, Dhanolti and Mussoorie Forest areas. The maximum survival (upto 85%) and optimum growth was observed in all selected sites when whole pseudobulbs were used as propagules. Seed propagation yielded very percentage of germination. The results demonstrate that orchids are inherently slow growers and their natural rate of multiplication is very low.

TISSUE CULTURE STUDIES:

Plant tissue culture technique has been accepted as a potential alternative method for mass scale propagation and conservation of rare, threatened and endangered orchids. Invention of in vitro propagation technique has saved the many naturally growing orchids and their collection from the wild has reduced.

Cheruvathur et al. [26] developed an efficient in vitro multiple shoot induction system of this species using intermodal explants sourced from sterile primary cultures. They used MS medium[27] supplemented with different concentrations of three cytokinins, i.e., 6-benzyladenine (BA), kinetin (Kn), and thidiazuron (TDZ) to induce adventitious shoot buds. From among the three cytokinins used, TDZ at 3 mg/l induced the highest frequency of response (86%), but each of the responding explants produced only single adventitious shoot

irrespective of the type and concentration of the cytokinin. Addition of 0.5 mg/l 1-Naphthaleneacetic acid (NAA) to the medium increased the number of adventitious shoots. A combined treatment of 3 mg/l TDZ and 0.5 mg/l NAA, resulted in 96% response with a mean number of 6.1 shoots per explant. Prolonged culture or subculture on the same medium did not promote further shoot production. However, addition of various concentrations of different polyamines (PAs), including spermine, spermidine, and putrescine to MS medium supplemented with 3 mg/l TDZ and 0.5 mg/l NAA, significantly increased mean shoot number per explant. Cent per cent adventitious shoot induction and mean shoot number of 14.6 per explant was observed on MS medium with 3 mg/l TDZ, 0.5 mg/l NAA, and 0.4 mM spermidine. Regenerated shoots were excised and subcultured on an elongation medium consisting of MS medium with 3 mg/l BA. The shoots were rooted and the highest frequency of rooting (96%) and mean number of roots per shoot (3.3) was observed on MS medium with 4 mg/l indole-3-butyric acid (IBA) and 1.5 mg/l activated charcoal (AC). Almost 90% of rooted shoots were successfully acclimatized and established in the green house.

In another study, the regeneration potential of pseudobulb segments of *M. acuminate* was tested on Mitra medium [28] and its various combinations with growth adjuncts [29]. In this study, juvenility of the explants and the chemical stimulus emerged as the important factors in eliciting response in the explants. The explants from relatively older pseudobulbs (>0.5cm in length) remained recalcitrant to regeneration whereas those representing younger ones (<0.5cm in length) responded positively. The response frequency, pathway of regeneration, and time taken for their development into complete plantlets was directly related to the growth stimulus in the nutrient regime. Shoot buds were induced in an individual treatment with BA/NAA (1 mg/l each) whereas their combination [BA (1.0 mg/l) + NAA (1.0 mg/l)] promoted protocorm-like body formation in the explants. Additional use of activated charcoal (AC) invariably proved beneficial in accelerating the morphogenetic processes leading to plantlet development. The combination of 1.0 mg/l each of BA and NAA along with 2 g/l of AC proved the best for early initiation, the highest regeneration frequency, proliferation of protocorm-like bodies (PLBs), and plantlet development. Plantlets were transferred to clay pots containing potting mixture (sand, soil, leaf compost) in the ratio of 1:1:1. Nearly 70-80% of plantlets survival was recorded. Histological investigation confirmed that PLBs were epidermal and multicellular in origin.

The effects of illumination, developmental stage of immature embryos, sucrose concentration, and quality and quantity of plant growth regulators were evaluated on seed germination of *M. acuminata* [30]. Seeds taken 7-8 wks after pollination (WAP) showed germination percentage of 85% and the germinability declined in older seeds (9 WAP). The seeds younger than 6 WAP either did not germinate or showed delayed germination. The germination of seeds was better under 50% diffused light as compared to full illumination. Among the different concentration of sucrose incorporated in the medium, 3% sucrose stimulated the optimum germination, while lower and higher concentration of sucrose supported lower rate of germination. Both BA and NAA proved beneficial in stimulating the growth of immature embryos but NAA was more effective for the purpose.

Deb and Arenmongla [31] successfully propagated this species using leaf explants from in vitro raised plantlets. The cultured leaf explants produced meristemetic loci within 4–5 weeks of culture on Murashige and Skoog, 1962 (MS) medium supplemented with 3% (w/v) sucrose and 3µM each of NAA and BA. The foliar explants were cultured in different orientations, and optimum response was recorded with the explants cultured horizontally. Under this condition, as many as 26 shoot buds developed. The resultant shoot buds converted into plantlets with well developed roots on MS medium supplemented with 3% (w/v) sucrose and 3µM each of NAA and BA, with average plant height, number of leaves and number of shoots 2.4 cm, 4.5, 18.0, respectively. The complete plantlets were hardened by transferring to community pots with potting mix [prepared by mixing charcoal pieces, chopped forest litters, coconut husk, sand and black soil at 1:1 ratio]. They showed 75% survival rate after two months.

CONCLUSIONS

The importance of *Malaxis acuminata* in Ayurvedic system of medicine is well documented. But the literature on phytoconstituents of this species is rather limited. Keeping in view its endangered status, several efforts have been made for in vitro propagation using seeds and pseudobulbs as explants. However, cost-effective and reproducible protocols for mass propagation of this medicinally important orchid species have to be developed further for its conservation and commercialization.

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