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Ex situ **conservation strategies for** ***Spathoglottis plicata*** **Blume- an orchid of ornamental and medicinal importance**

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Abstract

Spathoglottis plicata Blume is an important orchid having tremendous medicinal and ornamental values. The presence of numerous bioactive compounds such as alkaloids, flavonoids, terpenoids, saponins, phenols, tannins, etc., has been reported. In addition, anti-microbial and anti-inflammatory properties have also been reported in this species. S. plicata is extensively used in traditional medicines for curing various human ailments and diseases. Due to its significant role in traditional medicines and horticulture, it is highly sought after. As such, it has been extensively exploited and its natural population is decreasing at an alarming rate. Therefore, conservation of this remarkable orchid is of great importance. In the recent past, tissue culture techniques such as micropropagation and artificial seed production have been employed for its conservational purposes. The current review aims to provide a concise report on the ex situ conservation strategies employed for the conservation of S. plicata.

Keywords: *Spathoglottis plicata*, micropropagation, artificial seeds, protocorm-like bodies, conservation.

Introduction

The genus *Spathoglottis*, consisting of 49 terrestrial, sympodial species, spans a geographical range from India through Southeast Asia, reaching as far as Australia and the Pacific Islands, and occurs at altitudes from sea level to 3500 meters (Teoh 2022; Talkah et al. 2024). The generic name is derived from the Greek words: '*spathe*' (broad blade) and '*glotta*' (tongue) referring to the broad midlobe of the lip (Teoh 2016, 2022). The species belonging to this genus have tall, upright inflorescences with multiple flowers that open in succession on a comparatively short rachis and have multiple petiolate, elliptic, plicate, membranous leaves. The plant blooms for several months. *Spathoglottis* grows well under full sunlight among ferns and grasses.

Spathoglottis plicata Blume is a highly variable and widely distributed species. It is corm-like, above ground, 3 × 1.5–2 cm pseudobulbs are covered in sheaths. The linear-lanceolate, pleated leaves wrap around the pseudobulb and measure between 30 cm and 80 cm in length and 5-7 cm in breadth. Either inflorescences or new pseudobulbs arise from the pseudobulb's axillary buds (Teoh 2016, 2022). *S. plicata* is valued for both its aesthetic appeal and therapeutic qualities and blooms bright flowers all year long, which makes it a popular ornamental plant (Haque and Ghosh 2017; Bhowmik and Rahman 2020) (Fig.1a, b).

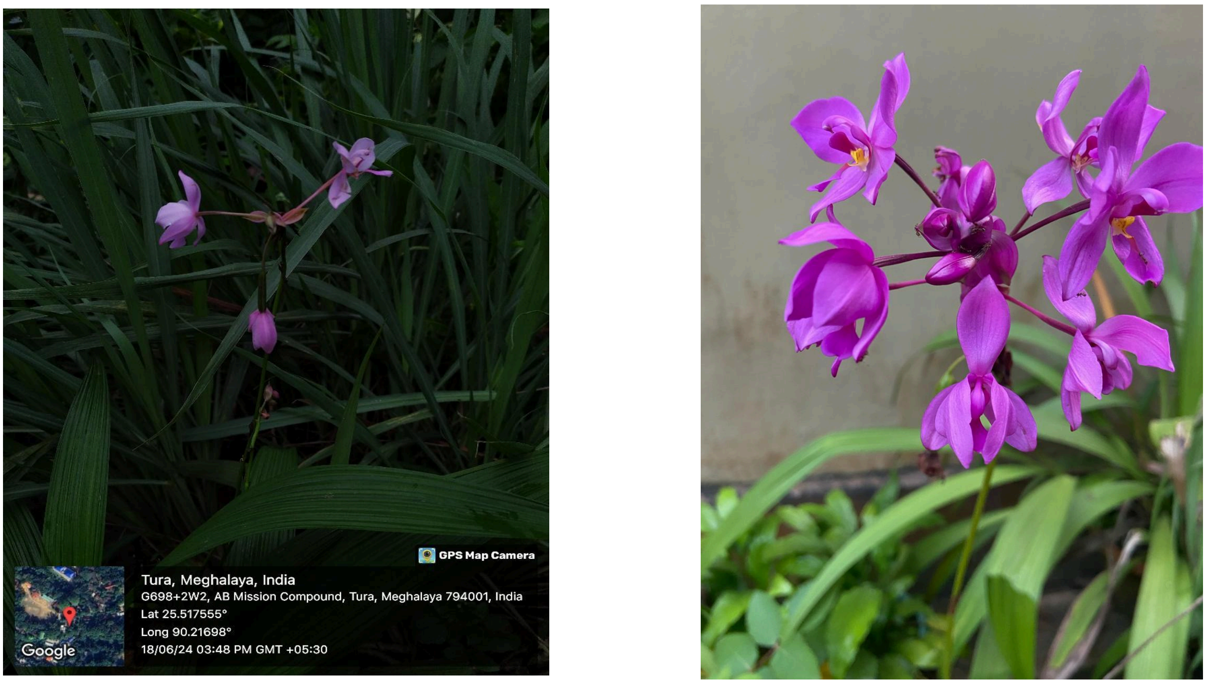

Fig. 1. a) Spathoglottis plicata in its natural habitat, b) Flowers of S. plicata.

Medicinal and economic importance

Numerous bioactive substances, including alkaloids, flavonoids, terpenoids, saponins, phenols and tannins, have been isolated from *S. plicata* through phytochemical screening, suggesting its potential medical properties (Bhowmik and Rahman 2020). Further, the presence of phenolic compounds, terpenoids, steroids and coumarins indicates that *S. plicata* shares a large number of phytochemicals that are frequently found in medicinal plants for their anti-microbial and anti-inflammatory activities (Bhowmik and Rahman 2020). The use of this orchid to treat different human ailments has been reported. In India, the decoction of *S.*

plicata is used in the treatment of rheumatism (Mollik *et al.* 2009). In Indonesia, it has been used in treating non-painful limb swelling (Teoh 2016). Dagar and Dagar (2003) have reported its use in alleviating earache. Even its powdered seeds are used in place of *bedak* (jasmine-perfumed rice flower) for lightening the complexion of children (Teoh 2016).

Declining population of *Spathoglottis plicata*

Several significant anthropogenic, biological and ecological factors influence the populations of *S. plicata*. Even though *S. plicata* is known to be capable of both outcrossing and self-pollination, self-pollination in isolated populations can eventually limit genetic variety (Ginibun *et al.* 2018). *S. plicata* usually multiplies by dividing pseudobulbs, however this mode of multiplication is slow and time taking. Also, habitat degradation such as construction, erosion, extensive collection and adverse environmental changes has made the species naturally fragile, causing its population to shrink in size (Sinha *et al.* 2009). Consequently, there is a need for rapid and reliable technologies to enable mass propagation of this orchid species effectively (Hossain and Dey 2013; Ginibun *et al.* 2018; Bhowmik and Rahman 2020).

Conservation Strategies

Conservation of plant resources can be achieved using both *ex situ* and *in situ* methods. While *in-situ* conservation deals with the protection of the plants in their natural habitats, *ex situ* methods of conservation involve the use of *in vitro* technologies for the mass multiplication and sustainable utilization. The *in situ* conservation technique makes it possible to conserve species, genetic and environmental variety in a highly comprehensive and economical way, but it is not achievable in regions with significant environmental and human constraints (Wolf 1999; Zegeye 2017). *Ex situ* conservation aims at preserving seeds and other germplasm materials for as long as possible in order to minimize the likelihood of genetic diversity loss (Dulloo and Borelli 2010). For the *in-vitro* propagation of *S. plicata*, different plant parts and nutrient media have been used. Studies have shown that explants such as nodes, internodes, root tips, leaves and capsules can be used to achieve successful *in vitro* multiplication of *S. plicata*. A variety of media such as Gamborg (B_5), Knudson C (KC), Murashige and Skoog (MS), Orchid Seed Sowing Medium (OSSM), and Phytamax (PM) along with the plant growth regulators 6-Benzylaminopurine (BAP), 2,4-Dichlorophenoxyacetic acid (2,4-D), kinetin (Kn), α-Naphthaleneacetic acid (NAA) and indole-3-acetic acid (IAA) have been tried for micropropagation of *S. plicata*.

Murthy *et al.* (2006) demonstrated that MS medium supplemented with 2 mg l^{-1} Kn, 0.5 mg l^{-1} NAA along with 100 mg l^{-1} casein hydrolysate and 10% coconut milk resulted in 85% seed germination in case of seeds from mature capsules of *S. plicata*. However, Thakur and Dongarwar (2012) reported enhanced seed germination in MS medium incorporated with 0.1mgl^{-1} BAP and 1.0 NAA mg l^{-1}. Hossain and Dey (2013), on the other hand, observed 95% seed germination of *S. plicata* on PM medium supplemented with 2% (w/v) sucrose and 2.0 g l^{-1} peptone. Sebastinraj and Muhirkuzhali (2014) also reported 90% seed germination on KC basal medium. Stella *et al.* (2015) observed induction of callusing in MS medium supplemented with 10 µM concentrations each of Kn and BAP. Aswathi *et al.* (2017) has reported the best medium for achieving 95% germination rate in *S. plicata* to be Gamborg B$_5$. Haque *et al.* (2017) recorded a total of 82% seed germination on ½ MS basal medium containing 0.5 mg l^{-1} Kn whereas Bhowmik and Rahman (2020) observed an 86.67% germination percentage on PM medium. Shekhawat *et al.* (2021) reported 93% germination rate on MS medium supplemented with 1.0 mg l^{-1} BAP. Using immature capsules of *S. plicata*, Hossain and Dey (2013) have reported around 70% germination in OSSM medium.

Further, nodal, root and leaf segments from the *in vivo* plants have also been utilized as explants for micropropagation of *S. plicata*. Teng *et al.* (1997) reported that ½ MS basal medium supplemented with BAP at 0.44 µM and 5.37 µM NAA resulted in the induction of protocorm-like bodies (PLBs) from nodal sections, leaf explants and root segments with 98.5%, 5-8% and 0.5% PLB induction efficiency respectively. Sinha *et al.* (2009) reported that micro-shoots could be induced in 66.6% explants without the intervening PLBs by culturing nodal segments of *S. plicata* in ½ MS medium supplemented with 2.0 mg l^{-1} BAP, 0.5 mg l^{-1} NAA, 3% sucrose, 10% coconut water, and 2 g l^{-1} peptone. Manokari *et al.* (2021) observed that 93.7% of somatic embryos per explant could be produced in leaf explants grown on MS media supplemented with 1.0 mg l^{-1} 2,4-D.

Also, the use of secondary explants from the *in-vitro* raised seedlings of *S. plicata* has been reported for its multiplication. Bapat and Narayanaswamy (1977) used the stem disc-bearing rhizomatous stock obtained from 6 weeks-old seedlings to generate callus masses on MS medium enriched with 6 mg l^{-1} NAA and 2 mg l^{-1} 2,4-D, along with 2 mg l^{-1} kinetin and 15% coconut milk. Hossain and Dey (2013) employed nodal segments of *in vitro* raised seedlings and found that the optimal combination for inducing PLBs (10.80 ± 0.44 PLBs per explant) was 1.0 mg l^{-1} NAA and 2.5 mg l^{-1} BAP. Sebastinraj and Muhirkuzhali (2014) reported a maximum induction of an average of 12.8 shoots per explant using 5 mm long *in vitro* pseudobulb explants on ½ MS medium supplemented with, 2.0 mg l^{-1} BAP, 0.6

mg l^{-1} NAA and 3% sucrose. Haque *et al.* (2017) reported the induction of callus in root, stem and leaf explants of *in vitro*-raised plantlets of *S. plicata* with a frequency of 30.0%, 43.3%, and 66.7% respectively when cultured in a modified MS medium supplemented with 1.0 mg l^{-1} 2,4-D, 3.0 mg l^{-1} NAA, and 1.0 mg l^{-1} Kn.

Ex vitro symbiotic seed propagation is another *ex situ* conservation technique which is advantageous over *in vitro* propagation since the seed germination process does not require severe axenic conditions or laboratory facilities (Aewsakul 2013; Mala and Nontachaiyapoom 2018). Aewsakul (2013) has reported that the seeds of *S. plicata* sown non-axenically on peat moss inoculated with the fungal strain Da-KP-0-1, resulted in enhanced protocorm growth and seed germination were most promoted.

The production of artificial seeds is an approach for conservation of desirable genotypes by encapsulation of suitable explants including somatic embryos in a suitable matrix such as agarose or calcium alginate. It can be a useful tool for large-scale multiplication projects as well as a useful way to re-establish plants in the wild for germplasm preservation (Kumar and Loh, 2012). Stella *et al.* (2015) developed "synseeds" of *S. plicata* by encapsulating the *in vitro* produced somatic embryos and protocorms in 3% sodium alginate matrix, without any hormonal supplementation. Further, Haque *et al.* (2017) reported the development of improved artificial seeds with 3% sodium alginate which had a germination percentage of 66.7% after 3 months of cold storage at 4°C. Manokari *et al.* (2021) reported a maximum of 97.4% conversion frequency of synthetic seeds formed in 3% sodium alginate and 100 mM calcium chloride which could be germinated on MS medium fortified with 2.0 mg l^{-1} BAP and 0.25 mg l^{-1} IAA.

Cryo-seed bank is used as an alternative to the conventional seed-bank for preserving orchid seeds at low liquid nitrogen temperatures while maintaining their high viability (Das *et al.* 2021). Ang *et al.* (2010) have reported successful preservation of seeds of *S. plicata* in liquid nitrogen at Singapore Botanic Gardens, Singapore with 90% viability post-storage.

Conclusion

S. plicata is a species of significant ornamental and medicinal importance, making its conservation and sustainable utilization a priority. Development of *ex vitro* and *in vitro* micropropagation techniques has yielded successful large-scale propagation strategies, providing a steady supply of desirable genotypes. These techniques have been critical in resolving propagation issues. Further, cryopreservation and artificial seed production offer viable ways to preserve the germplasm, and genetic uniformity of plants for the long term.

These biotechnological methods of conservation and multiplication may ensure the protection and sustainable use of *S. plicata*, an orchid of ornamental and medicinal importance.

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