Trends in Agriculutre Science
Popular Article
Vol.2 Issue 9 September 2023, Page: 718-720

Conservation of Orchids through Micro-propagation

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https://doi.org/10.5281/zenodo.8324988

Introduction

Orchidaceae is one of the most diverse and largest families comprising 25,000 to 35,000 species and 700 to 800 genera in the plant kingdom. The orchid flowers exhibit exquisite characters and command tremendous appreciation from botanists and others across the world. Orchids are widely distributed in a wide range of habitats of the world. These are not only valued for their diverse and distinctly beautiful flowers but also for their utilization in Ethnomedicine practices. In traditional healing systems, orchids have been used since ancient times in many parts of the world in the treatment of a number of diseases. India is an important country for its rich diversity of orchids. The state of Arunachal Pradesh (AP) is known as the 'Orchid Bowl of India'. Similarly, the Western Ghats of India are also well known for their rich diversity of orchids. Members of Orchidaceae are among the most popular cut flowers in the international market. *Dendrobium, Vanda, Aeridis, Bulbophyllum* etc. are some of the major genera of orchids. The most common threats affecting orchids are deforestation due to logging, fire, road construction and the expansion of forest plantations and agriculture, and over-collection for the ornamental, medicinal and food plant trades. Plant tissue culture plays an important role in conservation through micropropagation.

Preparation of explants

Mother plants of orchids are maintained in the greenhouse to obtain the explants throughout the year. For initiation, mature leaves are collected from the healthy plants. Leaves are cut into small pieces of about 5 cm length, and washed in running tap water for 30 min. Explants are then cut from all sides to reduce the size. To reduce the surface tension and thereby improve the efficiency of surface sterilization, the explants are soaked in Tween 20/ Tween 80 (mild detergent) solution for 10 min. Finally, the explants

are rinsed with tap water followed by three washings (5 min. each) with distilled water. Surface sterilization is carried out in the LAF under aseptic conditions.

Surface sterilization

The prepared explants are put into a glass bottle and mercuric chloride (0.1%) solution is poured in. Bottle containing explants is kept agitating by continuous shaking, in order to ensure proper sterilization. After 10 min., the sterilizing solution is removed and explants are rinsed with sterilized distilled water. Three to four washings, each of 10 min., should be given to remove all the traces of mercuric chloride.

Culture Initiation

Explants are cut from all sides using a sterilized scalpel and forceps to remove the dried portions, making the final inoculum of about 2 cm^2. The explants are then inoculated onto MS medium containing sugar (30 g/l), agar (4.5 g/l), and growth regulators *viz.* BAP (2 mg/l) and NAA (0.2 mg/l). Bottles, after initiation, are transferred to the incubation room maintained at 24 ± 2°C temperature and 16: 8 hours light: dark cycle.

Shoot induction and multiplication

Callus induction is noticed in the cultured explants after 3-4 weeks of initiation. The callus is subculture onto the fresh medium containing BAP (1.5 mg/l), Kinetin (1.5 mg/l) and NAA (0.1 mg/l). Generally, shoots are induced from the callus after 3-4 weeks of culture. Shoots may be subcultured on the same kind of media to obtain multiple shoots or kept for rooting. Cultures are observed regularly and contaminations, if any, are discarded immediately.

Rooting

Individual micro shoots are separated from the clumps and inoculated onto the half-strength MS medium containing IBA (1 mg/l) and NAA (0.5 mg/l) along with activated charcoal (2 g/l). The rooted plantlets are taken out of bottles and washed carefully with water to remove the adhering agar.

Acclimatization

For primary hardening, rooted plantlets are planted into the pro-trays containing coco peat. These trays are maintained in the mist house for two to three weeks. For secondary hardening, plantlets are transferred to plastic bags filled with red soil: sand: cocopeat in 1:1:2 proportion along with a few charcoal pieces. These plants are then kept in the greenhouse for two to three weeks. At this stage, the plants attain a height of around 10 cm and are ready for sale to the growers.

Conclusion

Sporadic studies have been made towards the exploitation of wild orchids for medicinal purposes. Species found endangered and that listed in IUCN are largely due to human activity. Hence, orchids can only survive with human assistance. Plant tissue culture Conservation of Ret Orchid Species of the Western Ghats Region will surely be one of the best alternative ways of reducing the pressure on the natural population.