TECHNICAL BULLETIN – SERIES 1

TISSUE CULTURE BASED PRODUCTION TECHNOLOGY

For Foxtail Orchid *(Rhynchostylis retusa)*

PUBLISHED BY

Research Education and Working Plan Wing

Environment and Forest Department

Govt. of Assam

Assam Forest Department

Azadi Ka Amrit Mahotsav

ACKNOWLEDGEMENT

Acknowledgements: Shri Amit Sahai, IFS PCCF& HOFF, Assam Forest Department is thanked for his encouragement and support. Dr. Sonali Ghosh, IFS CCF REWP, Shri Dibakar Deb, Silviculturist, Assam and the entire staff of silviculture division are thanked for their support and plan for bringing out a series of technical bulletins for orchid conservation. Shri Bankim Sharma, IFS and Shri Santanu Dey are thanked for providing their useful comments and photos.

SUGGESTED CITATION

Choudhury, R. (2022) Tissue culture based production technology for Foxtail Orchid *(Rhynchostylis retusa)*. Research, Education and Working Plan Wing, Environment and Forest Department. Govt of Assam.

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PREFACE

Research Education and Working Plan wing of the Assam Forest Department deals with various research activities relating to forest vegetation along with the factors affecting the forest vegetation., trainings and capacity building as well as preparation of working plans. The key mandate of Silviculture Division is as below:

i. Standardization of Nursery technique.

ii. Study of change of Vegetation.

iii. Study of growth of Species in different forest types.

iv. Development of method of bamboo propagation.

v. Development of method of Orchid Propagation.

vi. Cultivation method of Medicinal Plants.

The present technical publication is aimed to develop a protocol for mass propagation of *R. retusa*. This technical bulletin covers the wide aspects of botanical description, morphological description, Propagation through vegetative method and standardization of each step of Tissue culture methods from explant to complete plantlets and acclimatization for production of *Rhynchostylis retusa* plantlets.

It is hoped that this will serve as a handy reference manual for orchid growers, orchid entrepreneurs, plant breeders and researchers.

Introduction

Orchids are associated with the tradition and culture of Assam since times immemorial. Besides ornamental purposes some of the orchids are also used for their medicinal properties by the tribal groups.

Rhynchostylis retusa (L.) Blume (also called Foxtail Orchid) is an orchid, belonging to the Vanda alliance. It is an epiphyte growing on tree trunk in open forest or at forest margins at elevations of 300-1500 m (980 – 4920 ft). The State flower of Assam it is locally called (*Kapou phul* কপৌ ফুল) and is intrinsically linked with the culture of Assamese society. The entire inflorescence is used by the girls to adorn their hair during the spring festival, known as " Rongali Bihu".

It is regarded as a symbol of love among the youth. These days the entire plan are sold in market in large numbers along with the flowers during Spring festival time. Besides using in decoration, decoction of aerial roots of *Rhynchostylis retusa* is applied as drops for otorrhoea and other ailments. Paste of its flower is applied as emollient on face .To preserve these heritage orchid species and also to cultivate them extensively there is an urgent need to propagate and multiply them by tissue culture method for their commercial production.

Botanical description

Rhynchostylis retusa (L.) Blume (also called Foxtail Orchid) is an orchid, belonging to the Vanda alliance. *Rhynchostylis retusa* orchid is an epiphyte growing on tree trunks in open forest or at forest margins at elevations of 300 -1500 m (980 – 4920 ft). This plant has both medicinal and ornamental value. The inflorescence is a pendant raceme, consisting of more than 100 pink –spotted white flowers. The plant has a short, stout, creeping stem carrying up to 12, curved, fleshy, deeply channelled, keeled, retuse apically shaped leaves and blooms on an axillary pendant upto 60 cm (24in) long. The racemose, densely flowered, cylindrical inflorescence blooms in early spring in the month of Late March – April.

Scientific classification of *Rhynchostylis retusa*	
Kingdom	Plantae
Family	Orchidaceae
Subfamily	Epidendroideae
Genus	*Rhynchostylis*
Species	*Rhynchostylis retusa*

It occurs naturally in India, Bhutan, Cambodia, China (parts of Guizhou, Yunnan), Indonesia, Laos, Malaysia, Nepal, Philippines, Singapore, Sri Lanka, Thailand and Vietnam. In India, the plant is most common in North East, Orissa and Andhra Pradesh. As the Rhynchostylis retusais is intimately linked with the culture and tradition of local people, it has been recognized as the state flower of Assam and Arunachal Pradesh in India.

Morphological description

Epiphytes, to 40 cm tall; stems stout, woody. Leaves to 30 x 2 cm lorate, obliquely bilobed at apex with a mucro in between the lobules. Flowers are violet pink, closely packed in 30- 40 cm long, stout compact,

pendulous axillary raceme; sepals and petals similar; dorsal sepal ovate or ovate-oblong, obtuse; lateral sepals 8x7 mm, obliquely ovate, sub acute; petals 6 x 3 mm, oblong- ovate, obtuse; lip entir, oblon, pink; clawe; claw deflexed deeply inflexed, cuneiform; disc 2- lobe; spur 5 mm long, laterally compressed, tip emarginated; puberulus inside; column short; foot very short; pollinia 2, globose.

***Rhynchostylis retusa* in bloom**

PROPAGATION

Orchids are propagated by two methods.

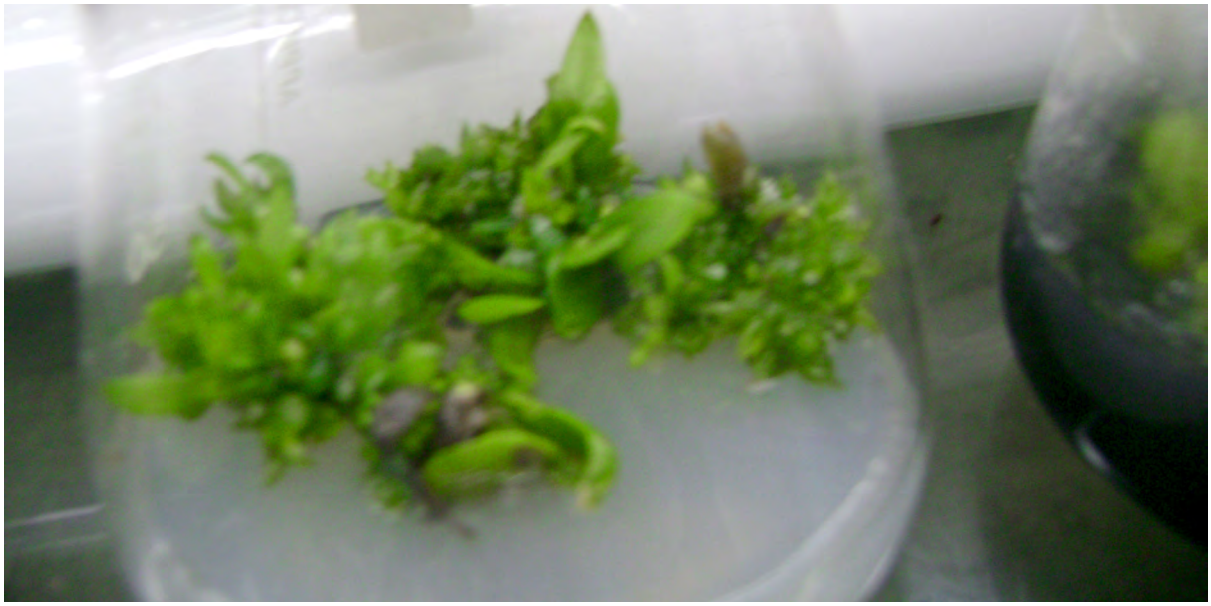
1. Vegetative method
2. Aseptic culture or Tissue culture method:
    - Seed culture
    - Meristem culture

Vegetative method

This method involves propagation of orchids by division. In case of *Rhynchostylis retusa*, it is a monopodial group of orchids, the stem continues to grow in a vertical direction producing flower spikes laterally and has generally no side branches. New roots are produced above the leafless stem, as the plant continues growing vertically. The stem can be cut below the new roots. The top part, with leaves and roots, can be propagated after proper care of the cut. The remaining stub can be left for a few days/ weeks. Again, new plants will be found growing out of the old stock. They can be separated out from the mother plant and propagated. Vegetative method of propagation is very slow. Only 1-2 plant can be separated out from the mother plant within one year period. Tissue culture/micropropagation has become a standard tool for producing a large number of plantlets in a short span of time.

Aseptic culture or Tissue culture

Mature but unripe green pods atleast 150 days old of *Rhynchostylis retusa* are used as explants for aseptic culture. The green pods are first washed with distilled water until it is free of any dirt and external impurities. After that pods are surface sterilized with 0.1 % Mercuric chloride solution for five minutes and again washed with sterilized distilled water. The pods are split open longitudinally with the help of sterilized blade under the Laminar Air Flow cabinet and thereafter seeds are inoculated in the culture vessels containing media.



Plb developed

The MS (Murashige and Skoog) modified medium supplemented with the concentration of kinetin and IBA (1ml/l) is used for germination and plb (protocorm) development. Ph of the MS medium is adjusted to 5.5 to 5.8 before autoclaving at 15 lb pressure at 121° c for 20 min.

Rooted plantlets in culture vessels

After inoculation culture vessels are incubated in growth room under 16 hr light period/day of 2000-2500 lux intensity where temperature is maintained at 25 ± 272 C.

Plb (Protocorm) develop after 35-40 days of inoculation and after 60-80 days of growth complete shoots also develop. Subsequently, subculture shoots multiply and rooting takes place. Around 120-150 days are required for development of complete plantlets in culture vessels.

Taking out plantlets from culture vessels (bottles)

For removing plantlets from the culture vessels, the cover of vessels are removed and spatula or forceps may be used for taking out the plants without causing any damage to the roots. The plantlets should be washed in distilled water 2-3 times to ensure that no traces of agar – medium are present on the plantlets.

Hardening of tissue culture plantlets

Fungicidal treatment

After washing, plantlets are soaked in a solution containing Bavistin (1.0 g/lt) for 10 min. Thereafter, plantlets are taken out and spread on paper towel for drying out excess moisture. The plants are now ready for transplanting.

Transplanting in to plastic trays /pots

The plants can be transplanted in pots or trays containing sterilized coconut husk. The pots are watered with nutrient solution and covered with clear transparent polythene film that are perforated with small holes for air circulation and maintaining sufficient humidity for 3-4 weeks period in the orchid house. After 3-4 weeks the plantlets should be exposed gradually to 50% shade and the polythene film can be removed when the plantlets have adopted to the microenvironment of the orchid house. For success of the plantlets and for transplanting from culture vessels to the pots, a hardening chamber is required

for primary hardening. Here the micro propagated plants get further strengthened with maintained temperature, light and relative humidity using intermitting misting as required.

In general, about 50 to 52 weeks are required for hardening after culture. After hardening completed, plantlets are ready for replanting in a bigger pot (with at least more than 3 holes in each pot) containing medium Charcoal : Bricks : Leaf mould (2:1:1). These plantlets take about 2.5 years for flowering.

Propagation of Rhynchostylis retusa plantlets through Tissue culture

At commercial scale, the success or failure of tissue culture technique often depends upon acclimatization and hardening of micro propagated plants. In their early stage, micro propagated plants are very sensitive to change in environmental conditions, but they perform after the roots become active.

Monitoring and caring for transplants

After transplanting the plantlets need to be monitored regularly for microclimatic conditions, moisture availability, wilting or drying symptoms, occurrence of diseases and pests. First 2-3 weeks are very crucial and great care is needed with respect to management of relative humidity and light.

Irrigation

The plantlets are irrigated after removing the polythene sheet at regular intervals considering the microclimatic conditions and the growth stage of plantlets.

Fertilizer application

Fertilizing the plantlets with macro and micronutrient solutions is beneficial. The fertilizers may be applied through micro sprays. The plantlets should be watered once a week with commercially available fertilizer solution depending upon the substrate is being used.

Disease and Pest management

No any major disease and pest has been recorded during hardening of *Rhynchostylis retusa*. Clean cultivation prevents spread of any disease and pest in the orchid house.The pots and benches should be sprayed with 5 per cent Formalin before keeping the pots in benches. Spraying with Mancozeb and Carbendazim @1g/l in water at once in a month is recommended to prevent fungal diseases.