

## ASYMBIOTIC SEED GERMINATION AND SYNTHETIC SEED PREPARATION OF *VANDA TESTACEA* (LINDL.) RCHB.F .

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### ABSTRACT

A protocol for asymbiotic seed germination and synthetic seed preparation of *Vanda testacea*(Lindl.) Rchb.f. from mature pods was developed. Seed germination was successfully established on half strength B<sub>5</sub> (Gamborg's medium) supplemented with 1.0mg/L GA<sub>3</sub>. Protocorm like bodies (PLB's) were formed after 90 days of culture and further artificial seed preparation of matured protocorm was successful using 5% sodium alginate and 100mM calcium chloride. The beads were inoculated on half strength B<sub>5</sub> medium supplemented with 1.0mg/L GA<sub>3</sub>.

**Keyword:** *Vanda testacea*, seed germination, artificial seed preparation, Protocorm.

### 1. INTRODUCTION

*Vanda testacea* (Lindl.) Rchb.f. is a species of orchid occurring from the Indian subcontinent to Indochina at the elevations of 500 to 2000 meters. It is an epiphytic perennial. In India orchids form 9% of flora and are the largest family among higher plants. It is estimated that about 1,300 species (140 genera) of orchids are found in our country with Himalayas as their main home and others scattered in eastern and Western Ghats. *Vanda testacea* (Lindl.) Rchb.f. has medicinal properties in sciatic nerve transaction (axotomy) induced peripheral neuropathy in rats (Santh Rani Thaakur and Swapna Pokkula, 2013). Root used to treat nervous disorders, piles, and inflammations as well as a potential anticancerous drug. Leaves, flowers in powder form are used as herbal medicines to cure rheumatism and bronchitis (Chauhan , 1990) . Asymbiotic seed germination by *in vitro* culture, which was first introduced and it has revolutionized the concept of orchid cultivation. Soumi Neha (2013).

*Vanda testacea* (Lindl.) Rchb.f. has a low rate of multiplication under natural/greenhouse conditions and like other monopodial orchids, survival of mother plant is not conducive to a shoot tip / meristem based micropropagation system. It is thus necessary to devise a rapid and efficient micropropagation. Orchid seeds became necessary for future sustainable harvesting system and maintaining orchid species to prevent from genetic erosion. Hence the present study was focused on seed germination and artificial seed preparation of *Vanda testacea* using matured pods.

### 2. MATERIALS AND METHODS

#### 2.1. Source of plant material

The mature pods of *Vanda testacea*(Lindl) Rchb.f. was provided by Dr.T.Muthukumar, Department of Botany, Bharathiar University, Coimbatore.

#### 2.2. Explant selection and mode of sterilization

Collected mature pods were surface sterilized by immersing in 70% ethanol for 10 sec. The sterilized pods were then washed 4-5 times with sterile double distilled water. Further capsules were cut longitudinally with a sterile scalpel and the seeds were inoculated on to half strength B<sub>5</sub> medium.

#### 2.3. Method of media preparation

Half B<sub>5</sub> (Gamborg's) medium was employed in the present study and the composition of the medium is given in Table-1. For media preparation, only analytical reagents of "Hi-media" chemicals and Borosil glassware's were used. Double distilled water was used for media preparation. Stock solutions were prepared separately for macronutrients, micronutrients, iron, potassium iodide and vitamins. All the chemicals were weighed accurately in electronic weighing machine. All the stock solutions were poured in to well stoppered sterilized bottles and preserved in a refrigerator at 4°C. Specific quantity of the stock solutions and growth regulators were pipetted on to a little beaker. Required source, other organic supplements and complex additives (optional) were added. The final volume was made up with distilled water and the pH was adjusted to 5.8 with either 1N NaOH or HCl using a pH meter (ELICO).

To the above said media, 0.8% agar (extra pure gelling point 32-35°C, Hi media, Bombay) was added, melted in a water bath and the medium was dispensed into culture bottles. The bottles after covering were autoclaved at 1.06 kg pressure/sq cm

for about 20min at 121°C. The autoclaved medium in the culture bottles were allowed to cool. The inoculation was done after 5 days to ensure that the bottles were free from contamination.

**Table 1. Composition of Gamborg's (1968) medium**

S. no.	Component	mg/l
MAJOR SALTS		
1	Na <sub>2</sub> HPO <sub>4</sub> .2H <sub>2</sub> O	150
2	KNO <sub>3</sub>	250
3	CaCl <sub>2</sub> .2H <sub>2</sub> O	150
4	MgSO <sub>4</sub> .7H <sub>2</sub> O	250
5	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	134
MINOR SALTS		
6	KI	0.75
7	H <sub>3</sub> BO <sub>3</sub>	3
8	MnSO <sub>4</sub> .4H <sub>2</sub> O	10
9	ZnSO <sub>4</sub> .7H <sub>2</sub> O	2
10	Na <sub>2</sub> MO <sub>4</sub> .2H <sub>2</sub> O	0.25
11	CuSO <sub>4</sub> .5H <sub>2</sub> O	0.025
12	CoCl <sub>2</sub> .6H <sub>2</sub> O	0.025
13	C <sub>10</sub> H <sub>12</sub> N <sub>2</sub> O <sub>8</sub> Fe Na	43
VITAMINS AND ORGANICS		
14	Meso-Inositol	100
15	Nicotinic acid	100
16	Pyridoxine HCl	100
17	Thiamine HCl	100
18	Cystine	10
19	Sucrose	20
20	Agar	8
21	Ph	5.8

#### 2.4. Growth regulator and its preparation

Growth regulator like gibberillic acid was used in the experiments.

#### 2.5. Preparation of Gibberillic acid (GA<sub>3</sub>)

The stock solution was prepared by dissolving 10 mg of GA<sub>3</sub> in 1mL of ethanol and the volume was made up to 100mL with sterile distilled water. This was used in two different concentrations (0.5 and 1.0 mg/L).

#### 2.6. Culture conditions

The cultures were maintained at 25±2°C under a 16hr photoperiod of 50-60µmol flux intensity provided by cool white fluorescent tubes.

#### 2.7. In vitro seed germination

Half B<sub>5</sub> (half strength Gamborg's medium) medium was used for seed germination.

#### 2.8. Artificial seed preparation

For encapsulation purpose 5% sodium alginate and 100mM calcium chloride (w/v) were prepared using sterile distilled water.

#### 2.9. Formation of beads

The protocorms were transferred to the sodium alginate solution. The protocorms along with sodium alginate matrix were dropped into a solution of calcium chloride and maintained for atleast 15 min to polymerize the beads. (When sodium alginate drops come in contact with calcium chloride solution surface complexation begins and form round beads). The beads were recovered by discarding the calcium chloride solution and later washed twice with sterile distilled water.

#### 3.0. Culture medium and conditions

The encapsulated protocorms were cultured on a half strength B<sub>5</sub> medium supplemented with 1.0 mg/L of GA<sub>3</sub>. All cultures were maintained in the culture room at 25±2°C under a 16hr photoperiod.

### 4. RESULTS AND DISCUSSION

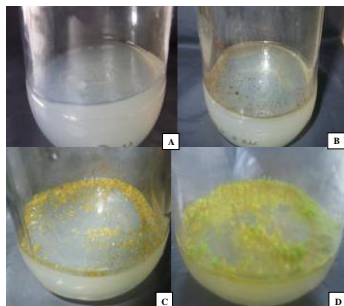
Orchids are faced with habitat destruction pressures due to extensive collections in the past. As a consequence the species has become rare and is restricted to very narrow pockets in its natural habitats (Kaur and Bhutani 2009). Thus to conserve this orchid from extinction and to increase the population size, plant tissue culture and micropropagation can play a significant role (Wochok, 1981). The increasing demand, and export values of orchids each year is driving the expansion of orchid growing areas (Kasikorn Research Center, 2008). A Significant number of identical clones can be raised from a single leaf through direct or callus mediated organogenesis (Arditti, 1977). Many reports have been made in growing the orchid pods in full strength MS medium and Knudson's medium. So the present study mainly focused on growing the seeds on half B<sub>5</sub> medium and to prepare synthetic seeds from protocorms.

The seeds of orchids produced in large numbers in each capsule are highly fragile and possess virtually no stored food material and endosperm. The epiphytic orchid *V.testace* has poor seed germination under natural condition. Seeds were cultured on Knudson's C medium, enriched with various concentrations of organic additives and plant growth regulators to study asymbiotic germination, seedling development and optimization of the cultural requirements (Mukhopadhyay and Roy, 1994).

The matured capsules are brown and thick in nature. The surface sterilized matured pods were opened under aseptic condition and the seeds were deposited on half strength B<sub>5</sub> (Half Strength Gamborg's Medium) medium supplemented with

1.0mg/L GA<sub>3</sub>. After 45 days of inoculation the seeds started germinating. Further the germinated seeds were allowed to grow in the same medium up to 90 days. Protocorm like bodies (PLB's) were formed after 90 days of culture (Plate-1). Seed germination and development of three *Vanda* hybrids were reported by Timothy Johnson (2007). Six asymbiotic orchid seed germination media were examined for their effectiveness in promoting germination and subsequent protocorm development of *Bletia purpurea* seeds was reported by Malmgren (1996).

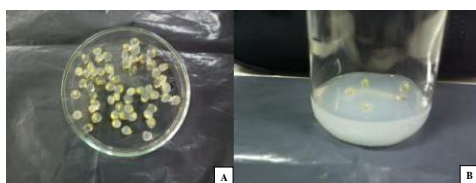
**Plate-1**



- A. Inoculated orchid pod
- B. Changes in colour after 30 days of inoculation
- C. Formation of protocorms after 45 days of inoculation
- D. Well grown protocorms after 60 days of inoculation

Further the fully developed protocorm like bodies (PLB's) were separated from the medium and subjected to artificial seed preparation. The mature protocorm like bodies (PLB's) were collected and mixed with 5% sodium alginate and dropped into 100mM of calcium chloride solution to form beads of synthetic seeds. Subsequently the beads were washed with sterile double distilled water and inoculated on half strength B<sub>5</sub> medium supplemented with 1.0mg/L GA<sub>3</sub>(Plate-2). The seedling development of *Oncidium sp.* was best on the MS medium supplemented with 2mg/l BA (Sharma, 1991). Several valuable species of *Dendrobium* have been reported to be propagated through asymbiotic germination via immature seeds (Vij *et al.*, 1981).

**Plate-2**



- A. Prepared synthetic seed
- B. Synthetic seed inoculated on half B<sub>5</sub> + 1mg/L GA

## 5. CONCLUSION

The findings of present study suggested that the asymbiotic seed germination was best on half B<sub>5</sub> medium supplemented with 1.0mg/L GA<sub>3</sub> and 5% sodium alginate and 100mM of calcium chloride was suitable for artificial seed preparation.

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