

## ***In vitro* Propagation of *Vanda testacea* (Lindl.) Reichb.f. – A Rare Orchid of High Medicinal Value**

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### **Abstract**

Foliar explants of *Vanda testacea* (Lindl.) Reichb. f. were cultured on Mitra (M) medium with 1.0 mg/l BAP, Kn each and 1.0 mg/l NAA individually and in combination for initiation of regeneration response, proliferation of regenerants and subsequent development of plantlets. Juvenility of the tissues and chemical stimulus were important factors in initiating the regeneration response in the explants. The relatively older leaf explants (>1cm in length) remained recalcitrant to regeneration the representing younger ones (<1cm in length) responded to certain chemical regimes. BAP, Kn individually in the medium should direct PLB regeneration whereas when used with NAA, the explants showed callus proliferation and further differentiated into PLBs. An individual treatment with NAA (1.0 mg/l) impaired the response frequency and delayed further morphogenetic processes leading to plantlet development. The best response in the explants (in terms of high regeneration frequency, early initiation, PLB proliferations, and plantlet development) was observed in 1.0 mg/l BAP alone/with 1.0 mg/l NAA + activated charcoal. Plantlets were transferred to pots containing epiphytic compost (1 charcoal : 1 brick pices : 1 bats). Nearly 75% of plantlets survival was recorded.

### **Introduction**

*Vanda testacea* (Lindl.) Reichb. f. (= *V. parviflora* Lindl.) known as Banda or Rasna, an alkaloid rich epiphytic species of vandaceous orchids, is widely known for its medicinal properties. Almost all plant parts (roots, leaves, flowers) in powder form or as an extract are used as herbal medicines to cure rheumatism, bronchitis, nervous disorders, piles, inflammations and also as a potential anti-cancerous drug (Chauhan 1990). This medicinally important orchid is faced with extensive collections and habitat destruction pressures. As a consequence its populations are depleted, the species has become rare and is restricted to very narrow pockets in its natural habitats. Herbal medicines are the precursors of

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many common drugs prescribed in clinical practice in many countries today. Furthermore, herbs and herbal products are still an important part of the primary health care systems in many parts of the world (Jawahar et al. 2008a). Medicinal plants are of great interest to the researches in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds (Chand et al. 1997). *In vitro* culture techniques offer a viable system for true-to-type rapid mass multiplication and germplasm conservation of rare, endangered, threatened, aromatic and medicinal plants (Arora and Bhojwani 1989, Sharma et al. 1991, Sudha and Seeni 1994, Sahoo and Chand 1998, Karuppusamy and Pullaiah 2007, Jawahar et al. 2008b).

*Vanda testacea* 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 protocol for obtaining true-to-type regenerants without detriment to the survival of mother/donor plant and saving its populations from getting rarer in nature. Ever since Morel (1960) suggested the use of shoot meristem culture, the technique has been applied in a large number of species to mass propagate them. Since the technique requires sacrifice of the only growing point in monopodial taxa, the utility of leaf explants is being increasingly realized as its excision does not endanger the growth and survival of the mother plant. In fact, significant number of identical clones can be raised from a single leaf through direct or callus mediated organogenesis (Arditti 1977). The regenerative potential of leaf explants has been positively tested in several orchid species representing diverse habits, habitats and taxonomic affinities (Tanaka and Sakanishi 1977, Fu 1978, 1979, Manorama et al. 1984, Mathews and Rao 1985a,b, Yam and Weatherhead 1991, Seeni and Latha 1992, 2000, Vij et al. 1994, Misra and Bhatnagar 1995, Kaur and Vij 2000, Vij and Aggarwal 2003, Tamjensangba and Deb 2005, Deb and Temjensabgba 2007). Presently, this communication reports the foliar explants based efficient *in vitro* propagation of *Vanda testacea* - a rare medicinal orchid species.

## Material and Methods

*Vanda testacea* (Lindl.) Reichb. f. (= *V. parviflora* Lindl.) leaves (1.0 cm long) sourced from 38 weeks old axenic cultures were used as explants. Mitra et al. 1976 (M) medium containing 2% sucrose was used as a source of nutrition. The medium was invariably gelled with 0.9% Agar powder (Qualigens). Individual and combined effects of plant growth regulators Kn, BAP and NAA were also assessed on culture initiation and various morphogenetic processes. In another set of experiments, 2% activated charcoal (AC) was consistently used in the

medium. The pH of medium was adjusted to 5.7 and autoclaved at 121°C at a pressure of 1.06 kg/cm<sup>2</sup> for 15 min. The inoculations were done under aseptic conditions in a laminar air flow cabinet. Cultures were incubated at 25 ± 2°C under 12 hr photoperiod of 3,500 lux light intensity (Flourescent tubes-40W; Philips India Ltd., Mumbai, India). The experiments were repeated twice. The observations were made regularly and data recorded accordingly. The results were tested using the one-way ANOVA test and were analyzed using Tukey Multiple Comparison using SPSS (Version 11.5) software package.

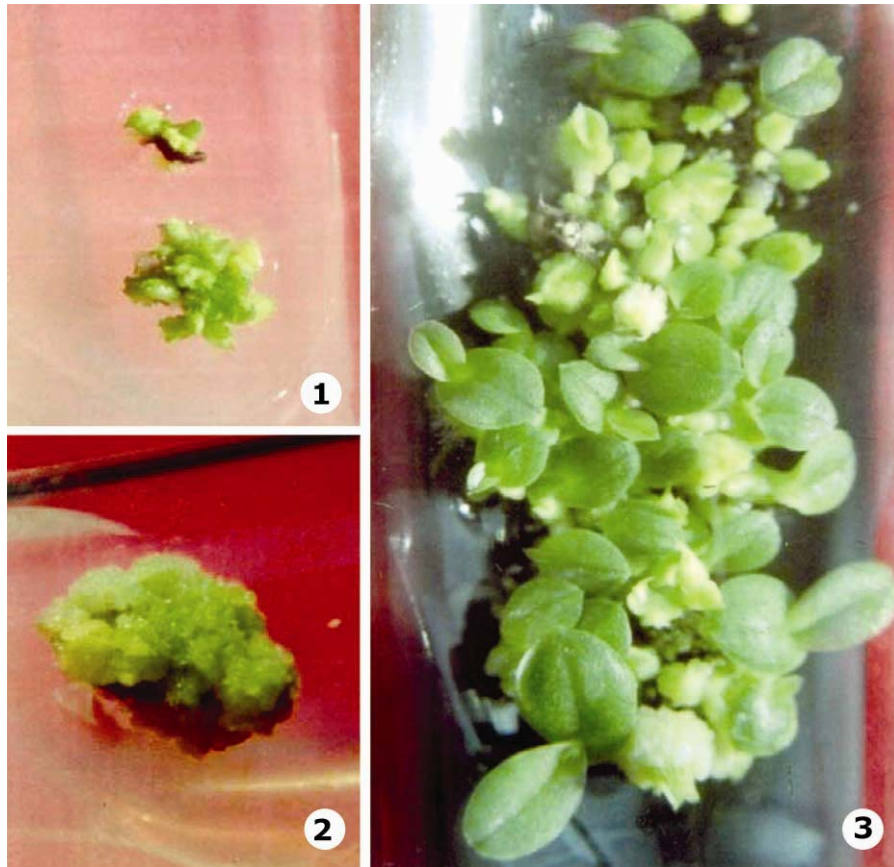
## Results and Discussion

Presently, the *in vitro* regeneration potential of foliar explants was positively tested in *Vanda testacea*. Culture initiation was markedly affected by the physiological status (juvenility) of tissues besides the chemical stimulus in the nutrient pool. Explants of more than 1.0 cm long leaves, remained recalcitrant to regeneration and those from less than 1.0 cm responded to certain selected media combinations. Juvenility of leaf tissues as an important factor promoting regeneration has been suggested in several orchids (Fu 1978, Vij and Pathak 1990, Arditti and Ernst 1993, Kaur and Vij 2000, Vij and Aggarwal 2003, Temjensangba and Deb 2005).

A better morphogenetic potential of juvenile (less differentiated) cells has been explained on the basis of their physiologically and biochemically more active state due to their less rigid cell walls (Misra and Bhatnagar 1995). In earlier experiments, apical and/or basal leaf segments were considered as meristematic loculae (Yam and Weatherhead 1991). Mathews and Rao (1985a,b) considered the leaf base as a decisive factor for regeneration in *Vanda* cultures. On the other hand, the tip region regenerated infrequently when excised and more frequently in an intact leaf. Almost similarly, the leaf base exhibited a greater proliferative potential than leaf tips in *Ascocenda* and *Vanda* (Fu 1978, 1979), *Coelogyne*, *Dendrobium*, *Oncidium* and *Phalaenopsis* (Abdul and Hairani 1990), *Renanthera* (Seeni and Latha 1992), and *Acampe* (Nayak et al. 1997).

In our present studies only the basal segments responded in cultures. This differential response of the juvenile leaves hints at the possibility of the influence of the genotype or the intrinsic factors of the mother plant upon regeneration potential. The study also determined the probability of control of meristematic potential by some factors emerging from the of leaf base tissues.

In our cultures, the explants regenerated along the adaxial surface only, in accord with similar earlier findings in *Acampe praemorsa* (Nayak et al. 1997) and *Renanthera imschootiana* (Seeni and Latha 1992). Literature studies also indicate activation of meristematic loci on both the adaxial and abaxial surfaces in some species (Manorama et al. 1984, Vij and Pathak 1990, Kaur and Vij 2000).



Figs. 1 - 3 : *In vitro* regeneration response of foliar explants of *Vanda testacea*. 1. PLB mediated regeneration response in M + BAP 1.0 mg/l. 2. Differentiation of callus into PLBs in M + BAP 1.0 mg/l + NAA 1.0 mg/l. 3 Plantlet formation in M + BAP 1.0 mg/l + NAA 1.0 mg/l + AC.

The role of growth adjuncts in initiating the meristematic activity in foliar explants and/or promoting proliferations thereof is well documented in orchids (Abdul and Hairani 1990, Arditti and Ernst 1993, Vij et al. 1994, Kaur and Vij 2000, Temjansangba and Deb 2005, Deb and Temjansangba 2007). In our studies, the regeneration response in the explants was completely dependent to the use of growth regulators in the culture medium (Table 1). BAP alone and with NAA were successfully used to enhance the regeneration frequency, early initiation of response and multiplication of regenerants, the explants followed PLB mediated and callus mediated pathway of regeneration respectively in the combinations (Figs.1, 2). Plantlets complete with two - three leaves and one - two roots developed within 20 - 25 weeks in activated charcoal supplemented medium (Fig. 3). The indispensability of cytokinins for regeneration of PLBs and multiplication thereof have been reported earlier in orchids (Vij et al. 2000). A

synergistic action of cytokinins and auxins is reported in several orchid species e.g. *Phalaenopsis* (Tanaka and Sakanishi 1977), *Vanda* hybrid (Mathews and Rao 1985 a, b), *Vanda cristata* (Vij et al. 1994), *Saccolabium papillosum* (Kaur and Vij 2000) *Cymbidium* hybrid (Vij and Aggarwal 2003). The percentage of responding explants was significantly impaired in NAA treated cultures; even the morphogenetic processes leading to plantlet development were also prolonged delaying the formation of complete plantlets to 25 weeks. The explants followed callus mediated development of PLBs in the responding explants. Earlier also in *Cleisostoma racimeferum* NAA proved poor for regeneration (Temjensangba and Deb 2005).

**Table 1. In vitro regeneration response of foliar explants of *Vanda testacea* in Mitra (M) medium and its combinations with growth adjuncts.**

| Additives      | % regeneration response | Initiation of response (weeks) | Regeneration pathway | Complete plantlets (weeks) |
|----------------|-------------------------|--------------------------------|----------------------|----------------------------|
| -              | 0.00 <sup>a</sup>       | 0.00 <sup>a</sup>              | -                    | 0.00 <sup>a</sup>          |
| AC             | 0.00 <sup>a</sup>       | 0.00 <sup>a</sup>              | -                    | 0.00 <sup>a</sup>          |
| BAP            | 48.75 <sup>g</sup>      | 4.75 <sup>f</sup>              | *PLB-pl              | 23.25 <sup>i</sup>         |
| BAP + AC       | 42.75 <sup>d</sup>      | 4.25 <sup>f</sup>              | "                    | 23.00 <sup>i</sup>         |
| Kn             | 42.25 <sup>d</sup>      | 5.00 <sup>fi</sup>             | "                    | 23.75 <sup>i</sup>         |
| Kn + AC        | 43.00 <sup>d</sup>      | 5.50 <sup>fi</sup>             | "                    | 22.75 <sup>g</sup>         |
| NAA            | 38.50 <sup>c</sup>      | 7.25 <sup>bcdfi</sup>          | C -PLB-pl            | 25.25 <sup>g</sup>         |
| NAA + AC       | 24.25 <sup>bi</sup>     | 5.50 <sup>i</sup>              | "                    | 26.00 <sup>efhi</sup>      |
| BAP + NAA      | 48.75 <sup>g</sup>      | 3.75 <sup>f</sup>              | **                   | 21.75 <sup>g</sup>         |
| BAP + NAA + AC | 48.75 <sup>g</sup>      | 3.00 <sup>defg</sup>           | **                   | 20.25 <sup>bcdg</sup>      |

Concentration of PGRs = 1.0 mg/l each; C = callus; PLB = Protocorm-like body; \*= PLB multiplication; pl = Plantlet. Values in a column with similar superscripts are not significantly different at  $p \leq 0.05$ .

All these data suggest that the initiation of meristematic activity in foliar explants is directly related to the juvenility of the donor tissues besides the chemical stimulus to which they are subjected. In general, the proximal segments respond better than distal ones. A careful selection of donor leaf (juvenile ones) and use of precise chemical stimulus can help devising an ideal propagation system without any detrimental effect to the mother plant. This protocol is simple and easy to carry for mass propagation and conservation of this rare valuable species.

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