

## Chemical growth regulator mediated propagation of *Dendrobium* 'Rinnapa' Lek Dee Dee and its comparative analysis with other methods of propagation

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### Abstract

The Orchids are the most fascinating and intriguing members of plant kingdom and form the backbone of orchid cut flower trade and sustainable means of livelihood for many. They are loved all over the world not only for their beautiful blooms but more importantly for their longevity. But orchid propagation and affordability has always been an important area of concern for all orchid growers. The present paper discusses some of the most easily adoptable and sustainable means of propagating *Dendrobium* hybrid orchids which are the highly valued flowers in cut flower markets. The paper highlights Benzyladenine (6-Benzylaminopurine) mediated propagation of a hybrid orchid *Dendrobium* 'Rinnapa' Lek Dee Dee and presents its comparative analysis with other methods of propagation. The response of the plants propagated using Benzyladenine in the present investigation suggests that this method of propagation may be an alternative to tissue culture and may be successfully employed by the small scale growers.

**Key words:** *Dendrobium* hybrid orchid, Benzyladenine, propagation, laonin paste

### INTRODUCTION

Orchids, the doyens among ornamentals are one of the most important global cut flower and pot plants, and their sheer beauty has enchanted and fascinated people since early times (da Silva 2013). *Dendrobium* genus is the second largest orchid genus in the world with more than 1,200 species and hybrid forms called "grexes" which are mostly used by the commercial orchid growers (Arditti 1992; da Silva 2013). Thriving in tropical forests of south East Asia and other tropical zones, *Dendrobiums* are epiphytic and can be easily cultured without soil as these orchids absorb nutrients and moisture from the surrounding environment. Although *Dendrobium* can be propagated by seeds, asexual propagation techniques are most often practiced by home growers only. *Dendrobium* alone accounts for 80% of the total micropropagated tropical hybrid orchids, usually by protocorms (Griesbach 2003; Saiprasad *et al* 2004) which unfortunately cannot be availed by all growers.

Some forms of orchid propagation are significantly easier than others; however, rapid multiplication of the plant of interest with high success rate is often an area of deep concern. Growing orchids from seed is somewhat complex, vegetative propagations are straightforward but tricky and propagation from keikis is relatively easy. Advanced techniques such as meristem propagation and other means of *in vitro* propagation are mostly expensive and employed by commercial growers only. Therefore, the hybrid orchids still continue to be beyond the reach

of a common man. The present paper aims to highlight some of the most commonly used methods of propagation which can be easily practiced by both the orchid enthusiasts and small scale growers (Orchid Growing in the Tropics, Times Editions; ISBN 981 204 108 7).

## METHODOLOGY

The amount of orchid hybrids has grown tremendously in the recent years due to the development of many new hybridization techniques and biotechnological methods making orchid cultivation a multibillion dollar industry (Alam *et al.* 2002). There are thousands of orchids found in all parts of the world. Therefore, it can be very intimidating for a person to take on the challenge of trying to grow a hybrid orchid as a hobby needless to mention about growing it on a commercial scale. With the right environment and the small amount of care they need, hybrid orchids too can be grown and propagated easily. The present study discusses different methods of propagating a hybrid orchid *Dendrobium* 'Rinappa' Lek Dee Dee [*Dendrobium darcie mikami* (seed parent) X *Dendrobium tomie drake* (pollen parent)]; Registrant name: MARDI, date of registration: 21.5.2008] originally grown from tissue cultured ex-agar plantlets produced in B.K. Biotech, Kolkata and makes a comparative analysis of various methods of propagation with Benzyladenine (6-Benzylaminopurine) mediated propagation with respect to flower quality and quantity, number of spikes produced, initiation of flowering, plant vigour etc.

**Propagation form Keikis:** Under low-light conditions, *Dendrobium* plants produce new plants or small plantlets called "keikis." Keikis grow as offshoots from a node on the mother stem. These offshoots produce roots while still attached to the mother plant. Propagation of *Dendrobium* 'Rinnapa' (Plate I B.) by keikis in the present study was done by severing the stem 1 to 2 inches below the node and above the node. Keikis were then planted in a porous medium or in a bed of jute fibres after the keiki developed three to four roots. The new plants were kept out of direct sunlight and placed in a relatively dry place and grown in a potting mixture of charcoal, cocoanut chips and brick pieces. .

**Asexual propagation by division of the rhizome:** This method of propagation involved severing the rhizome between sympodials, which are stem-and-leaf growths at the base of the plant. A sterilized knife or razor blade was used for severing the rhizome. The pseudobulb from the old pseudobulb was severed at the axis, making the cut close to the old pseudobulb. When there was no pseudobulb in the test plant, rhizome was cut into pieces with at least one live eye. The pseudobulbs or severed rhizomes so prepared were then placed on moist sphagnum peat in a low-light environment with good ventilation. Care was taken to provide mist twice a day and keep sphagnum moist but not soggy. When new growth developed, the new plants were moved to their natural environment and potted using the conventional potting mixture of charcoal, cocoanut chips and brick pieces.

**Propagation from old pseudobulbs:** The authors made an attempt to grow new plants from the old pseudobulb which appear to be dead but lying in dormant stage by simply pulling off the papery covering and dead leaves and placing the dormant pseudobulbs in moist sphagnum peat and coir fibres with the eyes facing up and kept in a warm area. When new growths were noticed, the plants were brought in the greenhouse environment and allowed to grow as usual in well-ventilated areas (Plate I A. & A1). Watering was done approximately twice a week, when the surface of the potting mixture was found to be dry. The plants were then moved into bright, indirect light and provided up to 12 – 14 hours of artificial light per day.

**Propagation from aerials:** Some species of *Dendrobium* form vegetative buds on the upper nodes of stems, and these new growths are referred to as 'aerials'. It is always wise

to allow the aerials to be mature before being separated. Propagation from aerial in the present study was done only when a second aerial growth was found to be present before removing the first one (Plate I C.).

**Propagation from seeds:** An attempt was made in the present study to germinate orchid seeds treated with dilute bleaching agent or any other surface sterilant in a bed of white *Sphagnum* moss.

**Propagation using benzyladenine:** Chemical growth regulator Benzyladenine (6-Benzylaminopurine) in the form of paste (0.05-0.5%) in lanolin was used to induce plantlet formation on the main stem and lateral buds in leaf axils. The paste was directly applied to the growth points after removing the covering bracts or sheaths.

**Meristem Propagation of Orchids:** Meristem propagation is a laboratory technique to clone a particular orchid cultivar in mass. Commercial orchid breeders often want to produce numerous copies of a particularly beautiful plant, so they use this technique. However, this method of propagation is relatively expensive and beyond the reach of a hobbyist or for a small scale grower.

After having tested and evaluated various commonly used methods of propagation, an attempt was made to perform a comparative analysis of three important methods, mainly the propagation by pseudobulbs, tissue culture and Benzyladenine (6-Benzylaminopurine) mediated propagation with respect to flower quality and quantity, colour intensity of the blooms, initiation of flowering, plant vigour etc for three consecutive flowering cycles. The plant vigour was assessed on the basis of overall health of the new plant and by comparing the increase in root and shoot length under the same environmental conditions (temperature of  $25\pm 3^{\circ}\text{C}$ , 60-70% RH and 10-12 hours of photoperiod. The experiment consisted of ten replicates with three plants in each treatment.

## RESULT AND DISCUSSION

The results of the present study clearly revealed that different methods of propagation can be safely practised for propagating even hybrid orchid like *Dendrobium* 'Rinnapa' Lek Dee Dee with varying degree of success rates (Table 1). The comparative analysis of different methods with Benzyladenine assisted methods of propagation revealed that the success rate of hormone assisted method was the highest (70 – 80 %), followed by propagation from relatively new pseudobulbs (50 – 60 %). The lowest success rate was recorded in case of propagation from the seeds (5 – 10 %). The hormone assisted method not only proved to be more successful than other methods but also produced plantlets of almost identical genetic characteristics as the mother plant. Chen & Chang (2001, 2004) have also reported the role of TIBA and hormones like auxin and cytokinines in direct somatic embryogenesis in *Oncidium* orchids. Saiprasad *et al.* (2003) have also mentioned the effect of growth regulators on production of PLBs and multiple shoots in orchid: *Dendrobium* 'Sonia'. The new plants produced in the present study, had more number of flowers per spike and flowers were of almost same colour intensity, size and keeping quality as the flowers harvested from plants grown from tissue cultured plantlets (Table 2). Pre and post harvest keeping quality of flowers harvested from plants propagated with Benzyladenine were found to be at par with the flower keeping qualities of flowers harvested from tissue cultured plants and the flower quality did not deteriorate even after three successive flowering cycles.

The observations and results of the present study further revealed that orchids have two growth patterns, monopodial and sympodial. Knowing whether the orchid of interest which we want to grow and propagate is important to grow and propagate successfully. Also

**Table 1.** Comparative Analysis of Benzyladenine assisted methods of propagation with other methods

Mode of Propagation	Success Rate (%)	Mean root length after 45 days (cm)	Mean Shoot length after 45 days (cm)	Plant vigour	Initiation of flowering
Pseudobulbs	50 – 60	3.2 ±0.9	2.1 ±1.3	++	3-4 years
Benzyladenine assisted	70 – 80	3.8 ±1.1	4.3 ±0.4	+++	2-3 years
Tissue Culture	80 – 90	4.0 ±0.8	4.6 ±0.7	+++	2-3 years

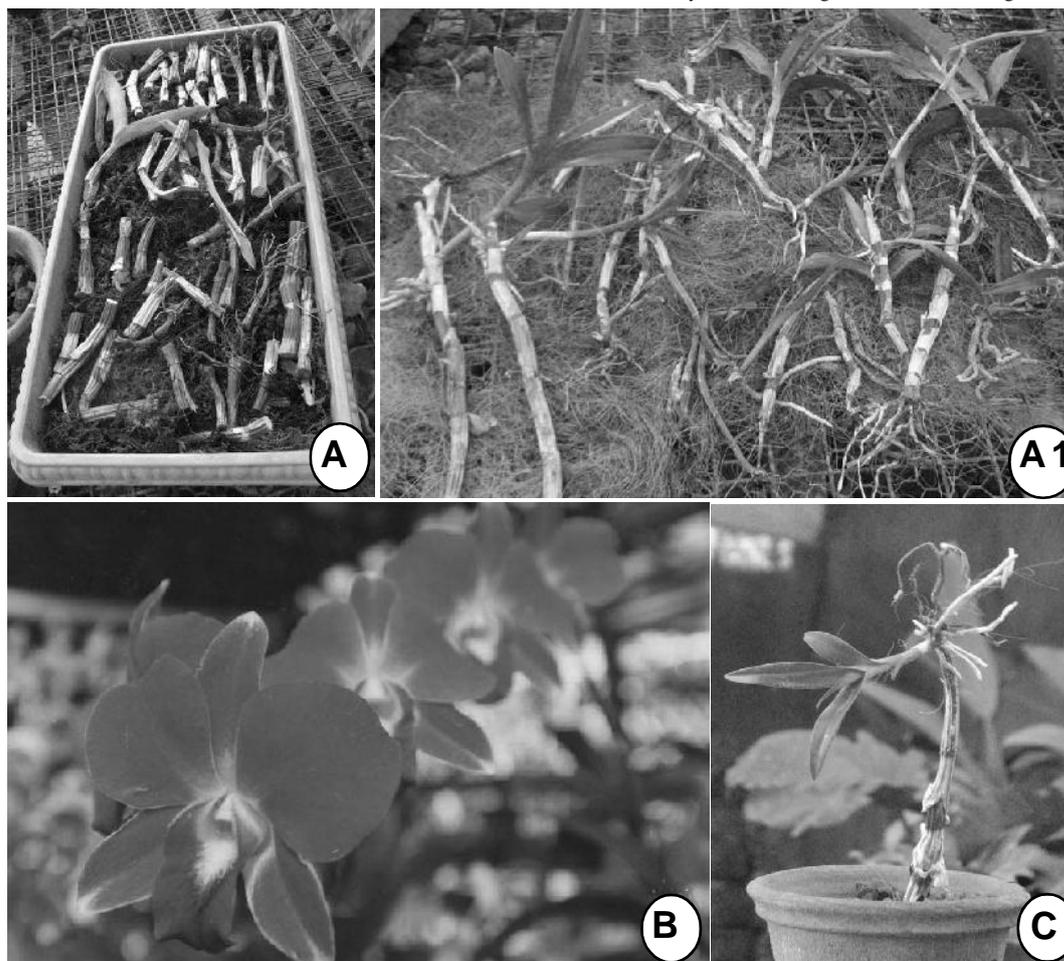
**Table 2.** Performance of plants produced by various methods of propagation in three consecutive flowering cycles [Good: +; Very Good: ++; Excellent: +++]

Mode of Propagation	Pre-harvest Flower quality			No. of spikes produced per plant			No. of flowers per spike			Colour intensity of the flowers			Post harvest keeping quality		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
Pseudobulbs	+	+	++	01	01	01	3	4	4	++	++	++	+	++	++
Benzyladenine assisted	++	+++	+++	02	02	03	6	7	7	+++	+++	+++	++	+++	+++
Tissue Culture	++	+++	+++	02	02	03	6	7	8	+++	+++	+++	++	+++	+++

it helps tremendously to know exactly the right time to propagate the orchid of interest. Present study revealed that propagation by division is best done at repotting time, when the plant has completely outgrown its pot.

Of all the tested methods of propagation, the method of forming keikis using keiki paste which has hormones Benzyladenine (0.5 %) to induce plantlet formation was most successful. Chang & Chang (2000) have also reported the role of a chemical like thidiazuron on bud development in species of *Cymbidium* and results of present study further corroborate their earlier findings. Once a keiki developed a strong root system, and at least a few leaves in a couple of weeks it could be carefully cut off the parent plant and planted as usual. The keiki, like a small orchid seedling, did not need particularly special care while it matured, but it was found to be more vulnerable to poor care than blooming-size orchids. Dividing orchids was also found to be an effective way to propagate them. As with keikis, the division produced plants that are genetically identical to the parent in many ways.

While it was occasionally possible to have a few orchid seeds germinate from the sterilized seeds, the rate of germination was found to be very low. Orchid propagation from



**PLATE-I: A & A1.** Propagation from pseudobulbs; **B.** *Dendrobium* 'Rinnapa' hybrid orchid; **C.** Propagation from aerial.

seed was found to be difficult. This may be due to the lack of stored nutrients in orchid seeds and failure of the seeds to encounter a symbiotic mycorrhizal fungus, which provides the nutrients the seeds need to germinate (Arditti & Ghani 2000). The authors therefore recommend growing orchids from seed in sterile laboratory flasks on agar, which provides enough nutrients for germination or rely on a good "orchid flasking service" as also suggested by Geetha & Shetty (2000) and Saiprasad & Polisetty (2003).

Propagation from entirely old growths such as backbulbs, was found to be a much slower process and less successful than a fresh bulb taking anywhere from 3 to 5 years. The findings of the present study therefore, clearly reveal that chemical growth regulator like Benzyladenine can definitely assist in the hormone assisted method of propagation if used in the right percentage dose and shows enough promise to be used as an alternative and cost effective means of propagation to in vitro propagation which can be safely practiced by all small scale growers for easy propagation of their prized possession. However, this method of propagation still needs further field trials and observations as the observations were recorded in the present study for only three consecutive cycles of flowering.

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