Mini Mission-1
Technical Bulletin No. 7

TECHNOGUIDE FOR

PROPAGATION OF CYMBIDIUMS THROUGH BACKBULBS

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(Indian Council of Agricultural Research)
Pakyong – 737 106, Sikkim, India

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TECHNOGUIDE FOR PROPAGATION OF CYMBIDIUMS THROUGH BACKBULBS

(Don't throw us Backbulbs)

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Technoguide for

Propagation of Cymbidiums through backbulbs

Compiled and Edited by

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Printed

January, 2011, 1000 copies
First print: 2011

Published by

Dr. R. P. Medhi, Director, National Research Centre for Orchids, Pakyong – 737 106, East Sikkim, Sikkim

Cover Photographs

Left : Cymbidium backbulb after shoot bud induction
Right : Removal of plantlet from the backbulb (Top)
Planting of plantlet after removal (bottom)

Printed at: M/S Hindustan Printers, Ground Floor, Gupta Market, Raja Rammohan Roy Road, Siliguri - 734 001
Phone No. 0353 2432776

FOREWORD

Cymbidiums occupy 6th position among top ten cut flowers traded in international market and also fetch highest price per spike among all floriculture crops grown for cut flowers. Cymbidiums are increasingly becoming popular among the growers as well consumers for cut flowers and potted flowering plants. The areas lying between elevations of 1500 - 1800 m MSL in northeastern Himalayas offer congenial climate for growing of these orchids. However, availability of planting material is one of the major constraints in expanding of orchid cultivation in this region. The infrastructure required for propagation of Cymbidiums through tissue culture is beyond the reach of common farmers. Hence, it was experienced that an effective propagation technique needs to be developed for multiplication of planting stock of Cymbidiums at farmers' level. The present technical bulletin is outcome of research conducted by Mr. Ram Pal who has been working on various aspects of micropropagation and conservation of orchids.

It gives me immense pleasure to bring out this bulletin on "Technoguide for Propagation of Cymbidiums through Backbulbs" where an innovative *in vivo* propagation technique has been described in a comprehensive manner. I am sure that the bulletin would be useful to orchid growers, extension personnel, students and researchers in understanding and propagating of Cymbidiums through backbulbs.

January 10th, 2011
NRC for Orchids, Pakyong,
Sikkim

(R. P. Medhi)
Director

PREFACE

The backbulbs of Cymbidiums are usually left over during repotting of outgrown plants. These backbulbs are either thrown or planted in potting compost. The planting of backbulbs in this way gives out usually one or none plant from the backbulbs. The modifications in existing protocol were made where the backbulbs can be re-used in the process of plantlet production. The "Technoguide for Propagation of Cymbidiums through Backbulbs" contains stepwise instructions on production of plantlets and their utilization for the production of cut flowers or potted flowering plants.

In producing this bulletin I have received valuable advice and encouragement from Dr. R. P. Medhi, Director, National Research Centre for Orchids, Pakyong, Sikkim and Prof. P. Tandon, Vice Chancellor, North Eastern Hill University and Chairman of 9th Research Advisory Committee (RAC) of the Institute. The same is gratefully acknowledged.

January 10th, 2011,
NRC for Orchids
Darjeeling Campus
Darjeeling

(Ram Pal)

CONTENTS

INTRODUCTION

Cymbidiums are popular orchids grown for cut flowers as well as production of potted flowering plants. These are commercially grown in Europe, North America, Africa and New Zealand and the highlands of Australia. In India the Cymbidiums are being cultivated in Sikkim, Arunachal Pradesh, Meghalaya, and Darjeeling district of West Bengal. The climatic conditions of north-eastern Himalayas are very congenial for growing of these orchids. Since most of the cultivars cultivated today have been bred from the wild species found in these areas; they grow and flower well. There is a great scope for further expansion in other cooler regions of the country.

The availability of quality planting is viewed as one of the major bottleneck in expanding Cymbidium cultivation in the country. Though meristem culture has its own advantage in propagation of planting stock but it is beyond the reach of individual farmer who want to multiply their desired planting stock. Conventionally, Cymbidiums are multiplied through division or through planting of backbulbs in sand or leaf mould.

The improved method of propagation of Cymbidiums through backbulbs has been found suitable for adoption at individual farmer level in the major Cymbidium growing areas of the country. The major advantages of this method over the traditional method are as follows:

- In conventional method, the backbulbs are planted on sand or leaf mould and after sprouting and plantlet development these are transferred to a pot

along with the backbulb. Thus, backbulbs can not be reused further in the production of plantlet. However in new method, the backbulbs can be reused for production of plantlets till its death.

- In conventional method, a backbulb produces one or very rarely two plantlets during its life span, whereas in new method backbulbs can be used twice in year and 4 - 6 times during its life span to produce 6 – 8 plantlets per backbulb.

The new method involves three major steps namely [a] sprouting of backbulbs, [b] the planting of sprouted backbulbs for developing into plantlets and [c] removal and planting of plantlets for cultivation. The technique is suitable for propagation of hybrids as well as species.

2. UNDERSTANDING CYMBIDIUM PLANT

Cymbidiums are herbaceous perennial plants having sympodial growth habit. The shoot buds (new growths) are initiated during the season of active growth from the base of pseudobulb. The new growths unfold and enlarge their leaves during this season. The initiation and development of roots also occurs during this period. The formation of shoot buds and enlargement of leaves occurs during warm part of the year, while accumulation of food reserves takes place during winters. Owing to accumulation of food reserves, the basal portion is thickened and swollen and known as pseudobulb. The flower spikes are likely to form on this pseudobulb during the flowering season and called as lead (see Fig. 1). Normally, the floral spikes are borne on previous season's growth (lead). However, some cultivars

in certain locality, also found to bear the flower spikes on the pseudobulbs that have produced flower spikes in the

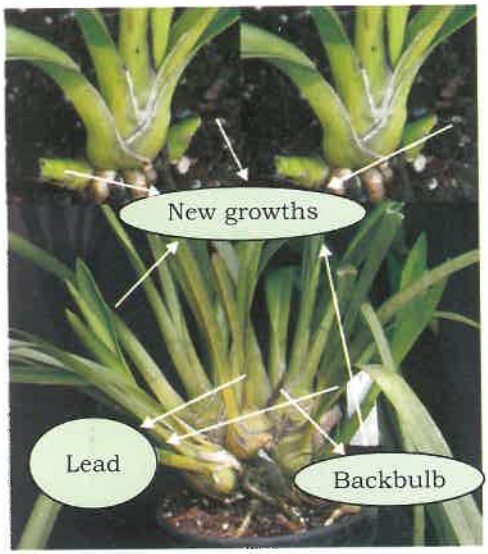

Fig 1. Showing the growth pattern in Cymbidium plants

preceding year. Depending upon the cultivar, the leads also produce 1 - 2 vegetative shoots during the season of active growth. The vegetative shoots may be produced during the initiation of floral spike or just after the completion of flowering. Generally, the pseudobulbs that have flowered once will not flower again termed as backbulbs. Thus each pseudobulb produces flower spikes only once in its life time. As the new growths take up the old bulbs (backbulbs) starts deteriorating and leaves fall down. In a considerable time a large numbers of backbulbs get accumulated in the pot. As long as backbulbs are attached to the mother plant, they do not produce new shoots. However, when detached and planted on sand or leafmould they experience bud break from the condensed part of the pseudobulb. It is may be

because of cumulative effects of growth inhibitory substances present in the backbulbs. However, when the backbulbs are detached and planted separately, the effect of growth inhibitory substances is reduced and they experience bud break from the base of backbulbs. These buds develop in to plantlet in due course of time.

3. BACKBULB MORPHOLOGY

Pseudobulbs are enlarged and thickened portion of the stem from which all leaves and inflorescences arise. Pseudobulb can be classified regardless to shape to be homoblastic (single node) or heteroblastic (many nodes) type on the basis of number of internodes for the pseudobulb (Hew et. al., 1994). The Cymbidium psudobulbs are heteroblastic and possess many nodes and internodes (Fig. 2). Once the flowering is over, gradually, the leaves on the pseudobulb dry up and leafless pseudobulbs are termed as backbulbs. These backbulbs are used for regeneration of new plants.The shape and size of the backbulbs may vary with the cultivars. Usually, miniatures and intermediate type of Cymbidium possess smaller pseudobulbs whereas standard type of Cymbidium possess larger backbulbs. The data collected from the 25 Cymbidium cultivars showed that backbulbs are 5.80 -12.75 cm long and 3.13 -8.47 cm wide and 10.63 - 24.54 cm in circumference. The backbulbs may be divided in to two parts, one basal which is thin, short, hard and contain many annular rings at a very short distance. The other, upper and swollen part of pseupbulb marked and annular rings at a larger distance. The backbulbs contain many annular rings and one dormant bud on each annular ring that

remains fully inhibited as long as the back bulbs are alive and remain attached to the plant. It is due to accumulation of growth inhibitory in the backbulbs. It has been found that BAP (6-benzylamino purine) stimulates the dormant buds in backbulbs of Cymbidium.

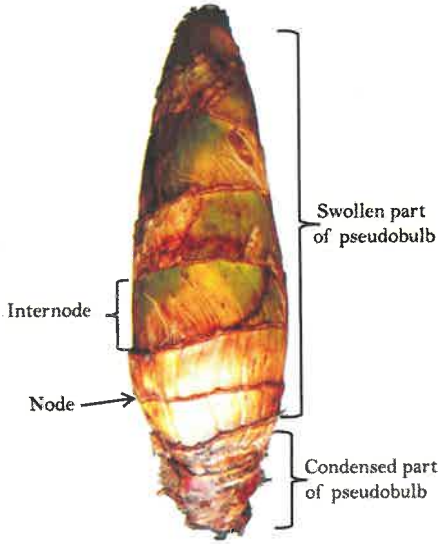

Fig 2. Backbulb of Cymbidium showing different parts; usually the condensed part of the backbulb produces one or two vegetative shoots during the season of active growth when the backbulb is detached from mother plant and planted separately on sand or pot mix

4. REVIEW OF PROPAGATION TECHNIQUES THROUGH BACKBULBS

Soon (2005) described a method for producing plantlets from backbulbs of Cymbidium. In this method, the backbulbs are hung up under shade and watered occasionally until new shoots develop when it can be

replanted usual way. If the bottom most bud is healthy, it will the one to develop into new shoot otherwise any bud along the length of pseudobulb may develop in to new plantlet. A slightly modified method for propagation of Cymbidium through backbulbs was suggested by Tibs (2008). In this method, washed backbulbs are placed in plastic bags and then the bags are hooked under the growing benches in a cool shade area. Bags are checked regularly, when the roots began to emerge these are potted in small pots. La Corix (2008) stated that loose sheaths of backbulb should be removed and allowed to dry a day or two then plant it about a third of its length in sharp sand or perlite and keep it moist and cool. In a few months a new shoot would appear and when roots have also developed, these can be potted up in the usual way. Pal (2001) suggested that backbulbs of Cymbidiums can be planted in polyethylene bags with moist sphagnum moss. The moist sphagnum moss provides moisture to backbulbs for sprouting the vegetative shoots. The shoots, when attain 3-4 leaves and 2-3 roots are severed and planted separately to develop into plant. The backbulbs are again placed in the polyethylene bags for shoot bud initiation/development. The higher moisture content of sphagnum moss causes rotting of backbulbs. The survey results indicated that farmers in Sikkim and Darjeeling also use backbulbs for regeneration of plantlets. They plant backbulbs in sand or leafmould individually or in group. The backbulbs planted singly are allowed to develop into plant in the same pot by changing pot mix every year. However, the backbulbs planted in groups are removed and planted individually when they have developed plantlets having 3-4 leaves and 2-3 roots.

5. SPROUTING OF BACKBULBS

5.1 Removal and section of backbulbs

Fig 3. Backbulbs collected from the overgrown plants of Cymbidiums

The backbulbs are obtained during repotting of overgrown plants. Repotting should be carried out just after the flowering is over. The delay in repotting may affect production of plantlets from the backbulbs as well as flowering in coming season. First of all, remove the plant from the pot and shake it off so that adhered potting mixture is removed. The plant should be divided by hand from its natural divisions keeping in mind each new plant should contain 3-5 green pseudobulbs and one back bulb. If necessary, use sterilized cutting tools for dividing the plant. The backbulbs from healthy planting stock are selected for propagation. The dried, shrivelled and diseased backbulbs are culled.The backbulbs should be removed in such a way that minimum damage occurs to the basal part of the backbulb.

5.2 Cleaning of backbulbs

The backbulbs obtained after repotting bear dried leaf sheaths and old and dead roots. The dried leaf sheaths and roots are removed with the help of sterilised knife. Wash the backbulbs in plain running tap water. Then make a suspension using Teepol/ hand wash /bathing soap. Soak the backbulbs for about 10 min.

and then wash 4 – 5 times with clean water to remove the soap. The backbulbs are air dried under shade. After air drying, the backbulbs are soaked in fungicide solution (Bavistn 2 g / litre) for 30 minutes. The backbulbs are taken out and dried in air under the shade (Fig. 4).

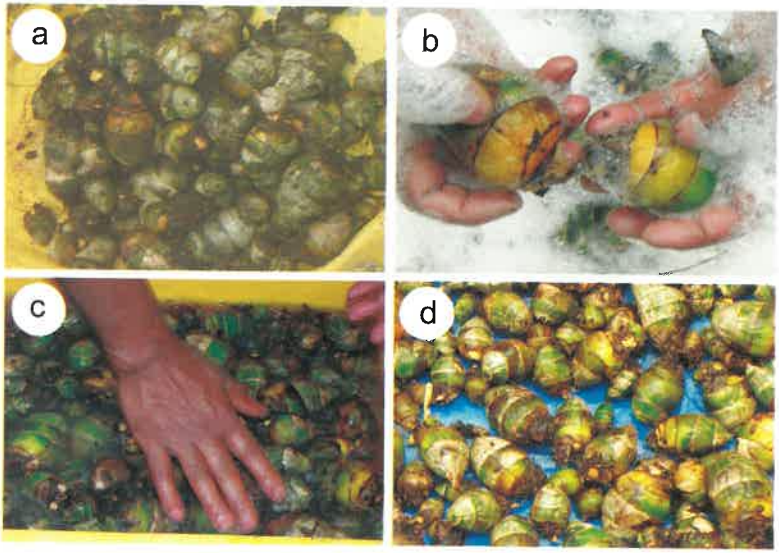

Fig 4. Cleaning of backbuls (a) backbulbs after removal of roots (b) washing of backbulbs (c) backbulbs soaked in Bavistin @ 2g/L (d) air drying of backbulbs

5.3 Backbulb treatment, packing and storage

Prepare a 100 ppm solution of 6 – BAP (6 – Benzyl amino purine) according to your requirement (for details of preparation see Appendix-I). Soak the backbulbs in the solution for 12 hours. Ensure that that all the bulbs are soaked well in the solution. Remove the backbulbs and air dry them. Once again ensure that no dried, decayed and injured backbulbs are in the lot.

Take the sawdust (we use *Cryptomeria japonica*,

Dhuppi) and moisten it so that it contains 50 - 60 % moisture. First of all, place moist saw dust in a polyethylene bag having nearly 5 % vents for the exchange of gases. Polyethylene sacs can also be used for the purpose. Insert the bacbulbs on sawdust as shown in Fig. 5. Fill the sawdust in the gaps loosely. Do not press, it will inhibit the air circulation. Again insert the backbulbs and fill it with moist sawdust. The process is repeated till the bag is filled. Once the bag is filled, tie it with a rope and label it properly by mentioning name of the cultivar and date of storage. Place the bags in cool (20 -25 ^{0}C) and dark place for 45 days. The backbulbs are likely to sprout within 45 days. Check once, if the backbulbs are not sprouted, leave them for some more time. The sprouting may differ with the cultivar and storage temperature. Keep in mind that backbulbs are stored in polyethylene bags/sacs only to induct the vegetative buds.

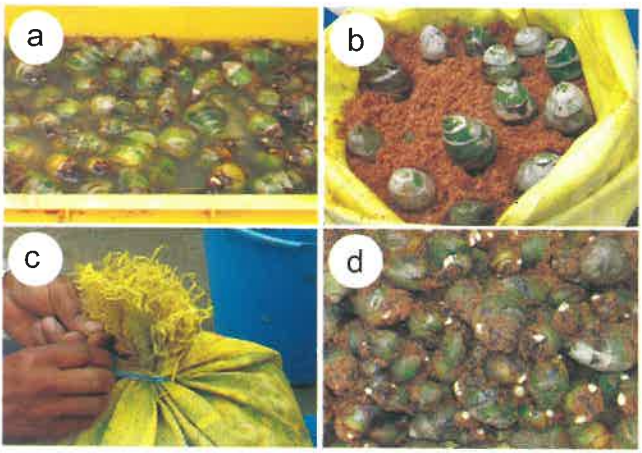

Fig 5. Packing of backbulbs after treatment (a) soaking of backbulbs in BAP solution (b) packing of backbulbs in moist sawdust and (c) tying and storage of backbulbs in dark (d) sprouted backbulbs 45 days after storage

6. HANDLING OF SPROUTED BACKBULBS

The back are likely to sprout within 45-60 days of treatment and packing depending upon cultivar and storage temperature. The sprouted backbulbs are planted separately for development of sprouted buds.

6.1 Unpacking and planting of sprouted backbulbs

Unpack the bag/sacs when the sprouts are small (Fig. 6). The delay in unpacking may result the backbulbs with larger and bent etiolated (white) shoots. These would have greater problem in their establishment. The sprouted backbulbs are separated for planting in beds and non - sprouted ones are packed again with sawdust. Now, the backbulbs are planted again in sawdust for the development of sprouts (Fig 7). Cover nearly the top of pseudobulb with moist sawdust.

Fig 6. Sprouted backbulbs showing shoot bud emergence from condensed as well as swollen regions of the backbulb 45 days after treatment

Fig 7. Planting of backbulbs in sawdust or any other suitable medium

6.2 Aftercare

Keep the sawdust moist by sprinkling water over it. Soon the sprouts start differentiating in to leaves and roots; plantlets are ready within 3 – 4 months. Though

the backbulbs contain enough food reserves to meet the requirement of developing sprouts, if required, they may be spayed with N: P: K mixture (10: 10: 10) @ 1 g/ litre at 15 days interval.

7. REMOVAL OF PLANTSLETS

Several buds (1-5) on the backbulbs may sprout simultaneously but only one grows very fast. It develops in to plantlet having 3-4 leaves and 2-4 roots within duration of 3-4 months. The plantlets are detached from the backbulbs and planted separately in pots. The backbulbs are recycled as described under para 7.2. The plantlets should be removed with the help of sharp, sterilised knife. These are removed in such a way that minimum injury is caused to the backbulbs. The wounded portion on backbulb and plant is treated with Metalaxyl (2 gm/ litre). Some cultivars are difficult to root and do not produce roots while sufficient leaves are present. Such plantlets are also removed from the backbulbs and planted separately to root out.

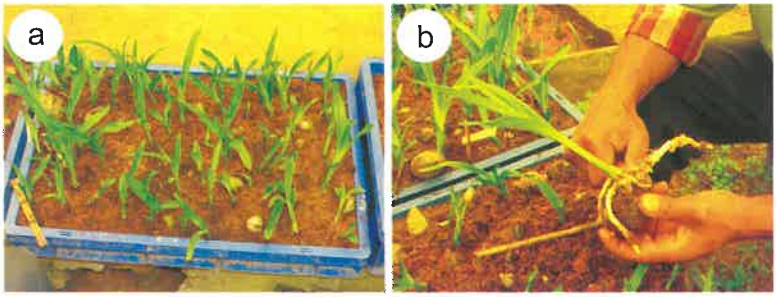

Fig 8. Removal of plantlets from the backbulbs (a) the backbulbs ready for removal of plantlets and (b) plantlets are being carefully removed from backbulbs with sterilized knife

7.1 Planting

The plantlets obtained from the backbulb are approximately 15 cm in height and may be planted in 10 cm diameter polybags/pots. A 5 cm thick layer of broken bricks is spread at the bottom (Fig.9). The plant is established by holding it over the polybag with one hand in such a way that roots are evenly spread inside the polybag and filling the potting mix with other hand. The upper portion of plantlet (the developing psedobulb) should remain exposed above the potting mixture (Fig.10). If needed, plant can be staked with small bamboo stick. The potting mixture can be made by mixing leaf mould, cocopeat, farmyard manure (FYM) and perlite in equal proportion. Every year the seedlings are transferred to a larger pot/ polybag. In the third year, plants can be transferred to a 15-20 cm diameter clay/plastic pots and in the fourth year transferred in 20-25 cm diameter pots. The plants are expected to

Fig 9. Pot is lined with brick pieces before planting

Fig 10. Plantlet after repotting and labeling

bloom this year but the best yield of flowers is obtained from fifth year onwards.

7.2 Recycling of backbulbs

The backbulbs from which the plantlets have been severed and unsprouted backbulbs can be recycled for the propagation. The diseased and shriveled backbulbs should be discarded and the remaining backbulbs should be washed in running tap water. Thereafter, soak the backbulbs in an aqueous solution of Bavistin @ 2 g /l for 30 min. After air drying the backbulbs, soak them in in solution containing 100 mg BAP/ litre for overnight; air dry the backbulbs and pack them in plastic bag preferably plastic sack with moist sawdust of Dhuppi, (Cryptomeria japonica). The plastic sacks preferably cement/fertilizer bags used for the packing of backbulbs as they ensure proper aeration and moist sawdust provides optimum moisture for sprouting of backbulbs. The packed pseudobulbs may be store cool dark place (20 - 25 °C) for sprouting. The rest of process is repeated as described earlier.

8. AFTERCARE

8.1 Fertigation

Cymbidiums are heavy feeder and like fertilizer to grow and bloom well. The plants should be fertilized with high nitrogenous fertilizer (30N:10P:10K) during growing season and high phosphorous containing fertilizers (10N:30P:10K) during flowering season. The fertilizers can be provided with irrigation water @ 1 g per litre once a week during summers and once in two weeks during winters. Over a period of time, increased concentration of

salts can build up, sometime to a level where the health of plant can be affected. It is essential that the pots should be thoroughly flushed out with clean water every month or so especially if regime of heavy fertilizer application has been followed.

8.2 Watering

The frequency and amount of water for irrigation depends on potting media and prevailing weather conditions. Plants require plenty of water from mid summer to early winter (April to July). Once the top of potting mix (2.5 to 5.0 cm) is dry the plants needs to be watered. The misting of plants underside of the leaves daily not only helps in maintaining temperature and humidity around the plants but also reduces the chances of red spider mite becoming established. The plants should be watered early in the morning so that the leaves are devoid of surface moisture before evening. Irrigation of plants with water containing salts should be avoided as build up salts may cause leaf tip to die back faster. The level of soluble salts in irrigation water should be 25-50 ppm but in any case it should not be more than 100 ppm.

8.3 Pest and disease management

Red spider mites: These are minute sucking type insects which suck the sap from under surface of newly emerged leaves and give rise a silvery appearance. The hot and dry weather favours in build up of red spider mite population. The incidence of these insects can be discouraged by providing humidity around the plants.

Scales: The scales stick on the under surface of leaves

and suck the sap from the cells. It is difficult to control these insects by applying chemical. These can be brushed off with the help of mild detergent solution.

Aphids: These are sucking insects accumulate on flowers and flower buds. They secrete honeydew on which shooty mould develops. The affected flower buds either fail to open or open in deformed shape. The shooty mould on flowers renders them unfit for market. Aphids can be controlled by spraying Imidochloprid or Azadirachitin.

Sludge and snails: These nocturnal creatures remain hiding in debris, under the benches or even in potting media during day and cause damage to plants by feeding on tender parts of the plants like buds, flowers and tender leaves during nights. They can be hand picked or trapped. The application of baits containing metaldehyde or iron phosphate is useful.

Tip burn: The leaves of Cymbidium first turn to light brown in colour and later dark brown. The exact reason of tip burn is not known but it is believed that accumulation of salts causes tip burn. Flush out the pots to remove the accumulated salts. The cultivars having *Cymbidium devonianum* in their back ground are susceptible to this disorder.

Black rot: A devastative disease of Cymbidiums caused by fungus which could get introduced through unsterilised potting ingredients especially leaf moulds. The water soaked symptoms develop on the aerial parts of the plants that turn brown. The affected shoots can be pulled out with slight pressure and rotting portion gives out a foul smell. The drenching of potting mixes with

Metalyxyl @ 2.0 g /litre is useful.

Viral diseases: Virus affected Cymbidiums show irregular or concentric yellow patches on the leaves and colour breaks in petal and sepals. The affected plants show stunted growth and produce less and poor quality of bloom on flower spike. This is a mechanically transmitted disease. Take every precaution for its spread

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APPENDIX-I

PREPARATION OF SOLUTION FOR BACKBULB TREATMENT

6 – BAP (6-benzylamino purine) is a synthetic analogue and effective as cytokinin. It is predominantly used in tissue culture of plants for induction of somatic embryos, adventitious shoots formation and callus proliferation. It is also used for breaking the bud dormancy in some perennial crops. It is non soluble in water but soluble in 0.1 N NaOH. The ppm denotes parts per million, hence for making 100 ppm solution, weigh 100 mg 6 – BAP and dissolve in 0.1N NaOH and make up the volume up to 1000 ml. The resultant solution would be of 100 ppm BAP. The amount of BAP required for making the required solution may be calculated accordingly. For example preparation of 5 liters of solution would be calculated as:

1 liter solution requires BAP = 100 mg
Hence, 5 liter solution would require = 100 mg x 5
= 500 mg

APPENDIX-II

SOURCES OF BACKBULBS AND BAP (6-BENZYLAMINO PURINE)

A. The backbulbs of the Cymbidiums may be obtained from following sources:

- M/S Himalayan Nursery,
  Takda Cantt. Takda, Darjeeling
  West Bengal; Ph. No. 09832559949
- Sri Govind Chhetri
  Village – Rambong; Bolck - Sukhia-Pokhari,
  Darjeeling, West Bengal
- Sri Milan Rai
  Tharbu Tea Estate, Mirik
  Darjeeling, West Bengal; Ph. No.09733274750
- Sri Dilip Rai
  President, Saureni Orchid Society, Mirik,
  Darjeeling, West Bengal
  Ph. No. 09593803259

B. The BAP (6-benzylamino purine) may be procured from the following sources:

- M/S Drugs & Chemicals
  P.N.B. Building, 2nd Floor(opposite to Meghdoot Cinema), H. C. Road, Siliguri – 734 001, Ph. No. 0353 - 2431109
- M/S Northern Scientific Mart
  Mia Garage Building (1st Floor), H. C. Road,
  Siliguri 734 001; Ph. 0353 -2432393

National Research Centre for Orchids
(Indian Council of Agricultural Research)
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