STANDARDISATION OF MEDIUM SUPPLEMENTS FOR SHOOT PROLIFERATION IN *Dendrobium*

By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree of

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STANDARDISATION OF MEDIUM SUPPLEMENTS FOR SHOOT PROPAGATION IN Rauvolfia

Dedicated to my parents ...

It's their hopes that
I strive to achieve today
and in their dreams lie
my tomorrows

DECLARATION

I hereby declare that this thesis entitled "**Standardisation of medium supplements for shoot proliferation in *Dendrobium***" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Vellanikkara,
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CERTIFICATE

Certified that this thesis entitled "Standardisation of medium supplements for shoot proliferation in *Dendrobium*" is a record of research work done independently by **Mr.R. Sudeep** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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We, the undersigned members of the Advisory Committee of **Mr.R.Sudeep** a candidate for the degree of Master of Science in Horticulture, agree that the thesis entitled "Standardisation of medium supplements for shoot proliferation in *Dendrobium*" may be submitted by Mr.R.Sudeep in partial fulfilment of the requirements for the degree.

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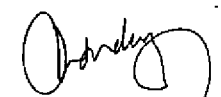

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ABBREVIATIONS

AC	- Activated charcoal
BA	- Benzyladenine
CH	- Casein hydrolysate
cv.	- cultivar
CW	- coconut water
2,4-D	- 2,4-dichlorophenoxy acetic acid
Na_2EDTA	- Disodium salt of ethylene diamine tetra acetic acid
GA/GA_3	- gibberellic acid
IAA	- indole-3-acetic acid
IBA	- indole-3-butyric acid
KC	- Knudson's C (1946) medium
KIN	- Kinetin; N^6-furfurl adenine
MS	- Murashige and Skoog's (1962) medium
NAA	- α-naphthalene acetic acid
ppm	- parts per million; mg/l
VW	- Vacin and Went's (1949) medium
v/v	- volume/volume
w/v	- weight/volume

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INTRODUCTION

Orchids, the most fascinating member of the modern flowers, belong to the largest family of flowering plants, orchidaceae, which comprises about 600-800 genera and 25,000-35,000 species (Chadha, 1992). They exhibit a wide range of diversity, from white through soft, delicate colours to the boldly gaudy and spectacular; from sweetly simple shapes to the sometimes incredibly fanciful and bizarre; from large plants with tremendous flowers to kinds with showers of small, gay blooms, and on down to miniatures. The blooms of most are long lasting, bewitchingly beautiful and are highly priced in the international market.

Orchids are represented in all the continents, except in Antarctica and majority of the cultivated orchids are native to tropical countries, occurring in their greatest diversity in humid tropical forests of South and Central America, Mexico, India, Ceylon, Burma, South China, Thailand, Malaysia, Philippines, New Guinea and Australia. About 1300 species have been reported from India (Maheshwari, 1980), scattered over N.E. Himalayas (600 species), N.W. Himalayas (300 species), Maharashtra (130 species), Andaman and Nicobar Islands (70 species) and Western Ghats (200 species). India, with its immense orchid wealth, is not contributing much to the international scenario and only marginal progress has been made in this respect.

The climatic condition of Kerala is highly congenial for growing orchids and is one of the leading States in India in the commercial cultivation of orchids. Tropical orchids like *Arachnis, Dendrobium, Cymbidium, Cattleya, Phalaenopsis* and *Vanda* thrive well under Kerala conditions. *Dendrobium*, one of the largest and most

diverse genera of orchids, with nearly 1000 species and innumerable hybrids, is widely exploited in orchid cut flower industry.

A major drawback in the orchid industry is the time taken for clonal propagation by the conventional method of back-bulb culture. This can be overcome by the technique of meristem culture which makes it possible to produce quality plants in large quantities by clonal multiplication.

Success in clonal propagation of *Dendrobium* through meristem culture has been reported earlier by Morel (1965). Studies were undertaken in the Department of Pomology and Floriculture also on the meristem culture of four species of *Dendrobium*, as influenced by media and explants. A multitude of factors determine the success of *in vitro* culture and it is well established that, apart from the inorganic constituents added to the media, some organic supplements also significantly help in the successful establishment of *in vitro* cultures. One argument against the use of organic supplements is that the response obtained is not stable since the constituents are undefined. On the other hand positive response of organic supplements in the tissue culture of orchids are also reported.

Hence it was felt desirable to explore the effect of different medium supplements on shoot proliferation in *Dendrobium*, which formed the main objective of this study.

REVIEW OF LITERATURE

The discovery in 1960's about the capacity of the apical meristems to regenerate new plants was a milestone in the development of orchid industry. Now *in vitro* cloning techniques have been standardised in orchids using different explants and tissue culture practices are followed throughout the world for orchid propagation. This major breakthrough triggered the multiplication of several quality plants and hybrids on a large scale, boosting the international orchid trade.

Irrespective of the quantum of material, in order that the tissue culture technique is efficiently exploited, it has to pass through a series of stages.

2.1. Explant selection

Selection of explant is very important in *in vitro* culture technique. All the tissues may not be equally potent in expressing themselves under *in vitro* conditions. Shoot tips, axillary buds, leaves, root tips, flower stalks etc. are usually employed for orchids. All the axillary buds may not develop into inflorescence, but some buds below the apex may remain quiescent due to apical dominance. These can be as or more responsive than shoot tip explants. *In vitro* multiplication through enhanced release of axillary buds has been standardised in *Dendrobium*. Sagawa (1961) and Scully (1965, 1966) used stem segments of *Phalaenopsis* with a node to initiate the culture. Johansen (1967) used both apical and lateral buds of *Cymbidium* and found that lateral buds were better than the apical buds. The percentage of survival of axillary buds of *Dendrobium phalaenopsis* was higher than that of the terminal bud explants, but the survival diminished with increasing height of nodes from which axillary buds were taken (Kim *et al.*, 1970). Intuwong and Sagawa (1973) could

produce plantlets directly in 40-55 days from nodal sections of *Calantha masuca, Dendrobium* and *Epidendrum radicans*. Node cultures were successfully used as a means for clonal propagation for *Dendrobium* by Ball and Arditti (1975) too.

Mosich *et al.* (1974) used axillary buds on node section from vegetative stems of *Dendrobium* cv. 'Hawaii' for producing shoots. Explants of 2-3 mm dimension taken from terminal and axillary buds of off shoots of *Dendrobium nobile* cv. 'Golden wave' and a related species were used (Intuwong and Sagawa, 1975), for obtaining protocorm like bodies (PLB's). Sagawa and Kunisaki (1982) reported that, with orchids of 12 genera, apical shoots or axillary buds were the best source of explants for successful clonal propagation, followed by inflorescences, leaves and roots. Wang (1988) used shoot meristems and axillary buds of *Cymbidium ensifolium* and *C. goeringii* for obtaining protocorm like bodies.

It was also reported that in Aranthera cv. 'James Storie', apical bud explants showed greater viability (averaging 87.5%) than axillary bud explants (47.9%). The proportion of diseased explants was also lower in apical (20.9%) than in axillary (52.1%) buds (Widiastoety, 1986).

2.2. Explant sterilization

The explant, before inoculating into the culture media, has to be sterilized to make it free from microorganisms present in the plant. During this procedure, care must be taken in that little damage is caused to the plant part. Usually strong oxidants like calcium hypochlorite or sodium hypochlorite are used to make the explant free from microbes. Mercuric chloride, chlorine water and alcohol are also used for sterilizing the explant. Ball *et al.* (1975) used 50% commercial bleach for *Phalaenopsis*

flower stalks. Sometimes, a combination of these are also employed. Raskauskas *et al.* (1989) reported that the best sterilization sequence for *Cymbidium* corm meristem was treatment in 70% ethyl alcohol for 10 minutes, in 0.1% diacid for 5 minutes and finally in 10% chloramine B for 10 minutes.

2.3. Culture media

The composition of the media play a vital role in determining the success of plant tissue culture. The most commonly employed media for orchids are Knudson's C (Knudson, 1946), Murashige and Skoog (1962), Morel (1965), Vacin and Went (1949) and White's (1943).

Bivins and Hackett (1969) reported that Knudson C medium was best for PLB formation and subsequent plantlet development in *Cymbidium* shoot tip culture. Mowe (1973) compared nine media (six of which were developed specially for orchid culture) for *Dendrobium* and found that five of the media were superior in promoting seedling growth. These were Chang's, Meyer's, Graeflinger's, Knudson's and Vacin and Went's basic media. Irawati *et al.* (1977) on the other hand reported that the best growth and survival rates were obtained in *Dendrobium* when cultured in modified Knudson C medium.

In trials with 12 media, Knudson C medium, modified with chelated iron, micro elements, coconut milk, banana and charcoal gave satisfactory seed germination and seedling growth with five genera, namely, *Cattleya, Cymbidium, Paphiopedilum, Phalaenopsis* and *Eulophidium* (Rosa *et al.*, 1977). Based on studies with *Aranda, Cattleya, Dendrobium, Ascocenda* cv. and hybrids in three different media, Fu (1978) found that the best medium for proliferation was that of MS salts.

Stewart and Button (1978) used Schenk and Hildebrandt medium and also a modified Ojima and Fujiwara medium for culturing root tips in *Epidendrum* spp. On both the media, callus formation followed a similar pattern. In another study all *Dendrobium* explants, cultured on modified Knudson C, Vacin and Went or modified Vacin and Went appeared expanded, but only the ones on modified Vacin and Went medium continued to grow (Fernando, 1979).

In *Dendrobium phalaenopsis*, seedling tip cultures produced better results in MS medium supplemented with both nitrate and ammonium than nitrate alone (Gandawidjaja, 1980). Kukulczanka and Wojciechowska (1983) used solidified Reinert and Mohr medium for developing callus which gave rise to protocorms allowing a clonal mass propagation of *Dendrobium phalaenopsis*.

Kerbauy (1984a) cultured micro inflorescence of *Oncidium varicosum* on liquid Knudson medium supplemented with 0.5 mg/l NAA under 16 hour photoperiod and 25°C and obtained PLB's. Paek and Chun (1985) obtained successful shoot formation from *Cymbidium elcapitan* cv. 'Conquistador'. Protocorms on KC medium were developed with 4 mM ammonium nitrate. Good formation and growth of roots were obtained on the same medium without added ammonium.

In *Cymbidium kanran* Makino, Hasegawa and Goi (1987) observed that shoot tip cultures produced rhizomes better when cultured in Linsmaier and Skoog medium containing organic nutrients at 1/5th of standard concentration plus 10 per cent coconut water. According to Shimasaki and Uemoto (1987), MS medium with a concentration of 1/8th gave best results in the axillary bud culture. Wang (1988) could obtain PLB's within 4-6 months from shoot meristems and axillary buds of

Cymbidium ensifolium and *C. goeringii* when cultured on MS or White media supplemented with 10 per cent CW and NAA 5 mg/l.

Gu and Yan (1989) cultured shoot rips of *Cymbidium grandiflorum* on VW liquid or solid medium with BA and 2,4-D. The protocorms developed were cut into several sections and cultured on the same liquid medium. Several protocorms formed on each section and each protocorm developed into a complete plantlet on KC solid medium. Seeds of *Dendrobium moschatum, D. farmeri, D. fimbriatum* var. Oculatum and *D. primulinum* were sown on four basal media, namely, VW, Burgeff, Nitsch and Nitsch and KC. Germination was 50-60 per cent higher in VW medium for *D. farmeri* and *D. primulinum* and in Nitsch and Nitsch medium for other two species, compared with the other media (Devi *et al.*, 1990). *Cymbidium forrestii* was clonally propagated in MS medium also using seed derived rhizomes as explants (Paek and Yeung, 1991).

2.4. Growth regulators

Plant growth regulators used in tissue culture include naturally occurring plant hormones such as Indole Acetic Acid, Gibberellins, Zeatin, Abscissic Acid, Ethylene etc. and also a number of synthetic chemicals that affect or control growth and development in plants. Their effects are determined by the kind of growth regulators, its concentration, presence or absence of other growth regulators and by the genetic make up and the physiological status of the target tissue.

When supplemented with 1 mg NAA or IAA, Bose and Mukherjee (1974) obtained shoots and roots simultaneously from callus cultures of Vanda, but with 2 mg, only roots developed with NAA and only shoots with IAA. About 80 per cent of

seedlings on the medium containing NAA developed thick healthy roots between 15 and 18 days after transfer, but shoot growth was less marked.

Yoneda *et al.* (1979) cultured protocorm of *Cattleya fabingiana* cv. Mikage on Hyponex basal medium modified with NAA, kinetin, 2,4-D, BA, GA or peptone. Plants showing the greatest average weight and height were obtained from the combinations of Hyponex + BA at 20 ppm + GA at 100 ppm and Hyponex + BA at 10 ppm + GA 100 ppm, respectively. A culture medium containing BA at 5 mg/l and NAA at 1 mg/l induced maximum proliferation of protocorms in *Cattleya* (Kusumoto, 1979). Shoot formation on propagules was stimulated mostly by a medium containing BA at 0.1 mg/l and 2,4-D at 0.1 mg/l. Kusumoto (1979) further reported that the medium most effective for plantlet growth of *Cattleya* contained 0.1 to 1 mg/l kinetin and 1 to 5 mg/l NAA or 0.1 to 0.5 mg/l kinetin and 0.1 mg/l 2,4-D

According to Kim and Kako (1982), *Cymbidium* x *Sazanami* cv. Haru-no-umi shoot apices produced many adventitious roots but no shoots when grown in MS medium with IAA, NAA, 2,4-D, BA, GA_3 and ABA. BA encouraged the formation of PLB's and shoots. Greisbach (1983) cultured inflorescence sections of *Phalaenopsis* cv. Betty Hausermann in MS medium with isoleucine (13 mg/l) and t -cinnamic acid (100 mg/l). Protocorm like bodies were obtained when the basal section of the shoots formed were grown with NAA (0.5 mg/l) + BA (2 mg/l) and these PLB's proliferated and when kept on a hormone free medium, differentiated and formed plants.

Kim and Kako (1984) further reported that shoot apices of *Cymbidium* x *Sazanami* cv. Haru-no-umi, when cultured on solid MS medium with BA 0.1 ppm, enhanced and NAA at 0.5 ppm inhibited leaf elongation, differentiation and forma-

tion of PLB's. Hasegawa *et al.*(1985) cultured rhizome tips of *Cymbidium faberi* in modified Kyoto solution supplemented with BA and found that the rate of shoot formation was significantly affected by BA concentration. According to Jambor-Benezer *et al.* (1986), buds from inflorescence stem of *Phalaenopsis amabilis* soaked in 100 ppm BA for 1-3 hours and cultured *in vitro* gave best results.

Flower stalk sections of *Phalaenopsis* and *Doritaenopsis* when cultured by Lin (1986) on modified VW medium with BA 1 or 5 mg/l, resulted in formation of PLB's on epidermis and cut surface in 30 days. Adventitious buds were formed and grew into plantlets after 60 days of culture.

Gu *et al.* (1987) reported that shoot tips from lateral buds of *Cymbidium* pseudobulbs formed multiple PLB's on a modified VW medium containing 1 mg/l BA. When transferred to VW medium supplemented with 0.4 mg/l BA, the PLB's formed shoots and eventually plantlets. In another study, plantlets were produced from root tips of *Bletilla striata* and *Cleisostoma fordii* cultured on a modified MS medium containing 1.0 mg NAA and BA 0.2 mg/l. Root tips elongated on a medium with a high cytokinin : auxin ratio and formed callus when the ratio was low (Yam and Weatherland, 1991).

Shimasaki and Uemoto (1991) reported that apical flower buds of *Cymbidium goeringii*, excised from inflorescences, when cultured on modified MS medium, supplemented with 0.1 mg BA and 10 mg NAA/l, was optimal for initiating rhizome development and subsequent plantlet regeneration.

2.5. Medium supplements

Medium supplements include certain complex organic additives which in-

fluence the establishment and growth of *in vitro* cultures. Apart from the inorganic constituents of the media which give consistent results, the organic medium supplements often do not give any definite results. Adenine, Adenine sulphate, Casein hydrolysate, Yeast extract, Peptone, Coconut water, Banana homogenate etc. are some of the complex substances added to the media, the salient works on which are reviewed under broad groups.

2.5.1. Cytokinin related substances

2.5.1.1. Adenine

Tanaka and Sakanishi (1985) reported that leaf segments of *Phalaenopsis amabilis* produced PLB's when they were excised from proximal unexpanded parts of the youngest leaf and cultured on a Murashige and Skoog medium supplemented with 1 ppm NAA, 10 ppm adenine and 10 ppm BA.

2.5.2. Amino acid supplements

2.5.2.1. Casein hydrolysate

Davidonis and Knor (1991) cultured shoot explants of *Vanilla planifolia* in MS medium with NAA, BA, 5 mg thiamine, 500 mg casein hydrolysate and 1000 mg inositol/l which resulted in good callus formation. Agrawal *et al.* (1992) devised a method of clonal propagation for *Vanilla walkeriae* with MS medium supplemented with 0.5 mg kinetin, 1.0 mg BA and 1000.0 mg CH/l which supported rapid proliferation of multiple shoots from stem node segments.

2.5.2.2. Yeast extract

According to Kusumoto (1979), yeast extract retarded organogenesis and

accelerated-propagation of protocorms in *Cymbidium*. Chung *et al.* (1985a) could successfully germinate *Cymbidium kanran* asymbiotically on a Murashige and Skoog medium with L-arginine, aspartic acid, yeast extract and sucrose which also gave good rhizome and seedling growth. Pseudobulb segments from axenic seedlings of *Dendrobium chrysanthum* were cultured on MS medium and shoots were regenerated only on media supplemented with yeast extract or urea. Activated charcoal proved beneficial for luxuriant growth and complete plantlets were formed in media with yeast extract and NAA.

2.5.2.3. Peptone

The most suitable medium for seed germination of *Aerides japonicum* was that with 3 g hyponex, 4 g peptone and 30 g sucrose, according to Chung *et al.* (1985b). After four weeks of subculture, meristem tissues of *Cymbidium vallambrosa* and cv. Vieu x Rose "Dell park" showed highest fresh weight and good shoot and root development on a media with pearl barley and peptone (Kukulczanka *et al.*, 1987; Kim *et al.*, 1988) reported that in *Cymbidium kanran* shoots were formed on a hyponex medium with peptone and BA, but growth and rooting were reduced. Addition of NAA and charcoal increased the growth and rooting but shoot formation was reduced. Kukulczanka *et al.* (1989) further reported that in Vuylstekeara 'Cambria' orchid, peptone stimulated meristematic tissue growth and adventitious shoot formation but it did not reduce root formation. The best regeneration and organogenesis were noted on MS medium provided that peptone interacted with Cytokinin (BA 0.2 mg/l and auxin (NAA 0.2 mg/l). According to Kim *et al.* (1990) protocorm of the epiphytic orchid *Aerides japonicum* cultured with NAA and BA showed the best growth on a medium with 3 g hyponex, 4 g peptone, 40 g sucrose, 35 g banana and

2 g charcoal/l. Based on another study, Vij *et al.* (1991) reported that the morphogenetic response of floral buds of *Dendrobium crepidatum* and *D. pierardi* when cultured *in vitro* in Mitra *et al.* medium, supplemented with 2 g/l peptone/yeast extract, 1-2.5 mg/l IAA/NAA and 1 mg/l BAP/kinetin, the older buds matured into flowers, whereas the younger ones reverted to vegetative growth.

2.5.3. Organic supplements

2.5.3.1. Coconut water

Superficial cell layers of the Singapore orchid (*Vanda hookeriana* x *Vanda teres*) became meristematic and formed protocorms in White's medium supplemented with coconut water or kinetin (4 ppm) or both (Payawal, 1976). The inner cortex, peripheral to the vascular strands, produced adventitious roots in the medium supplemented with coconut water and NAA. Kusumoto (1980) reported that in KC basal solution containing 15 g/l agar, *Cymbidium* protocorms proliferated best when 10-25% coconut milk was added. The highest increase in fresh weight was attained with 10% coconut milk.

Goh and Tan (1982) could obtain callus and protocorms of Renantanda orchid hybrid when cultured in VW medium with coconut water. Rapid proliferation of PLB's and plantlet formation in shoot tip cultures of *Dendrobium* cv. 'Jacqueline Thomas White' was observed by Soediono (1983) in VW medium with 15 per cent CW and 10 mg/l NAA. In *Oncidium varicosum* callus proliferation was highest in root tips cultured on a modified Vacin and Went medium with 15 per cent coconut water and 5 or 10 mg/l NAA. Callus masses transferred to VW medium with 15 per cent CW produced new PLB's (Kerbauy, 1984b). According to Homma and Asahira (1985) internodal sections of *Phalaenopsis* flower stalk produced PLB's on a medium

containing Thomale's macroelements, Ringe and Nitsch's minor elements and organic supplements, together with 10 per cent coconut milk, 5 mg/l NAA and 20 mg/l BA. Paek *et al.* (1989) reported that *Cymbidium* seeds germinated asymbiotically in Knudson C medium with 10 per cent coconut milk. According to Madhuri Sharon and Vasundhara (1990) shoot meristems of *Dendrobium* 'Joannie Ostenhault' initiated callus in liquid VW medium with 15 per cent coconut milk. Green protocorms were differentiated in four weeks and these failed to develop further unless transferred to agar medium with 15 per cent coconut water and 2 per cent sucrose. It was observed by Lam *et al.* (1991) that XE medium containing coconut water alone or with 2ip were equally effective in inducing the protocorm sections of a hybrid *Phalaenopsis* (cv. Michelle x Michelle) to form new protocorms.

2.5.3.2. Banana pulp

Cheah and Sagawa (1978) could successfully propagate Aranda Wendy Scott cv. Greenfield and Aranthera James Storei on VW medium supplemented with green banana 50 g/l.

In another study, Kerbauy (1984b) transferred PLB's of *Oncidium varicosum* to the medium supplemented with 60 g/l banana, 10 g/l sucrose, 0.8 per cent agar, 1 g/l activated charcoal and 27.8 mg/l Fe EDTA and flowering occurred after 8-9 months of culture when compared with plants growing under natural growing conditions, which usually flower in the 3rd year after germination.

Kim *et al.* (1990) reported that protocorms of the epiphytic orchid *Aerides japonicum* showed best growth when cultured on media containing 3 g hyponex, 4 g peptone, 40 g sucrose, 35 g banana, 2 g charcoal and 0.01 mg/l BA.

When protocorm sections of hybrid *Phalaenopsis* (cv. Michelle x Michelle) were cultured on XE medium, cytokinin alone proved to be lethal to explants, but the inclusion of banana homogenate reversed this effect, although it did not enhance protocorm production (Lam *et al.*, 1991).

2.5.3.3. Potato extract

Harvais (1982) reported that in *Cypripedium reginae* 1 per cent agar medium supplemented with 5 per cent potato extract gave the best germination and growth (for two years) when used with a modified Pfeffer solution containing 1400 mg ammonium nitrate, 19 mg ammonium citrate, 2 per cent dextrose, 10 mg niacin, 5 mg calcium pantothenate, 5 mg thiamine HCl, 1 mg kinetin and 0.1 mg NAA per litre.

2.5.3.4. Medicinal plant extracts

The essential oil from *Ixora coccinea* was reported (Yadav, 1989) to be highly active in inhibiting the growth of *Bacillus subtilis* and moderately active against *Salmonella richmond, Salmonella stanley* and *Aspergillus flavus*. Thakur *et al.* (1989) reported that six oils obtained from *Oscimum gratissimum, O. viride, O. canum, O. basilicum, Cymbopogon winterianus* and *C. martinii*, having predominantly thymol, linalool, methyl chavicol, citronellol and geranol respectively, could effectively check the growth of fungi in culture. The essential oils from foliage of *Cymbopogon martini* var. motia (Palmarosa), seeds of *Pimpinella anisum* (aniseed) and roots of *Vetiveria zizanioides* (vetiver) showed 70-80 per cent growth inhibition of *Aspergillus niger, A. flavus, Fusarium oxysporum* and *Penicillium* spp. (Gangrade *et al.*, 1991).

2.5.4. Carbon source

According to Hew *et al.* (1988), when apical meristems of *Dendrobium* were cultured in a VW liquid medium, fructose was more readily utilised than other sugars. Relative growth rate increased with increasing sugar concentration in the media and was highest with fructose as the carbon source. Hew and Mah (1989) reported that there was no difference in the preference for sugars when differentiated and undifferentiated tissues of *Dendrobium* were cultured in VW medium with 2 per cent glucose, 2 per cent fructose or 1-2 per cent sucrose. But fructose provided the best carbon source for both the tissues. In another study seeds of *Cymbidium elegans* and *Coelogyne punctulata* gave the best germination and seedling growth when sown on Knudson C medium with 20 or 30 g/L D-fructose, D-glucose or sucrose (Sharma and Tandon, 1990). Moderate growth was obtained with trehalose, maltose, D-mannose or raffinose.

2.6. Transplanting

Tissue culture plantlets are very tender and their transfer from the artificial environment of the culture vessel to the free living existence of a green house or similar environment and their establishment outside culture conditions is of great importance. The plantlet must be slowly allowed to acclimatise with the environmental conditions prevailing outside the culture vessel. The environmental conditions are so controlled that there is successful establishment of the *in vitro* grown plantlet.

Anderson (1980) reported that thorough washing of the plantlets to remove traces of nutrient medium and sterilizing the potting mixture eliminated serious problems of fungal infusion. Application of previcur-N (Propamocarb) 0.15-0.25 per cent

before transplanting *Cymbidium* hybrids from tissue culture increased plant survival and adjustment to green house conditions (Zimmer *et al.*, 1981). Ziu (1986) reported that the success in acclimatization of *in vitro* cultured plants is dependent not only on the post-transfer growth condition, but also on the pre-transfer culture condition.

Soebijanto and Widiastoety (1987) reported that vegetative growth was stimulated when Aranda seedlings growing in 12 cm pots were treated with IAA (0-60 mg/l) and the best results with regard to root number and plant weight were obtained with the highest concentration tried.

According to Weinright (1988), tissue cultured plants are very poorly adapted to resist the low relative humidity, higher light levels and more variable temperatures prevailing outside.

Kumar (1991) reported that maximum survival and optimal growth of shoots and roots of *Dendrobium* hybrid seedlings occurred in media having rubber seed husks, coconut shell pieces and gravel.

Thomas and Thomas (1992) transplanted Odontoglossums and their hybrids into perlite and rockwool media and found that orchids in perlite made slower growth initially, but after a year started to grow more rapidly with extensive root system.

MATERIALS AND METHODS

The present study, namely, standardisation of medium supplements for shoot proliferation in *Dendrobium* was carried out at the plant tissue culture laboratory attached to the All India Co-ordinated Floriculture Improvement Project (AICFIP), College of Horticulture, Vellanikkara, during 1991-93. The details of the experiment methodology, materials and the analytical techniques employed are presented in this chapter.

In vitro shoot proliferation by the enhanced release of axillary buds (Murashige, 1974) was attempted in the present investigation. It comprises of different stages which are illustrated in the fig. 1.

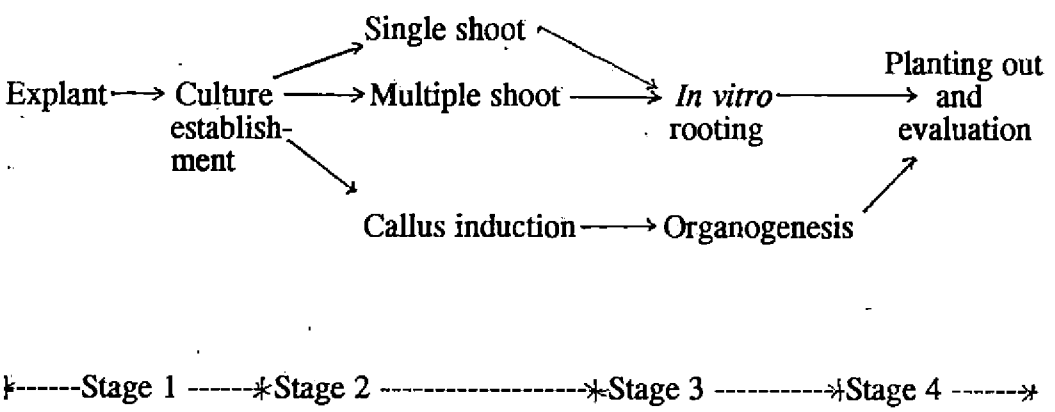
3.1. Explant

Dendrobium nobile is an important member in the genus and is often taken as a parent in many of the hybridization programme. Preliminary studies on the micropropagation of this species have already been conducted in the Department (Lakshmidevi, 1992). Based on this nodal segments from the young shoots and offshoots (keikis) of *Dendrobium nobile* were employed for the study.

3.1.1. Collection and preparation of the explant

Healthy plants in the orchidarium attached to the AICFIP at the College of Horticulture, Vellanikkara were selected for collection of explants. Shoots were excised from the plants using surgical blades and were brought to the laboratory immediately. All the leaves were removed and stem segments washed thoroughly in running tap water so as to remove all the dust and chemicals adhering to them.

Fig. 1. *In vitro* cloning procedure in orchids



Stage 1. Physiological preconditioning of the explant and explant establishment
Stage 2. Induction of axillary shoots/callus mediated organogenesis and rapid multiplication
Stage 3. *In vitro* rooting and acclimatization
Stage 4. Planting out and evaluation

Teepol solution was used for cleaning the stem segments. The stem segments were then immersed in emisan 0.1 per cent for 30 minutes, washed thoroughly with distilled water and then reduced to 2-3 noded sections. These nodal sections were scrubbed with cotton wool wetted with alcohol, followed by a 20 minutes dip in teepol solution to ensure effective surface sterilization.

3.1.2. Surface sterilization of the explant

Surface sterilization was carried out under perfect aseptic conditions in a laminar air flow cabinet. The 2-3 noded sections were put in 0.1 per cent mercuric chloride (sterilant) for 10 minutes. After that, the explants were rinsed four times with sterile distilled water to remove traces of sterilant from the surface. The explant size was further reduced to 1.0-1.5 cm by making two oblique incisions into the stem, one above and one below the axillary bud. The excess moisture remaining on the explants was removed by keeping the explants on sterile filter paper placed on sterile petri dishes.

3.2. Culture media

Two media were employed for the study, namely, MS (Murashige and Skoog, 1962) and VW (Vacin and Went, 1949). The composition of the media are given Appendix-I.

3.2.1. Media preparation

MS medium was prepared according to the standard procedures laid out by Gamborg and Shyluk (1981). Required quantities of major and minor nutrients were first dissolved in double glass distilled water and these stock solutions were stored in

amber coloured bottles under refrigerated conditions. The chemicals used for preparation of the media were of analytical grade from British Drug House (BDH), Sisco Research Laboratories (SRL), Merck or Sigma. The nutrient stock solutions were prepared fresh monthly and the vitamins, aminoacids and phytohormones were prepared fresh weekly. Specific quantities of the stock solutions and phytohormones were pipetted out into a 1000 ml standard flask. To this, the required amount of sucrose and inositol were added and the volume was made up with double glass distilled water. Then the pH of the made up solution was checked with a pH indicator paper and was adjusted to 5.8. For adjustment of pH one per cent NaOH/HCl was employed. After bringing the solution to the desired pH, agar was added and the final volume of the medium was made upto 1000 ml.

Agar was then dissolved in the media by heating the media at a temperature of 90-95°C. When all the agar had completely dissolved, the medium was poured hot into culture vessels which were priorly washed thoroughly, rinsed in distilled water and oven dried. The culture vessels were then closed tightly using sterilized non-absorbent cotton wool plugs.

For preparing VW media, the required quantity of chemicals were taken and were dissolved fresh in double glass distilled water. Sucrose and phytohormones were then added and the volume was made up after adjusting pH. Agar was then added and the final volume made up.

After preparation of media, sterilization of media was done by keeping the media in pressure cooker at a pressure of 1.1 kg/cm^2 at 121°C for 20-30 minutes. After sterilization, the culture tubes were immediately transferred to an airconditioned culture room.

3.3. Explant inoculation

The explants were inoculated under aseptic conditions in a 'Klenzaids' Laminar air flow cabinet. For this, the neck of the culture vessel was brought near the flame from a gas burner, cotton plug was removed, and the neck flamed over the gas burner. Then, using sterilized forceps, the explant was quickly transferred to the culture vessel and once again the neck of the culture vessel was flamed and then plugged tightly with cotton plug.

In order to provide the optimum conditions for growth and establishment of the explant, the culture vessels were transferred to a culture room where the temperature was maintained at 27 $\pm$ 2°C. White fluorescent tube lights were provided to meet the light requirements of the explant. Photo period was fixed as 16 hours per day which was regulated by a diurnal timer.

3.4. Culture establishment and shoot induction

Studies were conducted to find out the best medium for culture establishment and shoot initiation. The trials were carried out in the two media with NAA and BA each at 3 levels; NAA at 1.0, 1.5 and 2.0 mg/l and BA at 2.5, 3.0 and 3.5 mg/l. The number of days taken for bud initiation and elongation were recorded.

3.5. Shoot proliferation

The elongated buds were excised carefully under aseptic conditions and cultured to induce multiple shoots. Trials were conducted with VW and ½ MS media having NAA and BA, each at 2 levels; NAA 1.0 and 2.0 mg/l and BA at 3.0 and 5.0 mg/l. The percentage of cultures developing shoots and observations such as number

of shoots produced per culture, length of shoot and number of leaves per plant at 2 week interval were recorded.

3.6. Establishment of a continuous culture

The multiple shoots produced were excised carefully under aseptic conditions and subcultured into VW and ½ MS media with NAA 2.0 ppm and BA 5.0 ppm for further proliferation of shoots.

3.7. Effect of medium supplements on shoot proliferation

Studies were conducted to find out the effect of different supplements on shoot proliferation in ½ MS and VW media. The levels of different medium supplements tried are detailed below.

3.7.1. Cytokinin related substances

Adenine	20 ppm, 40 ppm
Adenine sulphate	50 ppm, 100 ppm

3.7.2. Amino acid supplements

Casein hydrolysate	50 ppm, 100 ppm
Yeast extract	1%, 2%
Peptone	20 ppm, 40 ppm

3.7.3. Organic supplements

Coconut water	5%, 10%
Tomato juice	5%, 10%
Banana (Robusta) pulp	5%, 10%
Orange (Mandarin) juice	5%, 10%

Palm liquid endosperm	2%, 4%
Potato extract	5%, 10%
Spathodea bud liquid	2%, 5%
Spathodea flower extract	2%, 5%
Oscimum leaf extract	5%, 10%

3.7.4. Carbon sources

Sucrose	3%, 5%
Glucose	3%, 5%
Table sugar	3%, 5%
Mannitol	3%, 5%

3.7.5. Observations

The following observations were recorded at 2 week intervals upto 6 weeks. However the data recorded after 6 weeks alone were considered for analysis.

i) Percentage of cultures developing shoots

ii) Number of shoots produced per culture

iii) Length of shoot

iv) Number of leaves per shoot

3.8. *In vitro* rooting

Studies on *in vitro* rooting were carried out in VW, KC and ½ MS medium. Individual shoots having 2-3 cm length excised from the shoot proliferating cultures were used. Trials were conducted to study the effect of different carbon sources like sucrose, glucose and table sugar (each at 3% level) on *in vitro* rooting.

The percentage of cultures developing roots and number of days taken for root initiation were recorded.

3.9. Planting out and acclimatization

After proper root development under *in vitro* conditions, the plantlets were taken out from the tubes taking care not to damage any roots. The nature of roots and the number of roots per plant were recorded. The roots were then washed gently under running tap water to remove the adhering medium. The plantlets were then transferred to small pots of 8 cm diameter and to styrofoam trays filled with potting media.

3.9.1. Potting media

The following potting media were tried.

i) Perlite

ii) Cocoa peat

iii) Brick-charcoal mix (1:1 ratio)

iv) Coconut husk

v) Charcoal

vi) Sphagnum moss

The potting media were used after autoclaving at a pressure of 1.1 kg/cm^2 at 121 $^{\circ}C$ for 30 minutes.

Coconut husks were cut into small pieces of one inch size, longitudinally split and after initial wetting of media, the plantlets were kept inside and tied. Plants were sprayed with Bavistin 0.1 per cent on alternate days.

3.9.2. Control of temperature and humidity

The temperature and humidity control during acclimatization was achieved by keeping the plantlets in a chamber covered with polythene sheets. Humidity was maintained by spraying cold water into the chamber daily using a hand sprayer. The temperature was maintained at 26 $\pm$ 2°C.

3.9.3. Observations

The following growth parameters were recorded at 2 week intervals.

i) Height of the plant

ii) Number of leaves

iii) Percentage survival

3.10. Statistical analysis

The data relating to the different aspects of the study were statistically analysed as per the techniques described by Panse and Sukhatme (1985).

RESULTS

The results generated from the study pertaining to the effect of different medium supplements on shoot proliferation in *Dendrobium*, conducted at the Plant tissue Culture Laboratory attached to the All India Co-ordinated Floriculture Improvement Project, College of Horticulture, Vellanikkara, are presented in this chapter.

4.1. Culture establishment

Data pertaining to the effect of different levels of NAA and BA in ½ MS and VW media, on culture establishment, are furnished in Table 1 and 2, respectively.

4.1.1. Effect in ½ MS medium

All the combination of NAA and BA tried were effective in inducing bud initiation (Table 1).

Significant difference could be observed among the treatments. Treatment T_2 (NAA 1.0 ppm + BA 3.0 ppm) recorded the least number of days for bud initiation (32.00) which was on par with T_1 (NAA 1.0 ppm + BA 2.5 ppm), T_3 (NAA 1.0 ppm + BA 3.5 ppm), T_4 (NAA 1.5 ppm + BA 2.5 ppm) and T_5 (NAA 1.5 ppm + BA 3.0 ppm) and was significantly superior to all other treatments. Treatment T_{10} (½ MS basal) took the maximum number of days (56.33) for bud initiation.

For bud expansion, T_3 (NAA 1.0 ppm + BA 3.5 ppm) took the minimum number of days (35.67) which was on par with T_1 (NAA 1.0 ppm + BA 2.5 ppm)

and T_2 (NAA 1.0 ppm + BA 3.0 ppm) and was significantly superior to all other treatments. T_6 (NAA 1.5 ppm + BA 3.5 ppm) recorded the maximum number of days for bud expansion (53.33) which was on par with T_9 (NAA 2.0 ppm + BA 3.5 ppm) and was significantly different from all other treatments.

4.1.2. Effect in VW medium

Data recorded on bud initiation and bud expansion to VW medium are presented in Table 2.

Significant difference could be observed among treatments. Treatment T_2 (NAA 1.0 ppm + BA 3.0 ppm) recorded the minimum number of days for bud initiation (31.67) which was on par with T_1 (NAA 1.0 ppm + BA 2.5 ppm) and T_3 (NAA 1.0 ppm + BA 3.5 ppm) and was significantly superior to all other treatments. T_9 (NAA 2.0 ppm + BA 3.5 ppm) recorded the maximum number of days (57.33 days) which was on par with T_{10} (control) and was significantly different from all others.

With regard to bud expansion also, T_2 recorded the minimum number of days (25.33) which was on par with T_1 and T_3 and was significantly superior to all others. On the other hand, T_7 (NAA 2.0 ppm + BA 2.5 ppm) recorded the maximum number of days (52.0) for bud expansion which was on par with T_{10}, T_9 and T_8 (NAA 2.0 ppm + BA 3.0 ppm) and these were significantly different from other treatments.

4.2. Effect of media supplements on shoot proliferation

The elongated buds were used for inducing multiple shoots. Of the two

Table 1. Effect of NAA and BA on the culture establishment in Dendrobium
Basal medium - ½ MS

Treatment	Period (days) for culture establishment	
	Bud initiation	Bud expansion
T_1 - NAA 1.0 ppm + BA 2.5 ppm	33.00	36.67
T_2 - NAA 1.0 ppm + BA 3.0 ppm	32.00	38.00
T_3 - NAA 1.0 ppm + BA 3.5 ppm	34.33	35.67
T_4 - NAA 1.5 ppm + BA 2.5 ppm	35.67	42.00
T_5 - NAA 1.5 ppm + BA 2.5 ppm	36.00	47.00
T_6 - NAA 1.5 ppm + BA 3.5 ppm	39.67	53.33
T_7 - NAA 2.0 ppm + BA 2.5 ppm	42.00	48.67
T_8 - NAA 2.0 ppm + BA 3.0 ppm	43.33	44.00
T_9 - NAA 2.0 ppm + BA 3.5 ppm	46.33	50.00
T_{10} - Control (Basal medium)	56.33	47.67
CD (0.05)	4.20	4.26
SEm±	2.02	2.04

Values are taken as average of three replications

Table 2. Effect of NAA & BA on the culture establishment in Dendrobium

Basal medium - VW

Treatment	Period (days) for culture establishment	
	Bud initiation	Bud expansion
T_1 - NAA 1.0 ppm + BA 2.5 ppm	32.67	28.00
T_2 - NAA 1.0 ppm + BA 3.0 ppm	31.67	25.33
T_3 - NAA 1.0 ppm + BA 3.5 ppm	36.00	29.00
T_4 - NAA 1.5 ppm + BA 2.5 ppm	38.00	37.00
T_5 - NAA 1.5 ppm + BA 2.5 ppm	42.33	31.67
T_6 - NAA 1.5 ppm + BA 3.5 ppm	45.33	41.33
T_7 - NAA 2.0 ppm + BA 2.5 ppm	45.33	52.00
T_8 - NAA 2.0 ppm + BA 3.0 ppm	46.33	48.33
T_9 - NAA 2.0 ppm + BA 3.5 ppm	57.33	48.67
T_{10} - Control (Basal medium)	55.00	51.33
CD (0.05)	5.29	4.30
SEm±	2.53	2.06

Values are taken as average of three replications

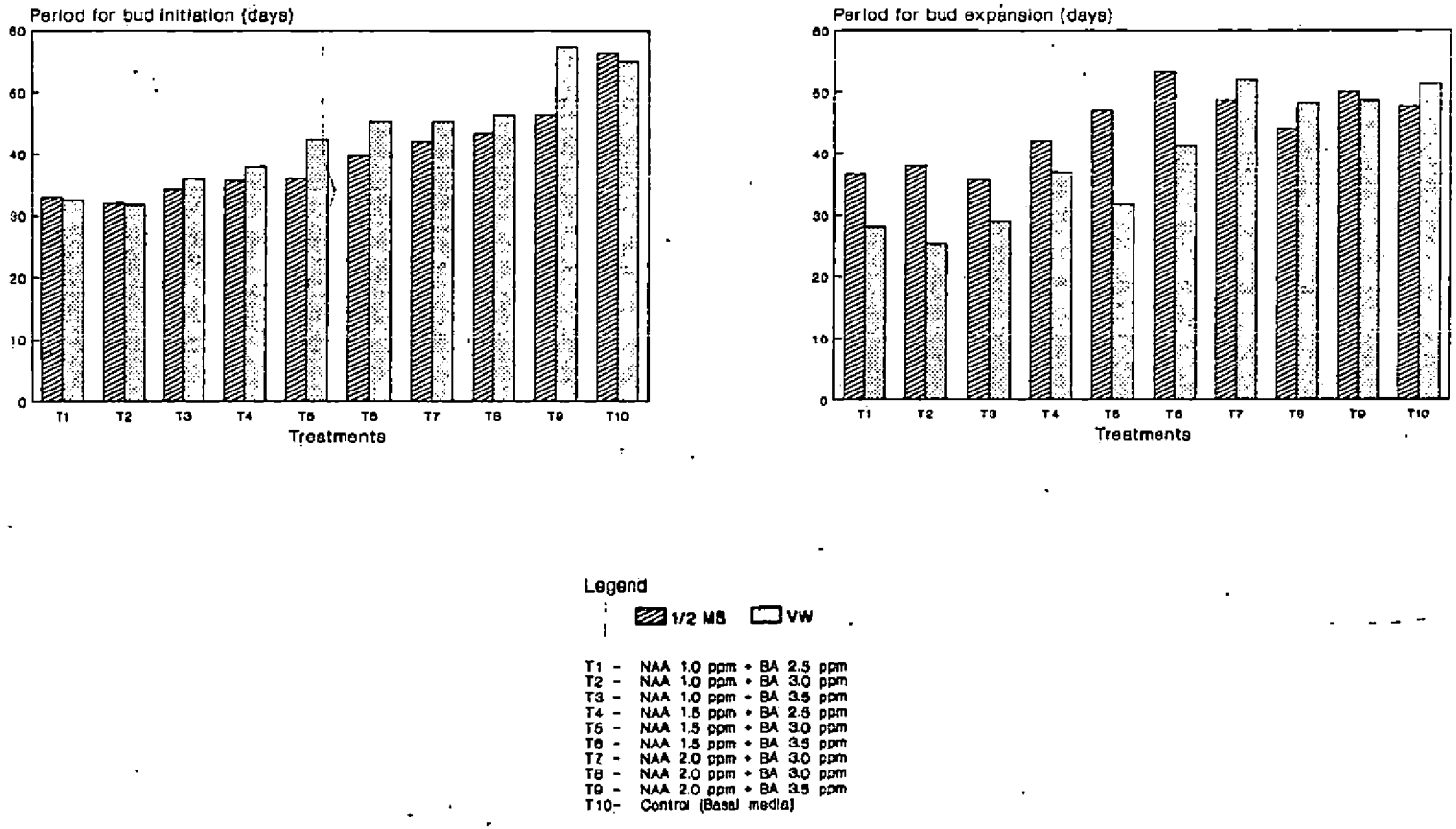


Fig.2 Effect of NAA and BA on the culture establishment in *Dendrobium*.

concentrations of auxins (NAA 1.0 and 2.0 ppm) and cytokinins (BA 3.0 and 5.0 ppm) tried, a combination of NAA 2.0 ppm and BA 5.0 ppm produced maximum number of shoots both in ½ MS medium (6.33) and VW medium (3.67) in a culture period of 6 weeks.

Studies were conducted to find out the effect of different media supplements on shoot proliferation in ½ MS and VW media either alone or in combination with NAA 2.0 ppm and BA 5.0 ppm. These treatments were also compared with the basal media (½ MS or VW) and the best combination of NAA and BA identified (2.0 ppm and 5.0 ppm, respectively), which were taken as control.

4.2.1. Cytokinin related substances

The influence of cytokinin related substances, viz., adenine, adenine sulphate and their combinations with NAA and BA were tried in ½ MS and VW media and the results are presented below.

4.2.1.1. Effect in ½ MS medium

Data recorded on the effect of cytokinin related substances in ½ MS medium are presented in Table 3.

On considering the number of shoots produced after 6 weeks, T_{10} (NAA 2.0 ppm + BA 5.0 ppm) produced the maximum number (6.33) and was significantly superior to T_1 (adenine 20 ppm), T_2 (adenine 40 ppm), T_3 (adenine 20 ppm + NAA 2.0 ppm + BA 5.0 ppm), T_4 (adenine 40 ppm + NAA 2.0 ppm + BA 5.0 ppm) and T_9 (control) and was on par with all other treatments. T_9 produced the minimum number of shoots (1.4) and was on par with all treatments, except T_{10} and

Table 3. Effect of cytokinin related substances on shoot proliferation in Dendrobium

Basal medium - ½ MS

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Adenine 20 ppm	100	2.67	3.60	3.00
T_2 - Adenine 40 ppm	100	3.00	3.43	4.00
T_3 - Adenine 20 ppm + NAA 2.0 ppm + BA 5.00 ppm	100	2.33	2.97	2.67
T_4 - Adenine 40 ppm + NAA 2.0 ppm + BA 5.0 ppm	100	2.00	3.50	2.67
T_5 - Adenine sulphate 50 ppm	100	4.00	3.93	4.33
T_6 - Adenine sulphate 100 ppm	100	3.67	2.87	4.67
T_7 - Adenine sulphate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm	100	4.33	2.10	3.67
T_8 - Adenine sulphate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm	100	4.00	3.06	4.33
T_9 - Control (Basal medium)	100	1.40	3.20	5.33
T_{10} - NAA 2.0 ppm + BA 5.0 ppm	100	6.33	4.23	4.67
CD (0.05)		2.68	0.90	1.49
SEm±		1.28	0.43	0.71

Values taken as average of three replications
Culture period - 6 weeks

T_7 (adenine sulphate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm).

Shoot length also showed considerable variation in response to different treatments. Treatment T_{10} (NAA 2.0 ppm + BA 5.0 ppm) was significantly superior to T_3 (adenine 20 ppm + NAA 2.0 ppm + BA 5.0 ppm), T_6 (adenine sulphate 100 ppm), T_7 (adenine sulphate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm), T_8 (adenine sulphate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm) and T_9 (control) but was on par with T_1 (adenine 20 ppm), T_2 (adenine 40 ppm), T_4 (adenine 40 ppm + NAA 2.0 ppm + BA 5.0 ppm) and T_5 (adenine sulphate 50 ppm) after 6 weeks in culture. T_7 recorded the shortest shoot length (2.1 cm) and was on par with T_3 and T_6.

With regard to number of leaves produced per shoot, after 6 weeks in culture, T_9 (control) recorded the maximum number of leaves (5.33) and was on par with T_2 (adenine 40 ppm), T_5 (adenine sulphate 50 ppm), T_6 (adenine sulphate 100 ppm), T_8 (adenine sulphate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm) and T_{10} (NAA 2.0 ppm + BA 5.0 ppm). The minimum number of leaves (2.67 each) was produced by treatments T_3 (adenine 20 ppm + NAA 2.0 ppm + BA 5.0 ppm) and T_4 (adenine 40 ppm + NAA 2.0 ppm + BA 5.0 ppm) which were on par with T_1 (adenine 20 ppm), T_2 and T_7 (adenine sulphate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm).

4.2.1.2. Effect in VW medium

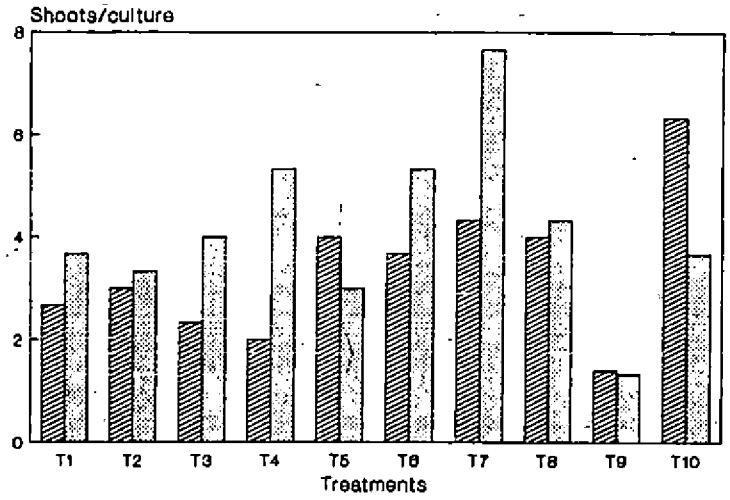
Data on the influence of cytokinin related substances on shoot proliferation in VW medium are furnished in Table 4.

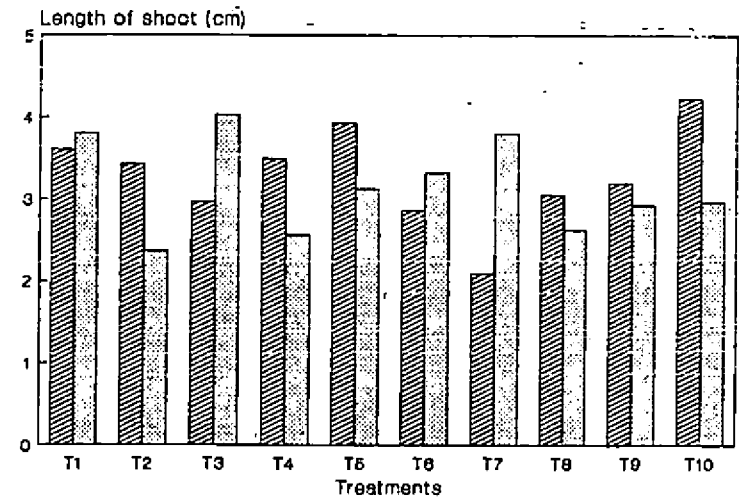
Observations recorded after 6 weeks showed that T_7 (adenine sulphate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm) was significantly superior to all other treat-

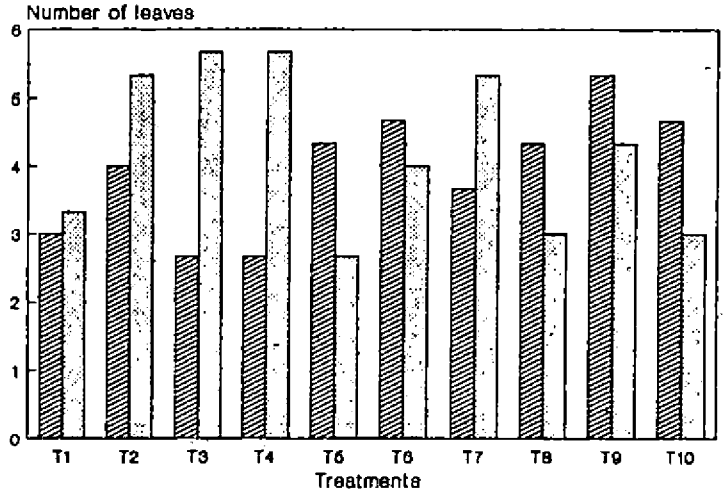
Table 4. Effect of cytokinin related substances on shoot proliferation in Dendrobium
Basal medium - VW

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Adenine 20 ppm	100	3.67	3.80	3.33
T_2 - Adenine 40 ppm	100	3.33	2.37	5.33
T_3 - Adenine 20 ppm + NAA 2.0 ppm + BA 5.00 ppm	100	4.00	4.03	5.67
T_4 - Adenine 40 ppm + NAA 2.0 ppm + BA 5.0 ppm	100	5.33	2.57	5.67
T_5 - Adenine sulphate 50 ppm	100	3.00	3.13	2.67
T_6 - Adenine sulphate 100 ppm	100	5.33	3.33	4.00
T_7 - Adénine sulphate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm	100	7.67	3.80	5.33
T_8 - Adenine sulphate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm	100	4.33	2.63	3.00
T_9 - Control (Basal medium)	100	1.33	2.93	4.33
T_{10} - NAA 2.0 ppm + BA 5.0 ppm	100	3.67	2.97	3.00
CD (0.05)		2.83	1.45	1.12
SEm±		1.36	0.69	0.54

Values taken as average of three replications
Culture period - 6 weeks







Legend

1/2 MS VW

T1 - Adenine 20 ppm
T2 - Adenine 40 ppm
T3 - Adenine 20 ppm + NAA 2.0 ppm + BA 5.0 ppm
T4 - Adenine 40 ppm + NAA 2.0 ppm + BA 5.0 ppm
T5 - Adenine sulphate 50 ppm
T6 - Adenine sulphate 100 ppm
T7 - Adenine sulphate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm
T8 - Adenine sulphate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm
T9 - Control (Basal media)
T10- NAA 2.0 ppm + BA 5.0 ppm.

Fig. 3 Effect of cytokinin related substances on shoot proliferation in *Dendrobium*.

ments except T_4 (adenine 40 ppm + NAA 2.0 ppm + BA 5.0 ppm) and T_6 (adenine sulphate 100 ppm) and produced 7.67 shoots on an average. T_9 (control) produced the lowest number of shoots (1.33) and was on par with T_1 (adenine 20 ppm), T_2 (adenine 40 ppm), T_3 (adenine 20 ppm + NAA 2.0 ppm + BA 5.0 ppm) and T_5 (adenine sulphate 50 ppm).

Treatment T_3 (adenine 20 ppm + NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum shoot length (4.03 cm) after 6 weeks and was on par with all treatments except T_2 (adenine 40 ppm) which produced the shortest shoot (2.37 cm) and T_4 (adenine 40 ppm + NAA 2.0 ppm + BA 5.0 ppm).

With regard to the number of leaves, observation recorded after 6 weeks revealed that treatment T_3 (adenine 20 ppm + NAA 2.0 ppm + BA 5.0 ppm) and T_4 (adenine 40 ppm + NAA 2.0 ppm) recorded the maximum leaf number (5.67) and was significantly superior to all treatments except T_2 (adenine 40 ppm) and T_7 (adenine sulphate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm) and was on par with these treatments. T_5 (adenine sulphate 50 ppm) recorded the lowest leaf number (2.67) which was on par with T_1 (adenine 20 ppm), T_5 (adenine sulphate 50 ppm), T_8 (adenine sulphate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm) and T_{10} (NAA 2.0 ppm + BA 5.0 ppm).

4.2.2. Amino acid supplements

Data on the results obtained from trials conducted to evaluate the various amino acid supplements in ½ MS and VW media are given in Table 5 and 6, respectively.

4.2.2.1. Effect in ½ MS medium

With regard to the number of shoots developing after 6 weeks the data revealed that T_3 (casein hydrolysate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm) produced the maximum number of shoots (9.33) and was significantly superior to all other treatments, except T_2 (casein hydrolysate 100 ppm) and was on par with T_2. The minimum number of shoots (1.00) was produced by T_{10} (yeast extract 2%) and T_{11} (yeast extract 1% + NAA 2.0 ppm + BA 5.0 ppm) and was on par with T_5 (peptone 20 ppm), T_{12} (yeast extract 2% + NAA 2.0 ppm + BA 5.0 ppm), T_9 (yeast extract 1%) and T_{13} (control).

Considering the length of shoots, T_{14} (NAA 2.0 ppm + BA 5.0 ppm) produced the longest shoot (4.23 cm) after 6 weeks of culturing and was on par with T_3 (casein hydrolysate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm) and T_4 (casein hydrolysate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm). The shoot with shortest length (1.83 cm) was produced by T_{10} (yeast extract 2%) and was on par with T_5 (peptone 20 ppm), T_6 (peptone 40 ppm) and T_{11} (yeast extract 1% + NAA 2.0 ppm + BA 5.0 ppm).

With regard to the number of leaves produced after 6 weeks, T_3 (casein hydrolysate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum number of leaves (6.0) and was on par with T_1 (casein hydrolysate 50 ppm), T_4 (casein hydrolysate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm) and T_{13} (control). T_5 (peptone 20 ppm), T_{10} (yeast extract 2%), T_{11} (yeast extract 1% + NAA 2.0 ppm + BA 5.0 ppm) and T_{12} (yeast extract 2% + NAA 2.0 ppm + BA 5.0 ppm) produced the lowest number of leaves (2.33) and was on par with T_6 (peptone 40 ppm).

Table 5. Effect of amino acid supplements on shoot proliferation in Dendrobium

Basal medium - ½ MS

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Casein hydrolysate 50 ppm	100	3.67	2.93	5.33
T_2 - Casein hydrolysate 100 ppm	100	7.33	3.53	4.67
T_3 - Casein hydrolysate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm	100	9.33	3.73	6.00
T_4 - Casein hydrolysate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm	100	6.33	3.90	5.67
T_5 - Peptone 20 ppm	100	2.00	2.47	2.33
T_6 - Peptone 40 ppm	100	3.67	1.87	3.00
T_7 - Peptone 20 ppm + NAA 2.0 ppm + BA 5.0 ppm	100	4.33	2.50	4.33
T_8 - Peptone 40 ppm + NAA 2.0 ppm + BA 5.0 ppm	100	4.67	2.53	3.67
T_9 - Yeast extract 1%	100	1.67	2.60	4.00
T_{10} - Yeast extract 2%	100	1.00	1.83	2.33
T_{11} - Yeast extract 1% + NAA 2.0 ppm + BA 5.0 ppm	100	1.00	1.90	2.33
T_{12} - Yeast extract 2% + NAA 2.0 ppm + BA 5.0 ppm	100	2.00	2.47	2.33
T_{13} - Control (Basal medium)	100	1.40	3.20	5.33
T_{14} - NAA 2.0 ppm + BA 5.0 ppm	100	6.33	4.23	3.67
CD (0.05)		2.57	0.79	1.32
SEm±		1.25	0.38	0.64

Values taken as average of three replications
Culture period - 6 weeks

4.2.2.2. Effect in VW medium

After 6 weeks of culturing, treatment T_8 (peptone 40 ppm + NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum number of shoots (16.67) in VW medium and was on par with T_1 (casein hydrolysate 50 ppm), T_2 (casein hydrolysate 100 ppm), T_3 (casein hydrolysate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm) and T_4 (casein hydrolysate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm). The minimum number of shoots (1.00) were recorded by T_9 (yeast extract 1%) and T_{10} (yeast extract 2%) and was on par with all treatments except T_8.

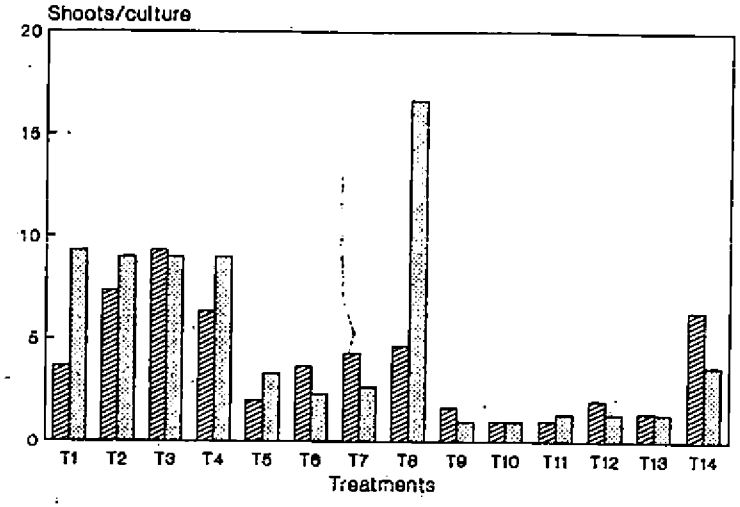
The maximum shoot length (3.97 cm) was recorded by T_1 (casein hydrolysate 50 ppm) after 6 weeks and was on par with T_3 (casein hydrolysate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm), T_4 (casein hydrolysate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm), T_5 (peptone 20 ppm), T_6 (peptone 40 ppm), T_{13} (control) and T_{14} (NAA 2.0 ppm + BA 5.0 ppm). The minimum shoot length (1.83) was recorded by T_{10} (yeast extract 2%) and was on par with T_2 (casein hydrolysate 100 ppm), T_7 (peptone 20 ppm + NAA 2.0 ppm + BA 5.0 ppm), T_8 (peptone 40 ppm + NAA 2.0 ppm + BA 5.0 ppm), T_9 (yeast extract 1%), T_{10} (yeast extract 2%), T_{11} (yeast extract 1% + NAA 2.0 ppm + BA 5.0 ppm), T_{12} (yeast extract 2% + NAA 2.0 ppm + BA 5.0 ppm) and T_{13} (control).

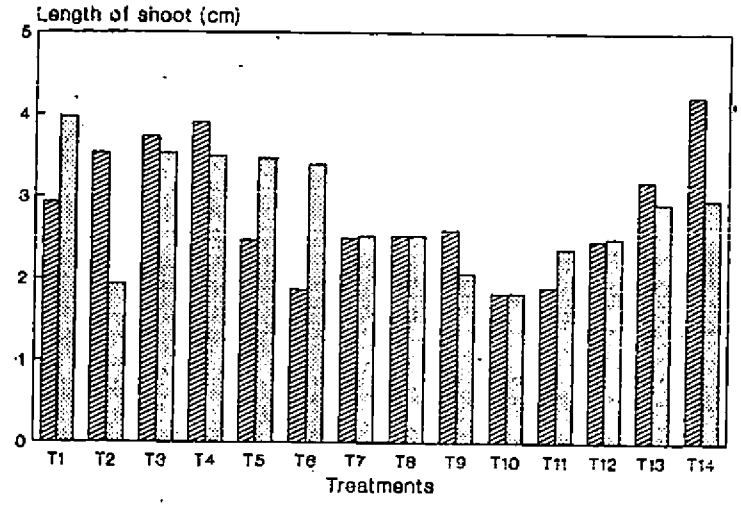
After a culture period of 6 weeks, T_4 (casein hydrolysate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm) remained significantly superior to all other treatments and produced the maximum number (6.0) of leaves. T_9 (yeast extract 1%) produced the lowest number (2.0) of leaves and was on par with T_3 (casein hydrolysate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm), T_5 (peptone 20 ppm), T_{10} (yeast extract 2%), T_{11}

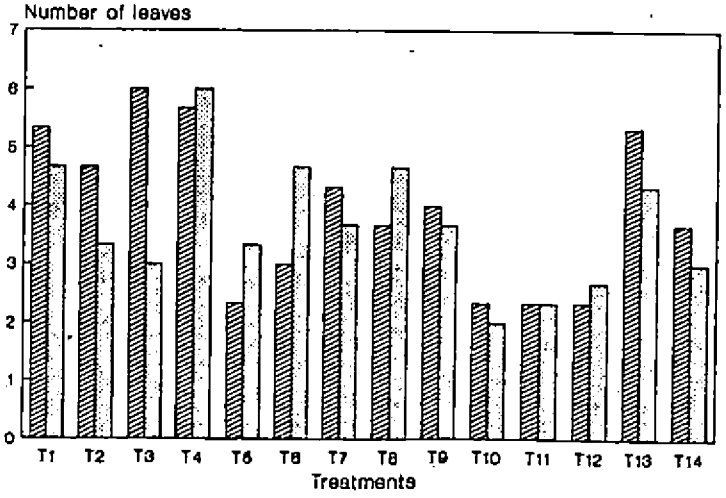
Table 6. Effect of amino acid supplements on shoot proliferation in Dendrobium
Basal medium - VW

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T1 - Casein hydrolysate 50 ppm	100	9.33	3.97	4.67
T2 - Casein hydrolysate 100 ppm	100	9.00	1.93	3.33
T3 - Casein hydrolysate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm	100	9.00	3.53	3.00
T4 - Casein hydrolysate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm	100	9.00	3.50	6.00
T5 - Peptone 20 ppm	100	3.33	3.47	3.33
T6 - Peptone 40 ppm	100	2.33	3.40	4.67
T7 - Peptone 20 ppm + NAA 2.0 ppm + BA 5.0 ppm	100	2.67	2.53	3.67
T8 - Peptone 40 ppm + NAA 2.0 ppm + BA 5.0 ppm	100	16.67	2.53	4.67
T9 - Yeast extract 1%	100	1.00	2.07	3.67
T10 - Yeast extract 2%	100	1.00	1.83	2.00
T11 - Yeast extract 1% + NAA 2.0 ppm + BA 5.0 ppm	100	1.33	2.37	2.33
T12 - Yeast extract 2% + NAA 2.0 ppm + BA 5.0 ppm	100	1.33	2.50	2.67
T13 - Control (Basal medium)	100	1.33	2.93	4.33
T14 - NAA 2.0 ppm + BA 5.0 ppm	100	3.67	2.97	3.00
CD (0.05)		10.50	1.16	1.29
SEm±		5.11	0.57	0.63

Values taken as average of three replications
Culture period - 6 weeks







Legend

1/2 MS VW

T1 - Casein hydrolysate 50 ppm
T2 - Casein hydrolysate 100 ppm
T3 - Casein hydrolysate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm
T4 - Casein hydrolysate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm
T5 - Peptone 20 ppm
T6 - Peptone 40 ppm
T7 - Peptone 20 ppm + NAA 2.0 ppm + BA 5.0 ppm
T8 - Peptone 40 ppm + NAA 2.0 ppm + BA 5.0 ppm
T9 - Yeast extract 1%
T10- Yeast extract 2%
T11- Yeast extract 1% + NAA 2.0 ppm + BA 5.0 ppm
T12- Yeast extract 2% + NAA 2.0 ppm + BA 5.0 ppm
T13- Control (Basal media)
T14- NAA 2.0 ppm + BA 5.0 ppm

Fig. 4 Effect of amino acid supplements on shoot proliferation in *Dendrobium*.

(yeast extract 1% + NAA 2.0 ppm + BA 5.0 ppm), T_{12} (yeast extract 2% + NAA 2.0 ppm + BA 5.0 ppm) and T_{14} (NAA 2.0 ppm + BA 5.0 ppm).

In general, the shoots growing in media supplied with casein hydrolysate was healthier when compared with other amino acid supplements.

4.2.3. Organic supplements

4.2.3.1. Palm milk

Data recorded on the influence of coconut water and palm endosperm liquid and also their combination with NAA and BA on shoot proliferation are presented below.

4.2.3.1.1. Effect in ½ MS medium

Data regarding the influence of palm milk in ½ MS medium is presented in Table 7.

After a culture period of 6 weeks, T_3 (coconut water 5% + NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum number of shoots (11.33) and was on par with T_2 (coconut water 10%) and T_4 (coconut water 10% + NAA 2.0 ppm + BA 5.0 ppm) but significantly superior to all other treatments. The least number of shoots (1.4) was produced by T_9 (control) and was on par with T_5 (palm endosperm liquid 2%), T_8 (palm endosperm liquid 4% + NAA 2.0 ppm + BA 5.0 ppm) and T_6 (palm endosperm liquid 4%).

At the end of 6 weeks of culturing, the maximum shoot length (4.23 cm) was recorded by T_{10} (NAA 2.0 ppm + BA 5.0 ppm) and was significantly superior to T_4 (coconut water 10% + NAA 2.0 ppm + BA 5.0 ppm), T_5 (palm endosperm liquid 2%), T_6 (palm endosperm liquid 4%) and T_8 (palm endosperm liquid 4% +

Table 7. Effect of palm milk on shoot proliferation in *Dendrobium*
Basal medium - ½ MS

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Coconut water 5%	100	7.33	4.07	5.00
T_2 - Coconut water 10%	100	9.33	3.00	3.00
T_3 - Coconut water 5% + NAA 2.0 ppm + BA 5.0 ppm	100	11.33	2.90	4.67
T_4 - Coconut water 10% + NAA 2.0 ppm +	100	9.67	2.47	2.67
T_5 - Palm endosperm liquid 2%	100	2.67	2.20	4.00
T_6 - Palm endosperm liquid 4%	100	1.67	2.33	3.00
T_7 - Palm endosperm liquid 2% + NAA 2.0 ppm + BA 5.0 ppm	100	7.00	3.00	3.00
T_8 - Palm endosperm liquid 4% + NAA 2.0 ppm + BA 5.0 ppm	100	4.67	2.77	4.33
T_9 - Control (Basal medium)	100	1.40	3.20	5.33
T_{10} - NAA 2.0 ppm + BA 5.0 ppm	100	6.33	4.23	4.67
CD (0.05)		3.75	1.31	1.49
SEm±		1.80	0.63	0.71

Values taken at average of three replications
Culture period - 6 weeks

NAA 2.0 ppm + BA 5.0 ppm) but was on par with other treatments. T_5 (palm endosperm liquid 2%) recorded the lowest shoot length (2.20 cm) and was on par with all treatments, except T_1 (coconut water 5%) and T_{10} and was significantly different from these two treatments.

After a culture period of 6 weeks T_9 (control) recorded the maximum leaf number (5.33) and was on par with T_1 (coconut water 5%), T_3 (coconut water 5% + NAA 2.0 ppm + BA 5.0 ppm), T_5 (palm endosperm liquid 2%), T_8 (palm endosperm liquid 4% + NAA 2.0 ppm + BA 5.0 ppm) and T_{10} (NAA 2.0 ppm + BA 5.0 ppm). The lowest leaf number (2.67) was recorded by T_4 (coconut water 10% + NAA 2.0 ppm + BA 5.0 ppm) and was on par with T_2 (coconut water 10%), T_5 (palm endosperm liquid 2%), T_6 (palm endosperm liquid 4%) and T_7 (palm endosperm liquid 2% + NAA 2.0 ppm + BA 5.0 ppm).

4.2.3.1.2. Effect in VW medium

Data recorded on the effect of palm milk on the number of shoots, length of shoot and number of leaves produced in VW medium are presented in Table 8.

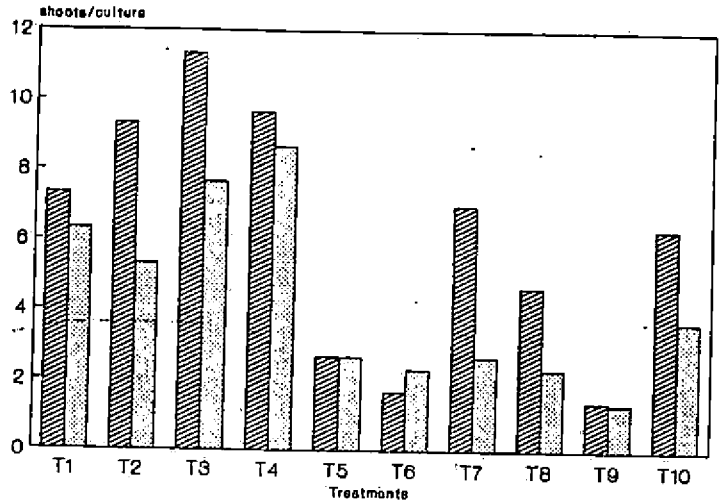
Considering the shoot number after 6 weeks, T_4 (coconut water 10% + NAA 2.0 ppm + BA 5.0 ppm) produced the maximum (8.67) and was significantly superior to all other treatments except T_1 (coconut water 5%) and T_3 (coconut water 5% + NAA 2.0 ppm + BA 5.0 ppm). T_9 (control) recorded the lowest shoot number (1.33) and was on par with T_5 (palm endosperm liquid 2%), T_6 (palm endosperm liquid 4%), T_7 (palm endosperm liquid 2% + NAA 2.0 ppm + BA 5.0 ppm), T_8 (palm endosperm liquid 4% + NAA 2.0 ppm + BA 5.0 ppm) and T_{10} (NAA 2.0 ppm + BA 5.0 ppm).

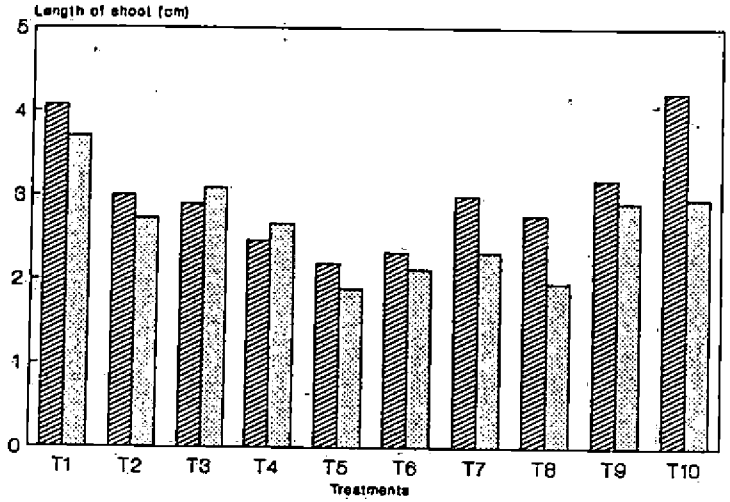
Table 8 . Effect of palm milk on shoot proliferation in Dendrobium

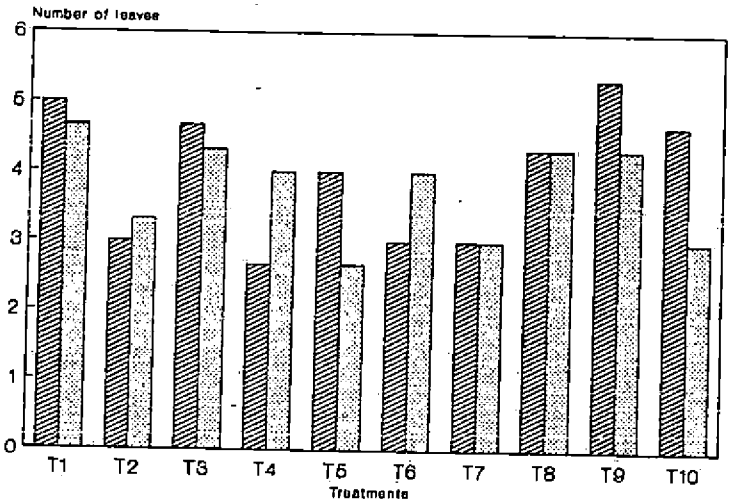
Basal medium - V W

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Coconut water 5%	100	6.33	3.70	4.67
T_2 - Coconut water 10%	100	5.33	2.73	3.33
T_3 - Coconut water 5% + NAA 2.0 ppm + BA 6.0 ppm	100	7.67	3.10	4.33
T_4 - Coconut water 10% + NAA 2.0 ppm + BA 5.0 ppm	100	8.67	2.67	4.00
T_5 - Palm endosperm liquid 2%	100	2.67	1.90	2.67
T_6 - Palm endosperm liquid 4%	100	2.33	2.13	4.00
T_7 - Palm endosperm liquid 2% + NAA 2.0 ppm + BA 5.0 ppm	100	2.67	2.33	3.00
T_8 - Palm endosperm liquid 4% + NAA 2.0 ppm + BA 5.0 ppm	100	2.33	1.97	4.33
T_9 - Control (Basal medium)	100	1.33	2.93	4.33
T_{10} - NAA 2.0 ppm + BA 5.0 ppm	100	3.67	2.97	3.00
CD (0.05)		3.16	0.89	1.52
SEm±		1.51	0.43	0.73

Values taken at average of three replications
Culture period - 6 weeks







Legend

1/2 MS VW

T1 - Coconut water 5%
T2 - Coconut water 10 %
T3 - Coconut water 5 % + NAA 2.0 ppm + BA 5.0 ppm
T4 - Coconut water 10 % + NAA 2.0 ppm + BA 5.0 ppm
T5 - Palm endosperm liquid 2 %
T6 - Palm endosperm liquid 4 %
T7 - Palm endosperm liquid 2 % + NAA 2.0 ppm + BA 5.0 ppm
T8 - Palm endosperm liquid 4 % + NAA 2.0 ppm + BA 5.0 ppm
T9 - Control (Basal medium)
T10- NAA 2.0 ppm + BA 5.0 ppm

Fig.5 Effect of palm milk on shoot proliferation in *Dendrobium*.

As regard the length of shoot, after a culture period of 6 weeks, the longest shoot (3.7 cm) was produced by T_1 (coconut water 5%) and was on par with T_3 (coconut water 5% + NAA 2.0 ppm + BA 5.0 ppm), T_9 (control) and T_{10} (NAA 2.0 ppm + BA 5.0 ppm). The shortest shoot (1.9 cm) was recorded by T_5 (palm endosperm liquid 2%) and was on par with T_2 (coconut water 10%), T_4 (coconut water 10% + NAA 2.0 ppm + BA 5.0 ppm), T_6 (palm endosperm liquid 4%), T_7 (palm endosperm liquid 2% + NAA 2.0 ppm + BA 5.0 ppm) and T_8 (palm endosperm liquid 4% + NAA 2.0 ppm + BA 5.0 ppm).

Considering the leaf development after 6 weeks, T_1 (coconut water 5%) produced the maximum number of leaves (4.67) and was on par with all other treatments, except T_5 (palm endosperm liquid 2%), T_7 (palm endosperm liquid 2% + NAA 2.0 ppm + BA 5.0 ppm) and T_{10} (NAA 2.0 ppm + BA 5.0 ppm). The minimum number of leaves (2.67) was produced by T_5 and was on par with T_2 (coconut water 10%), T_4 (coconut water 10% + NAA 2.0 ppm + BA 5.0 ppm), T_6 (palm endosperm liquid 4%), T_7 and T_{10}.

4.2.3.2. Fruit juice

Observations recorded on the influence of orange juice, tomato juice and their combinations with NAA and BA in ½ MS and VW medium are presented below.

4.2.3.2.1. Effect in ½ MS medium

Data relating to the effect of fruit juices on the number of shoots, length of shoot and number of leaves in ½ MS medium, are presented in Table 9.

Table 9. Effect of fruit juices on shoot proliferation in Dendrobium
Basal medium - ½ MS

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Orange juice 5%	100	4.33	2.23	3.33
T_2 - Orange juice 10%	100	3.00	2.33	3.67
T_3 - Orange juice 5% + NAA 2.0 ppm + BA 5.0 ppm	100	5.00	3.27	4.67
T_4 - Orange juice 10% + NAA 2.0 ppm + BA 5.0 ppm	100	3.67	3.07	3.67
T_5 - Tomato juice 5%	100	6.67	3.17	4.67
T_6 - Tomato juice 10%	100	3.00	2.10	3.00
T_7 - Tomato juice 5% + NAA 2.0 ppm + BA 5.0 ppm	100	8.67	3.03	6.00
T_8 - Tomato juice 10% + NAA 2.0 ppm + BA 5.0 ppm	100	3.67	3.53	2.67
T_9 - Control (Basal medium)	100	1.40	3.20	5.33
T_{10} - NAA 2.0 ppm + BA 5.0 ppm	100	6.33	4.23	4.67
CD (0.05)		2.74	0.77	1.39
SEm±		1.32	0.37	0.66

Values taken as average of three replications
Culture period - 6 weeks

As to the number of shoots, after a culture period of 6 weeks, T_7 (tomato juice 5% + NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum number (8.67), was on par with T_5 (tomato juice 5%) and T_{10} (NAA 2.0 ppm + BA 5.0 ppm) and was significantly superior to all other treatments. The minimum number of shoots (1.4) was recorded by T_9 (control) and was on par with T_2 (orange juice 10%), T_4 (orange juice 10% + NAA 2.0 ppm + BA 5.0 ppm), T_6 (tomato juice 10%), T_8 (tomato juice 10% + NAA 2.0 ppm + BA 5.0 ppm) and T_9 (control).

With regard to the shoot length after 6 weeks, the data revealed that T_{10} (NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum shoot length (4.23 cm) which was on par with T_8 (tomato juice 10% + NAA 2.0 ppm + BA 5.0 ppm) and was significantly superior to all other treatments. T_6 (tomato juice 10%) recorded the minimum shoot length (2.10) and was on par with T_1 (orange juice 5%) and T_2 (orange juice 10%).

After 6 weeks in culture, T_7 (tomato juice 5% + NAA 2.0 ppm + BA 5.0 ppm) produced the maximum number of leaves (6.00) and was on par with T_3 (orange juice 5% + NAA 2.0 ppm + BA 5.0 ppm), T_5 (tomato juice 5%), T_9 (control) and T_{10} (NAA 2.0 ppm + BA 5.0 ppm). The minimum number (2.67) was recorded by T_8 (tomato juice 10% + NAA 2.0 ppm + BA 5.0 ppm) and was on par with T_1 (orange juice 5%), T_2 (orange juice 10%), T_4 (orange juice 10% + NAA 2.0 ppm + BA 5.0 ppm) and T_6 (tomato juice 10%).

4.2.3.2.2. Effect in VW medium

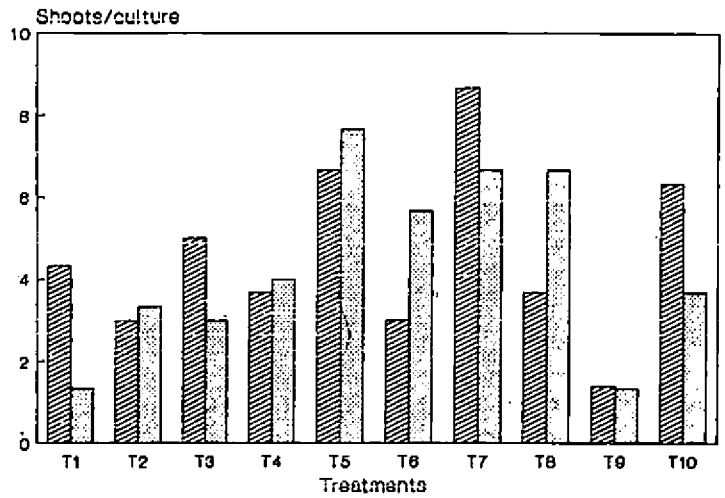
The data relating to the effect of fruits juice on the number of shoots, length of shoots and number of leaves produced in VW medium are presented in Table 10.

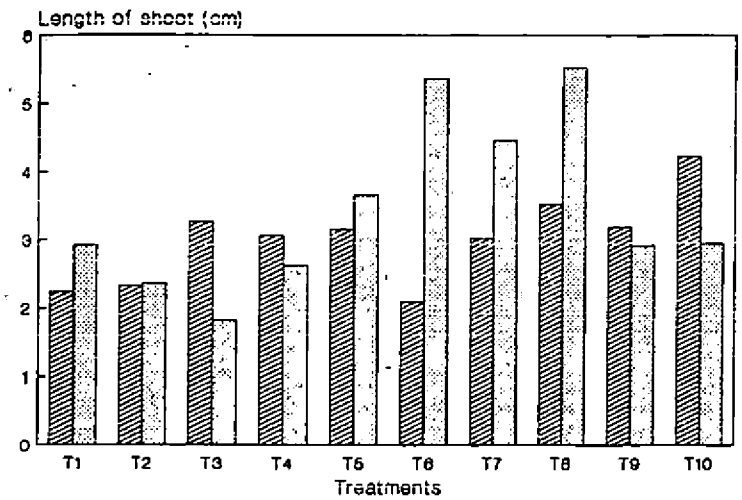
Table 10. Effect of fruit juices on shoot proliferation in *Dendrobium*

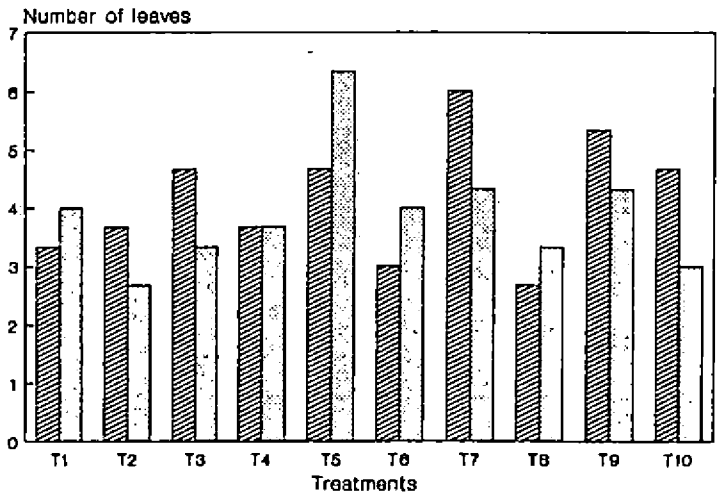
Basal medium - VW

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Orange juice 5%	100	1.33	2.93	4.00
T_2 - Orange juice 10%	100	3.33	2.37	2.67
T_3 - Orange juice 5% + NAA 2.0 ppm + BA 5.0 ppm	100	3.00	1.83	3.33
T_4 - Orange juice 10% + NAA 2.0 ppm + BA 5.0 ppm	100	4.00	2.63	3.67
T_5 - Tomato juice 5%	100	7.67	3.67	6.33
T_6 - Tomato juice 10%	100	5.67	5.37	4.00
T_7 - Tomato juice 5% + NAA 2.0 ppm + BA 5.0 ppm	100	6.67	4.47	4.33
T_8 - Tomato juice 10% + NAA 2.0 ppm + BA 5.0 ppm	100	6.67	5.53	3.33
T_9 - Control (Basal medium)	100	1.33	2.93	4.33
T_{10} - NAA 2.0 ppm + BA 5.0 ppm	100	3.67	2.97	3.00
CD (0.05)		2.56	0.97	1.12
SEm±		1.23	0.46	0.54

Values taken as average of three replications
Culture period - 6 weeks







Legend

1/2 MS VW

T1 - Orange Juice 5%
T2 - Orange Juice 10 %
T3 - Orange Juice 5% + NAA 2.0 ppm + BA 5.0 ppm
T4 - Orange Juice 10% + NAA 2.0 ppm + BA 5.0 ppm
T5 - Tomato juice 5%
T6 - Tomato Juice 10%
T7 - Tomato Juice 5% + NAA 2.0 ppm + BA 5.0 ppm
T8 - Tomato Juice 10% + NAA 2.0 ppm + BA 5.0 ppm
T9 - Control (Basal media)
T10- NAA 2.0 ppm + BA 5.0 ppm

Fig. 6 Effect of fruit juices on shoot proliferation in *Dendrobium*.

The maximum number of shoots (7.67) was produced by T_5 (tomato juice 5%) after 6 weeks and was significantly superior to all other treatments except T_6 (tomato juice 10%), T_7 (tomato juice 5% + NAA 2.0 ppm + BA 5.0 ppm) and T_8 (tomato juice 10% + NAA 2.0 ppm + BA 5.0 ppm). T_1 (orange juice 5%) and T_9 (control) recorded the minimum number (1.33) of shoots and were on par with T_2 (orange juice 10%), T_3 (orange juice 5% + NAA 2.0 ppm + BA 5.0 ppm) and T_{10} (NAA 2.0 ppm + BA 5.0 ppm).

When shoot length was considered, observation over a period of 6 weeks showed that T_8 (tomato juice 10% + NAA 2.0 ppm + BA 5.0 ppm) produced the maximum shoot length (5.53 cm) and was significantly superior to all treatments except T_6 (tomato juice 10%) with which it was on par. T_3 (orange juice 5% + NAA 2.0 ppm + BA 5.0 ppm) recorded the minimum shoot length (1.83 cm) and was on par with T_2 (orange juice 10%) and T_4 (orange juice 10% + NAA 2.0 ppm + BA 5.0 ppm).

With respect to the number of leaves, after a culture period of 6 weeks, T_5 (tomato juice 5%) recorded the maximum number (6.33) and was significantly superior to all other treatments. T_2 (orange juice 10%) produced the minimum number of leaves (2.67) and was on par with T_3 (orange juice 5% + NAA 2.0 ppm + BA 5.0 ppm), T_4 (orange juice 10% + NAA 2.0 ppm + BA 5.0 ppm), T_8 (tomato juice 10% + NAA 2.0 ppm + BA 5.0 ppm) and T_{10} (NAA 2.0 ppm + BA 5.0 ppm).

4.2.3.3. Flower extracts

The effect of Spathodea flower extracts, Spathodea bud liquid and their

combination with NAA and BA was studied in ½ MS and VW media and the observations recorded are presented below.

4.2.3.3.1. Effect in ½ MS medium

Data pertaining to the effect of flower extracts on the number of shoots, shoot length and number of leaves in ½ MS medium is presented in Table 11.

Considering the number of shoots over a period of 6 weeks, T_{10} (NAA 2.0 ppm + BA 5.0 ppm) produced the maximum number (6.33) and was significantly superior to all other treatments. T_9 (control) produced the minimum number (1.4) of shoots and was on par with T_1 (Spathodea bud liquid 2%).

Taking into consideration the length of shoot, observation over a period of 6 weeks revealed that T_6 (Spathodea flower extract 5%) produced shoots with maximum length (4.43 cm) and was on par with T_2 (Spathodea bud liquid 5%) and T_8 (Spathodea flower extract 5% + NAA 2.0 ppm + BA 5.0 ppm). T_3 (Spathodea bud liquid 2% + NAA 2.0 ppm + BA 5.0 ppm) recorded shortest shoots (3.10 cm) after 6 weeks and was on par with T_7 (Spathodea flower extract 2%) and T_9 (control).

When the leaf number was considered after a culture period of 6 weeks, T_9 (control) produced 5.33 shoots on an average and was on par with all treatments, except T_1 (Spathodea bud liquid 2%), T_3 (Spathodea bud liquid 2% + NAA 2.0 ppm + BA 5.0 ppm) and T_7 (Spathodea flower extract 2% + NAA 2.0 ppm + BA 5.0 ppm). The minimum number of leaves (3.0 each) was produced by T_1 and T_3 which was on par with T_7.

Table 11. Effect of flower extracts on shoot proliferation in Dendrobium

Basal medium - ½ MS

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Spathodea bud liquid 2%	100	2.00	4.07	3.00
T_2 - Spathodea bud liquid 10%	100	2.67	3.87	4.33
T_3 - Spathodea bud liquid 2% + NAA 2.00 ppm + BA 5.00 ppm	100	2.33	3.10	3.00
T_4 - Spathodea bud liquid 5% + NAA 2.00 ppm + BA 5.0 ppm	100	2.33	4.03	4.33
T_5 - Spathodea flower extract 2%	100	2.33	4.17	4.33
T_6 - Spathodea flower extract 5%	100	3.00	4.43	3.67
T_7 - Spathodea flower extract 2% + NAA 2.0 ppm + BA 5.0 ppm	100	3.00	3.40	3.33
T_8 - Spathodea flower extract 5% + NAA 2.0 ppm + BA 5.0 ppm	100	2.67	3.97	3.67
T_9 - Control (Basal medium)	100	1.40	3.20	5.33
T_{10} - NAA 2.0 ppm + BA 5.0 ppm	100	6.33	4.23	4.67
CD (0.05)		0.82	0.68	1.91
SEm±		0.39	0.32	0.92

Values taken as average of three replications
Culture period - 6 weeks

4.2.3.3.2. Effect in VW medium

Data on the effect of Spathodea flower extract and bud liquid and their combination with NAA and BA on the number of shoots, length of shoot and number of leaves are given in Table 12.

As regards the number of shoots produced after six weeks of culturing, T_4 (Spathodea bud liquid 5% + NAA 2.0 ppm + BA 5.0 ppm), T_8 (Spathodea flower extract 5% + NAA 2.0 ppm + BA 5.0 ppm) and T_7 (Spathodea flower extract 2% + NAA 2.0 ppm + BA 5.0 ppm) produced the maximum number of shoots (4.00) and was on par with all other treatments, except T_9 (control). T_9 (control) recorded the minimum number (1.33) of shoots and was on par with T_1 (Spathodea bud liquid 2%), T_2 (Spathodea bud liquid 5%), T_3 (Spathodea bud liquid 2% + NAA 2.0 ppm + BA 5.0 ppm), T_5 (Spathodea flower extract 2%) and T_6 (Spathodea flower extract 5%).

On considering the length of shoots T_3 (Spathodea bud liquid 2% + NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum (3.73 cm) after 6 weeks of culturing and was on par with all treatments except T_2 (Spathodea bud liquid 5%) and T_8 (Spathodea flower extract 5% + NAA 2.0 ppm + BA 5.0 ppm). T_2 (Spathodea bud liquid 5%) recorded the minimum shoot length of 2.60 cm and was on par with all treatments except T_3 (Spathodea bud liquid 2% + NAA 2.0 ppm + BA 5.0 ppm) and T_7 (Spathodea flower extract 2% + NAA 2.0 ppm + BA 5.0 ppm).

In respect of the number of leaves produced, data after 6 weeks revealed that T_7 (Spathodea flower extract 2% + NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum leaf number (5.00) and was on par with all treatments except T_4 (Spatho-

Table 12. Effect of flower extracts on shoot proliferation in Dendrobium

Basal medium - VW

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Spathodea bud liquid 2%	100	2.00	3.13	4.00
T_2 - Spathodea bud liquid 10%	100	3.33	2.60	4.00
T_3 - Spathodea bud liquid 2% + NAA 2.00 ppm + BA 5.00 ppm	100	3.00	3.73	4.33
T_4 - Spathodea bud liquid 5% + NAA 2.00 ppm + BA 5.0 ppm	100	4.00	2.80	3.00
T_5 - Spathodea flower extract 2%	100	3.33	3.07	4.00
T_6 - Spathodea flower extract 5%	100	3.00	2.97	4.33
T_7 - Spathodea flower extract 2% + NAA 2.0 ppm + BA 5.0 ppm	100	4.00	3.70	5.00
T_8 - Spathodea flower extract 5% + NAA 2.0 ppm + BA 5.0 ppm	100	4.00	2.63	3.33
T_9 - Control (Basal medium)	100	1.33	2.93	4.33
T_{10} - NAA 2.0 ppm + BA 5.0 ppm	100	3.67	2.97	3.00
CD (0.05)		2.10	0.96	1.46
SEm±		1.01	0.46	0.70

Values taken as average of three replications
Culture period - 6 weeks

dea bud liquid 5% + NAA 2.0 ppm + BA 5.0 ppm), T_8 (Spathodea flower extract 5% + NAA 2.0 ppm + BA 5.0 ppm) and T_{10} (NAA 2.0 ppm + BA 5.0 ppm). The minimum leaf number was recorded by T_4 (Spathodea bud liquid 5% + NAA 2.0 ppm + BA 5.0 ppm) and T_{10} and was on par with all other treatments except T_7.

4.2.3.4. Banana pulp

The influence of different levels of banana pulp and their combinations with NAA and BA in ½ MS and VW media were examined. The results recorded are presented below.

4.2.3.4.1. Effect in ½ MS medium

Data on the effect of banana pulp on the number of shoots, length of shoot and number of leaves in ½ MS medium are presented in Table 13.

After 6 weeks, T_6 (NAA 2.0 + BA 5.0 ppm) was on par with T_3 (banana pulp 5% + NAA 2.0 ppm + BA 5.0 ppm) and T_4 (banana pulp 10% + NAA 2.0 ppm + BA 5.0 ppm) and produced the maximum number (6.33) of shoots and was significantly superior to all other treatments. The minimum number of shoots (1.33) was recorded by T_2 (banana pulp 10%) and was on par with T_1 (banana pulp 5%) and T_5 (control).

With regard to the length of shoots, data over a period of 6 weeks revealed that T_6 (NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum length (4.23 cm) for shoots and was significantly superior to all other treatments. T_3 (banana pulp 5% + NAA 2.0 ppm + BA 5.0 ppm) recorded the minimum length (2.57 cm) and was on par with T_1 (banana pulp 5%) and T_5 (control).

Table 13. Effect of banana pulp on shoot proliferation in Dendrobium

Basal medium - ½ MS

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Banana pulp 5%	100	2.00	2.90	2.33
T_2 - Banana pulp 10%	100	1.33	2.83	3.00
T_3 - Banana pulp 5% + NAA 2.0 ppm + BA 5.00 ppm	100	5.00	2.57	4.33
T_4 - Banana pulp 10% + NAA 2.0 ppm + BA 5.0 ppm	100	4.67	3.60	3.67
T_5 - Control (Basal medium)	100	1.40	3.20	5.33
T_6 - NAA 2.0 ppm + BA 5.0 ppm	100	6.33	4.23	4.67
CD (0.05)		1.92	0.93	1.87
SEm±		0.88	0.42	0.86

Values taken as average of three replications
Culture period - 6 weeks

When the number of leaves was considered, observation recorded after 6 weeks showed that T_5 (control) produced the maximum (5.33) and was on par with T_3 (banana pulp 5% + NAA 2.0 ppm + BA 5.0 ppm), T_4 (banana pulp 10% + NAA 2.0 ppm + BA 5.0 ppm) and T_6 (NAA 2.0 ppm + BA 5.0 ppm). T_1 (banana pulp 5%) recorded the minimum number (2.33) of leaves and was on par with T_2 (banana pulp 10%) and T_4 (banana pulp 10% + NAA 2.0 ppm + BA 5.0 ppm).

4.2.3.4.2. Effect in VW medium

Data relating to the influence of banana pulp on the number of shoots, length of shoot and number of leaves in VW medium are furnished in Table 14.

After 6 weeks of culturing, T_4 (banana pulp 10% + NAA 2.0 ppm + BA 5.0 ppm) produced the maximum number (4.33) of shoots and was on par with all other treatments except T_5 (control). T_5 (control) recorded the minimum number of shoots (1.33) and was on par with T_1 (banana pulp 5%), T_2 (banana pulp 10%) and T_3 (banana pulp 5% + NAA 2.0 ppm + BA 5.0 ppm).

As to the length of shoot, data over a period of 6 weeks showed that T_4 (banana pulp 10% + NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum (3.83 cm) and was on par with all treatments, except T_1 (banana pulp 5%), which recorded the lowest length (2.70 cm).

Taking into consideration the number of leaves developing after 6 weeks, T_4 (banana pulp 10% + NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum number (5.33) and was on par with T_3 (banana pulp 5% + NAA 2.0 ppm + BA 5.0 ppm) and T_5 (control). T_1 (banana pulp 5%) recorded the minimum number of

Table 14. Effect of banana pulp on shoot proliferation in Dendrobium
Basal medium - VW

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Banana pulp 5%	100	2.67	2.70	1.67
T_2 - Banana pulp 10%	100	1.67	3.73	3.00
T_3 - Banana pulp 5% + NAA 2.0 ppm + BA 5.00 ppm	100	4.00	3.10	5.00
T_4 - Banana pulp 10% + NAA 2.0 ppm + BA 5.0 ppm	100	4.33	3.83	5.33
T_5 - Control (Basal medium)	100	1.33	2.93	4.33
T_6 - NAA 2.0 ppm + BA 5.0 ppm	100	3.67	2.97	3.00
CD (0.05)		2.69	1.04	1.03
SEm±		1.23	0.48	0.47

Values taken as average of three replications
Culture period - 6 weeks

leaves (1.67) and was significantly different from all other treatments.

4.2.3.5. Potato extract

The influence of potato extract at two levels, viz., 5 per cent and 10 per cent, and their combination with NAA and BA in ½ MS and VW media was studied with respect to the number of shoots, length of shoot and number of leaves produced and the results are presented below.

4.2.3.5.1. Effect in ½ MS medium

The data regarding the effect of potato extract in ½ MS medium are furnished in Table 15.

With respect to the number of shoots, after a period of 6 weeks, T_6 (NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum (6.33) and was on par with all treatments, except T_2 (potato extract 10%) and T_5 (control) and T_5 recorded the minimum (1.4).

Treatment T_6 (NAA 2.0 ppm + BA 5.0 ppm) recorded the highest value for shoot length (4.23 cm) after a period of 6 weeks and was significantly superior to all other treatments. T_1 (potato extract 5%) recorded the minimum number (2.23) of shoots and was on par with T_2 (potato extract 10%), T_4 (potato extract 10% + NAA 2.0 ppm + BA 5.0 ppm) and T_5 (control).

Maximum number of leaves (5.33) after 6 weeks was recorded by T_5 (control) and was on par with T_3 (potato extract 5% + NAA 2.0 ppm + BA 5.0 ppm) and T_6 (NAA 2.0 ppm + BA 5.0 ppm). T_1 (potato extract 5%) recorded the minimum leaf number (3.33) and was on par with all treatments, except T_5.

Table 15. Effect of potato extract on shoot proliferation in Dendrobium

Basal medium - ½ MS

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Potato extract 5%	100	4.33	2.23	3.33
T_2 - Potato extract 10%	100	3.00	2.33	3.67
T_3 - Potato extract 5% + NAA 2.0 ppm + BA 5.00 ppm	100	5.00	3.27	4.67
T_4 - Potato extract 10% + NAA 2.0 ppm + BA 5.0 ppm	100	3.67	3.07	3.67
T_5 - Control (Basal medium)	100	1.40	3.20	5.33
T_6 - NAA 2.0 ppm + BA 5.0 ppm	100	6.33	4.23	4.67
CD (0.05)		2.71	0.87	1.62
SEm±		1.25	0.39	0.75

Values taken as average of three replications
Culture period - 6 weeks

4.2.3.5.2. Effect in VW medium

Data on the effect of potato extract on the number of shoots, length of shoot and number of leaves produced in VW medium are presented in Table 16.

When the number of shoots was considered, after a period of 6 weeks, T_4 (potato extract 10% + NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum (6.67) which was on par with all others except T_5 (control). T_5 (control) recorded the minimum number of shoots (1.33) and was on par with T_2 (potato extract 10%).

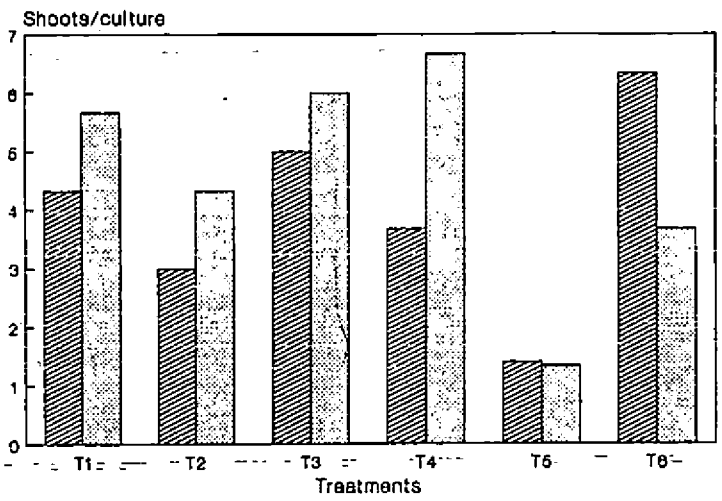
As regards the length of shoot, T_4 (potato extract 10% + NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum (3.83 cm) after 6 weeks and was on par with T_1 (potato extract 5%). The minimum length (2.27) was recorded by T_2 (potato extract 10%) and was on par with T_1 (potato extract 5%), T_3 (potato extract 5% + NAA 2.0 ppm + BA 5.0 ppm), T_5 (control) and T_6 (NAA 2.0 ppm + BA 5.0 ppm).

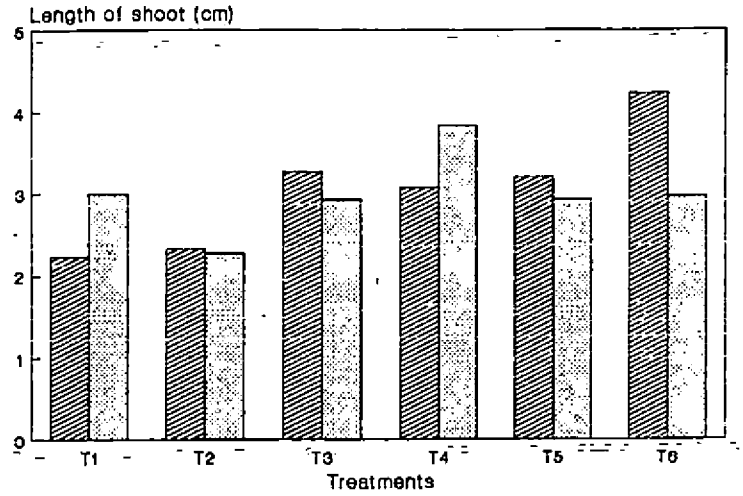
The treatment T_1 (potato extract 5%) and T_3 (potato extract 5% + NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum number of leaves (5.33) after a period of 6 weeks and was on par with T_4 (potato extract 10% + NAA 2.0 ppm + BA 5.0 ppm) and T_5 (control). The minimum number of leaves was recorded by T_6 (NAA 2.0 ppm + BA 5.0 ppm) and was on par with T_2 (potato extract 10%). In general, the growth of shoots in potato extract supplemented medium was very vigorous and healthy.

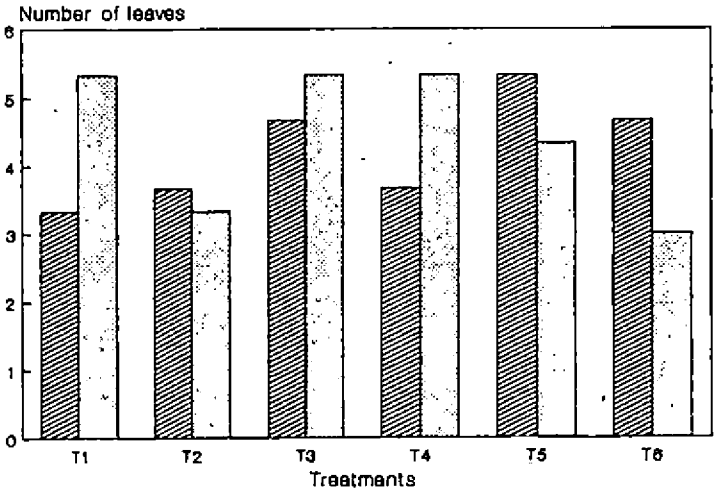
Table 16. Effect of potato extract on shoot proliferation in Dendrobium
Basal medium - VW

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Potato extract 5%	100	5.67	3.00	5.33
T_2 - Potato extract 10%	100	4.33	2.27	3.33
T_3 - Potato extract 5% + NAA 2.0 ppm + BA 5.00 ppm	100	6.00	2.93	5.33
T_4 - Potato extract 10% + NAA 2.0 ppm + BA 5.0 ppm	100	6.67	3.83	5.33
T_5 - Control (Basal medium)	100	1.33	2.93	4.33
T_6 - NAA 2.0 ppm + BA 5.0 ppm	100	3.67	2.97	3.00
CD (0.05)		3.30	0.85	1.19
SEm±		1.52	0.39	0.54

Values taken as average of three replications
Culture period - 6 weeks







Legend

1/2 MS VW

T1 - Potato extract 5%
T2 - Potato extract 10%
T3 - Potato extract 5% + NAA 2.0 ppm + BA 5.0 ppm
T4 - Potato extract 10% + NAA 2.0 ppm + BA 5.0 ppm
T5 - Control (Basal media)
T6 - NAA 2.0 ppm + BA 5.0 ppm

Fig. 7 Effect of potato extract on shoot proliferation in *Dendrobium*.

4.2.3.6. Medicinal plant extract

Oscimum leaf extract was tried at 5 per cent level and 10 per cent level and their combinations with NAA and BA were also attempted. The results are presented below.

4.2.3.6.1. Effect in ½ MS medium

Data relating to the influence of Oscimum leaf extract in ½ MS medium are presented in Table 17.

As to the number of shoots after a culture period of 6 weeks, T_6 (NAA 2.0 ppm + BA 5.0 ppm), recorded the maximum number (6.33) and was significantly superior to all other treatments. The lowest shoot number (1.4) was recorded by T_5 (control) and was on par with T_2 (Oscimum leaf extract 10%), T_3 (Oscimum leaf extract 5% + NAA 2.0 ppm + BA 5.0 ppm) and T_4 (Oscimum leaf extract 10% + NAA 2.0 ppm + BA 5.0 ppm).

With regard to the shoot length, treatment T_6 (NAA 2.0 ppm + BA 5.0 ppm) was significantly superior to all treatments after 6 weeks and recorded the maximum length for shoots (4.23 cm). The minimum shoot length (1.60 cm) was recorded by T_4 (Oscimum leaf extract 10% + NAA 2.0 ppm + BA 5.0 ppm) and was on par with T_2 (Oscimum leaf extract 10%).

Considering the leaf number, T_5 (control) recorded the maximum (5.33) after 6 weeks and was on par with T_1 (Oscimum leaf extract 5%) and T_6 (NAA 2.0 ppm + BA 5.0 ppm). T_2 (Oscimum leaf extract 10%) and T_3 (Oscimum leaf extract 5% + NAA 2.0 ppm + BA 5.0 ppm) recorded the minimum number (3.33) of

Table 17. Effect of medicinal plant extract on shoot proliferation in *Dendrobium*

Basal medium - ½ MS

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Oscimum leaf extract 5%	100	3.00	2.90	5.00
T_2 - Oscimum leaf extract 10%	100	2.67	2.23	3.33
T_3 - Oscimum leaf extract 5% + NAA 2.0 ppm + BA 5.00 ppm	100	2.67	2.57	3.33
T_4 - Oscimum leaf extract 10% + NAA 2.0 ppm + BA 5.0 ppm	100	2.33	1.60	3.67
T5 - Control (Basal medium)	100	1.40	3.20	5.33
T_6 - NAA 2.0 ppm + BA 5.0 ppm	100	6.33	4.23	4.67
CD (0.05)		1.51	0.93	1.57
SEm±		0.69	0.43	0.72

Values taken as average of three replications
Culture period - 6 weeks

leaves and was on par with T_4 (Oscimum leaf extract 10% + NAA 2.0 ppm + BA 5.0 ppm) and T_6 (NAA 2.0 ppm + BA 5.0 ppm).

4.2.3.6.2. Effect in VW medium

The data on the effect of Oscimum leaf extract on the number of shoots, length of shoot and number of leaves produced in VW medium are presented in Table 18.

After a culture period 6 weeks, T_6 (NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum number of shoots (3.67) and was on par with all other treatments. T_5 (control) recorded the lowest number of shoots (1.33).

When the shoot length was considered, after a culture period of 6 weeks, T_6 (NAA 2.0 ppm + BA 5.0 ppm) recorded the maximum (2.97 cm) and was on par with T_5 (control) and T_3 (Oscimum leaf extract 5% + NAA 2.0 ppm + BA 5.0 ppm). The minimum shoot length (2.0 cm) was recorded by T_4 (Oscimum leaf extract 10% + NAA 2.0 ppm + BA 5.0 ppm) and was on par with T_1 (Oscimum leaf extract 5%) and T_2 (Oscimum leaf extract 10%).

Regarding the number of leaves produced after 6 weeks the data showed that T_5 (control) was significantly superior to all other treatments and produced the maximum number of leaves (4.33). All the other treatments were on par and the minimum number of leaves was produced by T_1 (Oscimum leaf extract 5%).

4.2.4. Carbon sources

Trial on the influence of carbon sources like glucose, sucrose, table sugar, mannitol and their combinations with NAA and BA were carried out in ½ MS and

Table 18. Effect of medicinal plant extract on shoot proliferation in Dendrobium

Basal medium - VW

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T_1 - Oscimum leaf extract 5%	100	1.67	2.03	2.67
T_2 - Oscimum leaf extract 10%	100	3.33	2.17	3.00
T_3 - Oscimum leaf extract 5% + NAA 2.0 ppm + BA 5.00 ppm	100	1.67	2.27	3.33
T_4 - Oscimum leaf extract 10% + NAA 2.0 ppm + BA 5.0 ppm	100	3.00	2.00	3.00
T_5 - Control (Basal medium)	100	1.33	2.93	4.33
T_6 - NAA 2.0 ppm + BA 5.0 ppm	100	3.67	2.97	3.00
CD (0.05)		2.58	0.79	0.73
SEm±		1.18	0.36	0.33

Values taken as average of three replications
Culture period - 6 weeks

VW media and the results are presented below.

4.2.4.1. Effect in ½ MS medium

Data in respect of the effect of carbon sources on the number of shoots, shoot length and number of leaves in ½ MS medium are presented in Table 19.

Six weeks after culturing, T_{11} (sucrose 3% + NAA 2.0 ppm + BA 5.0 ppm) produced the maximum number (6.33) of shoots and was significantly superior to all treatments but was on par with T_2 (glucose 5%) and T_9 (glucose 3% + NAA 2.0 ppm + BA 5.0 ppm). The minimum number of shoots (1.0) was recorded by T_6 (table sugar 5%) and was on par with T_1 (glucose 3%), T_3 (sucrose 3%), T_4 (sucrose 5%), T_5 (table sugar 3%), T_7 (mannitol 3%), T_8 (mannitol 5%), T_{10} (glucose 5% + NAA 2.0 ppm + BA 5.0 ppm). T_{13} (table sugar 3% + NAA 2.0 ppm + BA 5.0 ppm), T_{14} (table sugar 5% + NAA 2.0 ppm + BA 5.0 ppm), T_{15} (mannitol 3% + NAA 2.0 ppm + BA 5.0 ppm), T_{18} (NAA 2.0 ppm + BA 5.0 ppm), T_{16} (mannitol 5% + NAA 2.0 ppm + BA 5.0 ppm) and T_{17} (control).

When the shoot length was considered, six weeks after culturing, the maximum length (4.23 cm) was recorded by T_{11} (sucrose 3% + NAA 2.0 ppm + BA 5.0 ppm) and was significantly superior to all treatments. T_{13} (table sugar 3% + NAA 2.0 ppm + BA 5.0 ppm) recorded the minimum shoot length (1.63 cm) and was on par with T_2 (glucose 5%), T_4 (sucrose 5%), T_5 (table sugar 3%), T_7 (mannitol 3%), T_{10} (glucose 5% + NAA 2.0 ppm + BA 5.0 ppm), T_{14} (table sugar 5% + NAA 2.0 ppm + BA 5.0 ppm), T_{15} (mannitol 3% + NAA 2.0 ppm + BA 5.0 ppm) and T_{17} (control).

With regard to the number of leaves after six weeks, T_3 (sucrose 3%)

Table 19. Effect of carbon sources on shoot proliferation in *Dendrobium*

Basal medium - ½ MS

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T1 - Glucose 3%	100	3.00	3.00	4.00
T2 - Glucose 5%	100	4.33	2.47	3.00
T3 - Sucrose 3%	100	1.40	3.20	5.33
T4 - Sucrose 5%	100	1.33	2.50	3.33
T5 - Table sugar 3%	100	2.67	2.53	3.00
T6 - Table sugar 5%	100	1.00	3.07	3.33
T7 - Mannitol 3%	100	2.33	2.10	3.67
T8 - Mannitol 5%	100	2.00	2.77	5.00
T9 - Glucose 3% + NAA 2.0 ppm + BA 5.0 ppm	100	4.67	3.23	3.33
T10 - Glucose 5% + NAA 2.0 ppm + BA 5.0 ppm	100	3.00	1.83	2.67
T11 - Sucrose 3% + NAA 2.0 ppm + BA 5.0 ppm	100	6.33	4.23	4.67
T12 - Sucrose 5% + NAA 2.0 ppm + BA 5.0 ppm	100	4.00	2.70	3.33
T13 - Table sugar 3% + NAA 2.0 ppm + BA 5.0 ppm	100	1.33	1.63	4.67
T14 - Table sugar 5% + NAA 2.0 ppm + BA 5.0 ppm	100	1.67	1.73	4.00
T15 - Mannitol 3% + NAA 2.0 ppm + BA 5.0 ppm	100	3.00	2.30	4.33
T16 - Mannitol 5% + NAA 2.0 ppm + BA 5.0 ppm	100	2.00	2.17	3.00
T17 - Control (Basal medium without carbon source)	100	1.80	2.20	3.33
CD (0.05)		2.10	0.82	1.41
SEm±		1.07	0.42	0.72

Values taken as average of three replications
Culture period - 6 weeks

recorded the maximum number (5.33) and was on par with T_1 (glucose 3%), T_8 (mannitol 5%), T_{11} (sucrose 3% + NAA 2.0 ppm + BA 5.0 ppm), T_{13} (table sugar 3% + NAA 2.0 ppm + BA 5.0 ppm), T_{14} (table sugar 5% + NAA 2.0 ppm + BA 5.0 ppm), T_{15} (mannitol 3% + NAA 2.0 ppm + BA 5.0 ppm) and T_{18} (NAA 2.0 ppm + BA 5.0 ppm). The minimum number (2.67) was recorded by T_{10} (glucose 5% + NAA 2.0 ppm + BA 5.0 ppm).

4.2.4.2. Effect in VW medium

Data in respect of the effect of different carbon sources on shoot proliferation in VW medium are presented in Table 20.

After a culture period 6 weeks, T_9 (glucose 3% + NAA 2.0 ppm + BA 5.0 ppm) and T_4 (sucrose 5%) produced 5.00 shoots on an average and was on par with T_2 (glucose 5%), T_3 (sucrose 3%), T_6 (table sugar 5%), T_8 (mannitol 5%), T_{10} (glucose 5% + NAA 2.0 ppm + BA 5.0 ppm), T_{11} (sucrose 3% + NAA 2.0 ppm + BA 5.0 ppm), T_{12} (sucrose 5% + NAA 2.0 ppm + BA 5.0 ppm), T_{13} (table sugar 3% + NAA 2.0 ppm + BA 5.0 ppm) and T_{14} (table sugar 5% + NAA 2.0 ppm + BA 5.0 ppm). T_3 (sucrose 3%) and T_{17} (control) produced 1.33 shoots on an average and was the minimum.

Considering the shoot length, T_1 (glucose 3%) recorded the maximum (4.30 cm) after a period of 6 weeks and was on par with T_5 (table sugar 3%) and T_{12} (sucrose 5% + NAA 2.0 ppm + BA 5.0 ppm). The minimum shoot length (2.33 cm) was recorded by T_{16} (mannitol 5% + NAA 2.0 ppm + BA 5.0 ppm).

With regard to the number of leaves, data recorded after 6 weeks showed that T_{12} (sucrose 5% + NAA 2.0 ppm + BA 5.0 ppm) produced 5.67 leaves which

Table 20. Effect of carbon sources on shoot proliferation in Dendrobium
Basal medium - VW

Treatment	Percentage of culture developing shoots	Shoots/ culture	Length of shoots (cm)	Number of leaves
T1 - Glucose 3%	100	2.33	4.30	4.67
T2 - Glucose 5%	100	3.33	2.43	3.67
T3 - Sucrose 3%	100	1.33	2.93	4.33
T4 - Sucrose 5%	100	5.00	3.43	3.33
T5 - Table sugar 3%	100	1.67	3.97	3.00
T6 - Table sugar 5%	100	3.00	3.47	3.33
T7 - Mannitol 3%	100	1.67	3.10	5.00
T8 - Mannitol 5%	100	2.67	2.73	4.00
T9 - Glucose 3% + NAA 2.0 ppm + BA 5.0 ppm	100	5.00	3.07	4.00
T10 - Glucose 5% + NAA 2.0 ppm + BA 5.0 ppm	100	4.33	2.80	4.00
T11 - Sucrose 3% + NAA 2.0 ppm + BA 5.0 ppm	100	3.67	2.97	3.00
T12 - Sucrose 5% + NAA 2.0 ppm + BA 5.0 ppm	100	4.33	3.73	5.67
T13 - Table sugar 3% + NAA 2.0 ppm + BA 5.0 ppm	100	3.00	2.93	4.33
T14 - Table sugar 5% + NAA 2.0 ppm + BA 5.0 ppm	100	3.33	3.13	3.33
T15 - Mannitol 3% + NAA 2.0 ppm + BA 5.0 ppm	100	1.67	3.50	4.33
T16 - Mannitol 5% + NAA 2.0 ppm + BA 5.0 ppm	100	2.00	2.33	3.00
T17 - Control (Basal medium without carbon source)	100	1.33	2.93	3.67
CD (0.05)		2.50	0.78	1.46
SEm±		1.28	0.40	0.75

Values taken as average of three replications
Culture period - 6 weeks

leaves each, which was the lowest.

4.3. *In vitro* rooting

Studies were also carried out to find out the *in vitro* rooting effect of carbon sources, the data relating to which are presented in Table 21. Three media, viz., ½ MS, VW and KC were employed for the study.

Of the different treatments employed, T_8 (KC + glucose 3%), took the minimum number of days for rooting (24.00) and was significantly superior to all treatments. The longest time (55.33 days) was taken by T_2 (½ MS + glucose 3%) and was significantly different from all treatments.

4.4. Planting out and acclimatization

In vitro rooted plantlets were removed from culture, roots washed thoroughly with distilled water and then transferred to potting media. Data on the survival percentage of plantlets in the different potting media are presented in Table 22.

4.4.1. Potting media

The different potting media employed were sphagnum moss, charcoal, perlite, cocoapeat, coconut husk : charcoal mix (1:1), brick : charcoal mix (1:1) and coconut husk. Data were recorded over a period of 8 weeks. The maximum survival percentage (28%) after 8 weeks was observed in plantlets kept in coconut husk followed by brick : charcoal (1:1) mix (26%) and coconut husk : charcoal (1:1) mix

Table 21. Effect of carbon sources in in vitro rooting of Dendrobium

Media	Treatment	Root initiation percentage	Days for root initiation	Nature of roots
½ MS	T_1 - Sucrose 3%	100	36.67	Short, thin, white roots
	T_2 - Glucose 3%	100	55.33	Long, thin, white roots
	T_3 - Table sugar 3%	100	46.67	Short, thin, white roots
VW	T_4 - Sucrose 3%	100	46.67	Short, thin, white root
	T_5 - Glucose 3%	100	30.67	Long, thin, white roots
	T_6 - Table sugar 3%	100	47.67	Very short, thin, white roots
KC	T_7 - Sugar 3%	100	45.00	Short, thin, white roots
	T_8 - Glucose 3%	100	24.00	Long, thin, white roots
	T_9 - Table sugar 3%	100	46.33	Short, thin, white roots
	CD (0.05)		2.16	
	SEm±		1.03	

Values taken as average of three replications

Table 22. Influence of potting media on the survival of *in vitro* plantlets in Dendrobium after planting out

Potting media	Survival percentage			
	2 weeks after	4 weeks after	6 weeks after	8 weeks after
Sphagnum moss	44	23	14	7
Charcoal	20	11	4	-
Perlite	23	12	8	3
Cocoapeat	35	14	6	4
Brick charcoal mix (1:1)	44	32	28	26
Coconut husk	67	43	32	28
Coconut husk charcoal mix (1:1)	40	35	28	22

Table 23. Influence of hardening treatments on the survival of *in vitro* plantlets

Post-transfer treatment	Survival percentage
Keeping in mist chamber covered with ventillated plastic sheet	20
Keeping plants and pots under microscope cover	11
Covering plants and pots with ventillated plastic cover	12
Keeping in open	28

(22%). Charcoal alone as the potting media did not support any plant growth after a period of 8 weeks.

4.4.2 Hardening treatments

Hardening treatments also influenced the survival percentage of plantlets. Data pertaining to the trial conducted to find out the best hardening treatment are given in Table 23. The plants were sprayed thrice daily with distilled water for the first two days followed by spraying with 1/10 MS solution for 1 week and then spraying with magnesium sulphate and urea mixture each @ 500 mg/1000 ml. Keeping plants in open with coconut husk as the potting media recorded the highest survival percentage followed by keeping in a mist chamber covered with ventilated plastic sheets.

DISCUSSION

The present investigations on the standardisation of medium supplements for shoot proliferation in *Dendrobium* were carried out at the Plant Tissue Culture Laboratory, attached to the All India Co-ordinated Floriculture Improvement Project (AICFIP), College of Horticulture, Vellanikkara during 1991-93. The results of the study are discussed in this chapter.

Orchids, the super ordinate among ornamentals, are very distinctive plants. Taxonomically they represent the most highly evolved family (Orchidaceae) among monocotyledons, and the largest of all flowering plant families with 600-800 genera and 25000-35000 species (Garay, 1960; Sheehan, 1983; Chadha, 1992). They account for 7 per cent of the total species of flowering plants of the world (Van der Pijl and Dodson, 1966). They are perennial herbs exhibiting an incredible range of diversity in size, shape and colour of flowers.

Since most of the genera and species of orchids are cross compatible, hundreds of hybrids are added to orchid wealth every year. However, breeding of orchids is often a slow and costly undertaking and the conventional methods of propagation through symbiotic seed germination and division of back bulbs, off-shoots and keikis are very slow (Blowers, 1964). Depending on the kind, it takes even a decade to cultivate from six to a dozen good-sized propagules. The use of micropropagation techniques has revolutionised the commercial orchid industry which made it possible to multiply unlimited number of desirable clones.

India has a very large variety of orchids and accounts for about 1300 species, a majority native to the tropics. Most of the species belong to the genus *Den-*

drobium and in the commercial sphere also *Dendrobium* hybrids occupy a major area. This also performs excellently well under the humid tropical climatic conditions of Kerala. In the present studies, *Dendrobium nobile*, an important species widely used in the breeding programme, was used.

Node cultures as a means of clonal propagation for *Dendrobium* was reported earlier by Ball and Arditti (1975). In a study conducted recently (Lakshmidevi, 1992), among the different explants tried for initiating enhanced release of axillary buds in four species of *Dendrobium*, axillary bud explant was found to give 100 per cent response under culture conditions. Hence axillary bud explants were employed for the study.

Among the various characteristics associated with micropropagation techniques, the frequency of culture Survival as well as development into a reculturable structure has been directly related to the initial explant size (Murashige, 1974). Explants of size 0.5-1.5 cm were used for the present investigation.

As the plants are grown in field conditions, chances of it accumulating soil and dust are very high and chances for microbial contamination in the cultures is also immense. This necessitates a thorough and effective surface sterilization of explants before culturing. Lakshmidevi (1992) reported that, of the various sterilants tried, treating the explants with 0.1 per cent mercuric chloride for 10 minutes gave the maximum percentage of explant survival with least contamination rate for *Dendrobium*. Hence, mercuric chloride 0.1 per cent for 10 minutes followed by four rinses in sterile distilled water was followed for surface sterilization of the explants which was found to give the least contamination.

A number of media are reported to be ideal for the *in vitro* cultures of different orchids. The VW medium (Vacin and Went, 1949) was suggested to be the best for culturing different species of *Dendrobium* by many investigators (Gilliland, 1958; Nimote and Sagawa, 1961; Sagawa and Valmayor, 1966; Sagawa *et al.*, 1967; Kim *et al.* 1970; Singh and Sagawa, 1972; Sanguthai and Sagawa, 1973 and Valmayor, 1974). Fernando (1979) also reported that the best medium for the clonal propagation of *Dendrobium* Caeser Red Lip was modified VW. In general, VW medium was found to have a better influence on the culture establishment and was best for *Dendrobium fimbriatum* and *D. moschatum* too (Lakshmidevi, 1992). The success in the use of VW medium indicated that requirements of mineral elements for most of the orchids are relatively simple, compared to those for higher plants.

MS medium (Murashige and Skoog, 1962) was found to be more effective than KC medium for orchid tissue culture. KC medium was also suggested by various workers (Sagawa and Shoji, 1969; Irawati *et al.*, 1977) for clonal propagation of *Dendrobium*. However, Lakshmidevi (1992) reported that KC medium took the longest duration for culture establishment compared to MS and VW media.

Shoots formed in MS medium often produced necrotic leaves and on reducing the concentration of inorganic salts in the medium to half, the symptoms disappeared (Lakshmidevi, 1992). Several other workers also made such observations and they have recommended the use of half or one fourth strength of MS inorganic salts (Anderson, 1980; Mikami *et al.*, 1985; Ilahi and Jabeen, 1987). Taking into account all the aspects, VW medium and half strength MS medium were employed for the present study.

The favourable effects on axillary bud bursting and multiple shoot produc-

tion by cytokinin has been reported by Murashige (1974). Auxins, along with cytokinin, could induce bud break and in the present study NAA 1.0 ppm together with BA 3.0 ppm was found to be the best for bud initiation in both half strength MS and VW media. Also, application of BA resulted in an increased production of shoots in the present study. Higher level of cytokinin has proved to have deleterious effect on shoot growth. Auxin added to the medium has already been reported to nullify the suppressive effect of high cytokinin content on axillary shoot growth (Lundergran and Janick, 1980).

Matsui *et al.* (1970) reported that increase in number of shoots in *Cymbidium* was observed when cultured in a medium containing NAA and BA. A higher concentration of NAA and BA was reported to induce maximum proliferation of shoots in *Cattleya* (Kusumoto, 1979). Multiple shoot formation was the best in VW media with NAA and BA when compared with MS medium, in *Dendrobium nobile* (Lakshmidevi, 1992). In the present study more shoots were obtained in ½ MS medium than with VW medium, when NAA 2.0 ppm and BA 5.0 ppm were employed. Longer shoots with more number of leaves could also be obtained in half strength MS medium.

The necessity of modifying the original composition of tissue culture media slightly with complex additives has been reported by Arditti (1977). Adenine, a nitrogenous base of DNA, and adenine sulphate have been widely used in tissue culture media for their growth regulatory effects. Davis *et al.* (1977) reported the possible role of adenine in enhancing apical dominance. Synergistic effect of adenine on cytokinins has been suggested by Nitsch *et al.* (1967). In the present study also adenine sulphate 50 ppm along with NAA 2.0 ppm and BA 5.0 ppm produced more

shoots than using adenine or adenine sulphate alone. Start and Cumming (1976) also obtained similar results and they found that adenine added to a medium in conjunction with BA and NAA increased direct shoot initiation from African Violet leaf sections, whereas in the basal medium with only adenine sulphate, shoot formation was delayed and roots alone were formed.

Amino acid supplements are often incorporated into the tissue culture media for obtaining better proliferation of shoots. Casein hydrolysate, a mixture of several amino acids, peptone and yeast extract were used in the present study. In half strength MS medium, casein hydrolysate and its combination with NAA and BA were the best for multiple shoot production as compared to peptone or yeast extract. With regard to the number leaves produced also, casein hydrolysate and its combination with NAA and BA proved to be the best in half strength MS medium. Agrawal *et al.* (1992) has also reported that casein hydrolysate along with kinetin and BA resulted in rapid proliferation of multiple shoots in *Vanilla walkeriae*.

In the present study it was further observed that in VW medium peptone along with NAA and BA produced the highest number of multiple shoots and was better than yeast extract and casein hydrolysate. The results obtained by Kukulczanka *et al.* (1989) were also on similar lines who reported that MS medium enriched with peptone stimulated the development of adventitious shoots of Vuylstekeara 'Cambria' orchid.

Yeast extract was not found to be beneficial either in VW or in half strength MS medium. When compared with casein hydrolysate or peptone. Retarding nature of yeast extract has been reported earlier by Kusumoto (1978) based on his studies on *Cymbidium*. In general, the media supplemented with casein hydrolysate

produced shoots healthier than those with peptone or yeast extract.

Coconut water has been proved to promote growth and differentiation of excised tissues and organs of several crops. The beneficial effect of coconut water has been attributed to the presence of cytokinin like substances (Straus and Rodney, 1960). Coconut water also contains a number of cell division factors and free amino acids (Shantz and Steward, 1952). Addition of coconut water to the culture medium was reported to have a beneficial effect in the production of PLBs and plantlet formation in *Dendrobium* (Kim *et al.*, 1970; Soediono, 1983).

In the present study, it was observed that, in half strength MS medium coconut water in combination with NAA and BA was superior than using coconut water alone. Homma and Asahira (1985) obtained PLBs in *Phalaenopsis* on a medium containing 10 per cent coconut milk, when used together with Thomale's macro elements and Ringe and Nitsch's minor elements, NAA and BA. Kerbuay (1984b) also reported that callus proliferation in *Oncidium varicosum* was the highest in root tips on modified VW medium with 15 per cent coconut water and 5 or 10 ppm NAA. In general, when supplied with coconut water, NAA and BA, half strength MS medium was better than VW medium in producing multiple shoots as observed from the present study. An attempt was also made to study the effect of palm endosperm liquid in half strength MS and VW media. However, in both the media, coconut water was found to be better than palm endosperm liquid in producing multiple shoots.

Other complex additives which are employed by some laboratories include fruit juices too. In *Aranda* Deborah cultures, both leaf and root formation were inhibited by tomato juice and only a slight increase in fresh weight was observed in cultures on 15 per cent tomato juice enriched medium (Loh *et al.*, 1978). Hence the influence

of tomato juice and orange juice on shoot proliferation was studied. It was observed that, tomato juice at 5 per cent level alongwith NAA 2.0 ppm and BA 5.00 ppm was better in half strength MS medium. In VW medium also tomato juice was proved to be better than orange juice on shoot proliferation. In general, shoot growth was comparatively faster in both the media enriched with tomato juice. Arditti (1967) had reported that in orchid seed germination, the few protocorms which survived in tomato juice enriched media developed normally at a very fast rate.

The exceptional property of the intra calicinal liquid of *Spathodea* in stimulating growth of normal tissues in the same way as IAA has been reported as early as in 1953 by Kovoor. In the present study, in which Spathodea bud liquid and Spathodea flower extract were tried, no significant growth regulating property was observed in half strength MS media. However, in VW medium a marginal increase in shoot number was obtained when Spathodea bud liquid at 5 per cent level and flower extracts at 2 per cent and 5 per cent levels were tried in combination with NAA 2.0 ppm + BA 5.0 ppm. Further studies are required to standardise the season, age of flower, concentration and the like factors which affect the composition of floral parts.

Banana pulp at different levels was tried by some investigators for its growth regulatory properties. Cheah and Sagawa (1978) could successfully propagate Aranda Wendy Scott 'Greenfield' and Aranthera James Storie on VW medium, supplemented with green banana 50 g/l. However, in the present study the effect of banana pulp in producing multiple shoots was only marginal in both half strength MS and VW media.

Potato extract is also suggested by some workers for the germination of

seeds, growth of seedlings and shoot proliferation. In the present study it was observed that the effect of potato extract on producing multiple shoots was marginal in half strength MS medium. However, the growth of shoots in the media supplemented with potato extract was very vigorous. In VW medium, potato extract alone and also in combination with NAA and BA produced more number of shoots than those produced by NAA and BA alone. Harvais (1982) based on his studies on *Cypripedium reginae* also reported that potato extract at 5 per cent level gave the best germination and growth.

Medicinal plant extract were also used by some investigators to check contamination. *In vitro* antifungal activity of *Cymbopogon martini*, *Pimpinella anisum* and *Vetiveria zizanioides* has been reported by Gangrade *et al.* (1991). In the present study, Oscimum leaf extract was tried at two different levels and also in combination with NAA and BA to find out whether it has any effect on multiple shoot production. It was observed that no significant increase in shoot number could be obtained with Oscimum leaf extract in either half strength MS or in VW medium. Further research should be carried out in this area to find out the correct concentration that would be beneficial for *in vitro* growth and development.

Cells in culture are fully heterotrophic or partially autotrophic and require a carbon energy source for their growth and development. Specific carbohydrate requirement may be related to the specified carbohydrate metabolism through which water relations and indigenous phytohormones are regulated. This is an important factor which can determine the success of plant tissue culture by influencing morphogenesis and growth. Sucrose is almost universally used for micropropagation purposes. It is the sugar of transport in intact plants and it is not surprising that this disac-

charide and its constituent hexoses, glucose and fructose generally best serve the carbon nutritional requirement of cells in culture. Of late, table sugar is also being used by workers with the objective of reducing the cost on carbon source. In cardamom tissue culture, sucrose and glucose were reported to give comparable performance (Reghunath, 1989). Hew *et al.* (1988) observed that fructose was the best carbon source for apical meristem culture of *Dendrobium* in VW medium. Hew and Mah (1989) reported that there was no difference in preference for sugars when differentiated and undifferentiated tissues of *Dendrobium* were cultured in VW medium with glucose, fructose or sucrose. seeds of *Cymbidium elegans* and *Coelogyne punctulata* gave best germination and seedling growth when sown on KC medium with fructose, glucose or sucrose (Sharma and Tandon, 1990).

In the present investigation it was observed that sucrose 3 per cent along with NAA 2.0 ppm and BA 5.0 ppm produced the maximum number of shoots in half strength MS medium whereas glucose was the best when used alone in ½ MS media. In VW medium when used alone, glucose at 3 per cent level was better than sucrose at the same level, but at 5 per cent level, sucrose was better than glucose, When used in combination with NAA and BA. Glucose at 3 per cent level was the best and at 5 per cent level they performed equally with respect to number of shoots produced.

In vitro regeneration of adventitious roots from the shoots produced was also studied. The effect of carbon sources on *in vitro* rooting was examined in three media, viz., half strength MS, VW and KC.

Sucrose had already been recognised (Chong and Pua, 1985) as a source of energy as well as a factor for osmoregulation for optimising the rooting response.

However, glucose at 3 per cent level in KC medium was found to be the best for rooting. Lakshmidevi (1992) reported that keeping the plantlet in the same medium for another two weeks after root initiation resulted in better survival percentage than transferring of the plantlets into the potting medium soon after the production of all roots. This was confirmed in the present investigation.

Lakshmidevi (1992) further reported that potting medium having charcoal and brick pieces was found to be better than coconut husk. Similar observations were also made by Kumar (1991). Thomas and Thomas (1992), based on their studies on *Odontoglossums* and their hybrids, reported that the plants transplanted into perlite made slower growth initially but after a year grew more rapidly. Kumar (1991), on the other hand reported that maximum survival of shoots and roots of *Dendrobium* hybrid occurred in media having rubber seed husks, coconut shell pieces and gravel. In the present study, among the different potting media tried, coconut husk was found to be slightly better than brick-charcoal (1:1) mixture.

Hardening the *in vitro* raised plantlets so as to acclimatize them to the out-side environment is very important. According to Weinright (1988) tissue cultured plants are very poorly adapted to resist the low relative humidity, higher light levels and more variable temperatures prevailing outside. Of the various hardening methods tried in the present study, keeping plantlets in the open under shade, with intermittent water sprays was found to be the best. Covering plantlets with a transparent plastic cover and providing intermittent cold water sprays was also effective (Rajmohan, 1985; Reghunath, 1989). However, in the present investigation this method was not found to be advantageous for the establishment of the plantlet.

The problem encountered in using organic additives in tissue culture is the

lack of consistency, unlike inorganic chemicals, as influenced by the source of the material. However, their role in the shoot proliferation, seed germination and seedling growth of orchids cannot be ruled out. The present studies also reveal the beneficial effects of many organic supplements. Further research trials are however required to correctly establish the types of organic additives that could be safely used to enhance the *in vitro* culturing; combination and concentration of different additives etc. are other aspects that require further attention.

SUMMARY

In order to study the effect of various medium supplements on shoot proliferation in *Dendrobium nobile*, investigations were carried out at the Plant Tissue Culture Laboratory attached to the All India Co-ordinated Floriculture Improvement Project (AICFIP), College of Horticulture, Vellanikkara.

Dendrobium nobile is an important species coming under the much renowned epiphytic orchid genus. Axillary buds of the plants of this species were cultured in ½ MS and VW media. The explants were surface sterilized with 0.1 per cent mercuric chloride for 10 minutes and inoculated. The results of the study are summarised below.

For culture establishment, NAA 1.00 ppm + BA 3.0 ppm was the best which took 32 days and 31.67 days for bud initiation in ½ MS and VW medium, respectively.

For bud expansion, in ½ MS medium a combination of NAA 1.0 ppm + BA 3.5 ppm was the best and took 35.67 days, whereas in VW medium, NAA 1.0 ppm + BA 3.0 ppm was the best which took only 25.33 days.

Addition of cytokinin related substances, viz., adenine and adenine sulphate, was found to have no significant influence on shoot proliferation either in ½ MS or in VW medium. In ½ MS medium these substances could not influence the shoot length and number of leaves produced. But in VW medium the maximum shoot length (4.03 cm) was recorded by the combination of adenine 20 ppm + NAA 2.0 ppm +

BA 5.0 ppm and maximum leaf number (5.67, each) was recorded by the combinations of adenine 20 ppm + NAA 2.0 ppm + BA 5.0 ppm and adenine 40 ppm + NAA 2.0 ppm + BA 5.0 ppm.

On considering the effect of amino acid supplements, in half strength MS medium, the combination of casein hydrolysate 50 ppm + NAA 2.0 ppm + BA 5.0 ppm recorded the maximum number of shoots (9.33) and also leaves (6.0). Yeast extract and peptone could not produce any significant effect on shoot proliferation in half strength MS medium. In VW medium, peptone 40 ppm + NAA 2.00 ppm + BA 5.0 ppm produced the maximum number of shoots (16.67). Casein hydrolysate 50 ppm recorded the maximum shoot length (3.97 cm) whereas the maximum number of leaves (6.00) was produced by casein hydrolysate 100 ppm + NAA 2.0 ppm + BA 5.0 ppm.

Of the two different palm milks employed, viz., coconut water and palm endosperm liquid, in half strength MS medium, the combination of CW 5 per cent + NAA 2.0 ppm + BA 5.0 ppm recorded the maximum number of shoots (11.33). Neither coconut water nor palm endosperm liquid significantly influenced the shoot length or number of leaves. In VW medium also CW, in combination with NAA and BA significantly influenced the production of multiple shoots and the maximum number of shoots (8.67) was produced by CW 10% + NAA 2.0 ppm + BA 5.0 ppm. CW 5 per cent produced the longest shoot (3.70 cm) and the maximum number of leaves (4.67).

Considering the effect of orange juice and tomato juice, in half strength MS medium, the treatment combination of tomato juice 5 per cent + NAA 2.0 ppm + BA 5.0 ppm produced the maximum number of shoots (8.67) and

maximum number of leaves (6.0). Neither of the fruit juice could significantly influence shoot length. In VW medium, multiple shoot production was significantly influenced by tomato juice and 5% produced the maximum number of shoots (7.67) and the maximum shoot length was recorded by the treatment combination of tomato juice 10 per cent + NAA 2.0 ppm + BA 5.0 ppm. The maximum number of leaves (6.33) was produced by tomato juice at 5 per cent.

Among the floral extracts tried, viz., Spathodea bud liquid and Spathodea flower extract, no significant effect could be seen in respect of multiple shoot production or leaf production, in half strength MS medium. But Spathodea flower extract at 5 per cent produced the longest shoots (4.43 cm) in half strength MS medium. In VW media, no significant influence was seen on multiple shoot production, but Spathodea bud liquid 2 per cent + NAA 2.0 ppm + BA 5.0 ppm recorded the maximum shoot length (3.73 cm). On the other hand, maximum leaf number (5.0) was recorded in Spathodea flower extract 2 per cent + NAA 2.0 ppm + BA 5.0 ppm treatment.

In half strength MS medium, banana pulp did not produce any significant effect on multiple shoot production, shoot elongation or leaf production. In VW medium banana pulp 10 per cent combination with NAA 2.0 ppm + BA 5.0 ppm produced the maximum number (4.33) of shoots followed by banana pulp 5 per cent + NAA 2.0 ppm + BA 5.0 ppm. The maximum shoot length (3.68 cm) and the maximum leaf number (5.33) were also produced by banana pulp 10 per cent + NAA 2.0 ppm + BA 5.0 ppm.

Potato extract, in half strength MS medium, did not induce multiple shoots, shoot elongation or leaf production significantly. But in VW medium potato extract

tried at both the levels enhanced shoot proliferation. Potato extract 10 per cent + NAA 2.0 ppm + BA 5.0 ppm produced the maximum (6.67) number of shoots and shoot length (3.38 cm). Maximum leaf number (5.33) was recorded in three treatments, viz., potato extract 5 per cent, potato extract 5 per cent + NAA 2.0 ppm + BA 5.0 ppm and potato extract 10 per cent + NAA 2.0 ppm + BA 5.0 ppm.

Oscimum leaf extract was found to have no significant influence on multiple shoot production, shoot elongation or leaf production, either in half strength MS or in VW medium.

When the different carbon sources were compared, sucrose 3 per cent along with NAA 2.0 ppm + BA 5.0 ppm produced the maximum number of shoots (6.33) and the maximum shoot length (4.23). In VW medium glucose 3 per cent in combination with NAA 2.0 ppm + BA 5.0 ppm and sucrose 5 per cent produced the maximum (5.00) shoots. The longest shoots (4.30 cm) were produced by glucose 3 per cent and the maximum number of leaves (5.67) by sucrose 5 per cent combined with NAA 2.0 ppm + BA 5.0 ppm.

For *in vitro* rooting, among the three media employed, viz., half strength MS, VW and KC, KC medium was superior. Of the different carbon sources employed, glucose 3 per cent was the best which took only 24 days for rooting in KC medium.

Survival of the *in vitro* rooted plantlets was the highest when kept in coconut husk medium. Among the various hardening treatments tried, keeping the plantlets in open was found to be the best.

REFERENCES

Agrawal, D.C., Morwal, G.C. and Mascarenhas, A.F. 1992. *In vitro* propagation and slow growth storage of shoot cultures of *Vanilla walkeriae* Wight - an endangered orchid. *Lindleyana*. 7(2):95-99

*Anderson, W.C. 1980. Mass propagation by tissue culture: Principles and techniques. *Proc. Conf. Nursery Production of Fruit Plants through Tissue Culture*. Applications and feasibility. U.S.D.A., Manylana. pp.1-10

Arditti, J. 1967. Factors affecting the germination of orchid seeds. *Bot. Rev.* 33:1-97

Arditti, J. 1977. Clonal propagation of orchids by means of tissue culture - a manual. *Orchid Biology - Reviews and Perspectives*. Cornell University Press, U.S.A., pp.203-293

*Ball, E.A. and Arditti, J. 1975. Node cultures as a means of clonal propagation in *Dendrobium. Proc. of Eighth World Orchid Conference*. Palmengarten, Frankfurt. pp.367-371

Ball, E.A., Reisinger, D. and Arditti, J. 1975. Clonal propagation of *Phalaenopsis. Malay. Orchid. Rev.* 12:6-9

Bivins, J.L. and Hackett, W.P. 1969. The effect of medium and wounding techniques in aseptic culture of *Cymbidium* orchids from shoot apices. *Plant Propagator* 15(2):9-14

Blowers, J.W. 1964. Meristem propagation and possible effects. *Orchid Rev.* 72:407-408

Bose, T.K. and Mukherjee, T.P. 1974. Effect of growth substances on seedling growth and differentiation from callus of vanda *in vitro* culture. *Orchid Rev.* 82(7):148-149

Chadha, K.L. 1992. The Indian Orchid Scenario. *J. Orchid Soc. India* 6(1,2):1-4

Cheah, K.T. and Sagawa, Y. 1978. *In vitro* propagation of *Aranda* Wendy Scott and *Aranthera* James Storei. *HortScience* 13(6):661-662

Chong, C. and Pua, E.C. 1985. Carbon nutrition of Ottawa-3 apple rootstock during stages of *in vitro* propagation. *J. Hort. Sci.* **60**:285-290

Chung, J.D., Chun, C.K., Kim, S.S. and Lee, J.S. 1985a. Factors affecting rhizome growth and organogenesis of Korean native *Cymbidium kanran*. *J. Kor. Soc. Hort. Sci.* 26(3):281-288

Chung, J.D., Chun, C.K. and Kim, S.S. 1985b. Asymbiotic germination of *Aerides japonicum*. II. Effect of plant growth regulators, sucrose, agar and pH value on seedling growth. *J. Kor. Soc. Hort. Sci.* 26(4):365-374

Davidonis, G. and Knor, D. 1991. Callus formation and shoot regeneration in *Vanilla planifolia*. *Food Biotech.* 5(1):59-66

Davis, M.K., Baker, R. and Hannan, J.J. 1977. Clonal multiplication of carnation by micropropagation. *J. Am. Soc. Hort. Sci.* 102:48-53

Devi, J., Nath, M., Devi, M. and Deka, P.C. 1990. Effect of different media on germination and growth of some North-East Indian species of *Dendrobium*. *J. Orchid Soc. India.* 4(1-2):45-49

Fernando, K.K.S. 1979. Clonal propagation of *Dendrobium* Caesar Red Lip by meristem culture. *Trop. Agriculturist* 135:111-113

Fu, F.M.L. 1978. Clonal propagation of Aranda, Ascocenda and Cattleya by leaf tissue culture. *Gardens Bull.* 31(2):132-138

Gamborg, O.C. and Shyluck, J.P. 1981. Nutrition, media and characteristics of plant, cell and tissue culture. In: Thorpe, P.A. (Ed.). *Plant Tissue Culture Method and Application in Agriculture*. Academic Press, New York. pp.21-44

*Gandawidjaja, D. 1980. Effects of nitrate and ammonium on growth of tissue culture of *Dendrobium phalaenopsis* Fitzg. *Annales Bogorienses* 7(2):63-69

Gangrade, S.K., Shrivastava, R.D., Sharma, O.P., Jain, N.K. and Trivedi, K.C. 1991. *In vitro* antifungal effect of the essential oils. *Indian Perfumer* 35(1):46-48

Garay, L. 1960. On the origin of orchidaceae. *Proc. 3rd World Orchid Conf.* pp.172-195

Gilliland, H.B. 1958. On the symmetry of the orchid embryo. *Proc. Centennary and Bicentennary Conf. of Bio.* Singapore. pp.276-279

Goh, C.J. and Tan, H. 1982. Clonal propagation from leaf explants in Renantanda orchid hybrid. *Orchid Rev.* 90(1067):295-296

Greisbach, R.J. 1983. The use of Indole acetyl amino acids in the *in vitro* propagation of *Phalaenopsis* orchids. *Scientia Horticulturae* 19(3/4):363-366

Gu, Z., Higaki, P.C., Nishida, M.M., Arditti, J. and Nyman, L.P. 1987. The effect of benzyl adenine, 2,4-D, IAA on shoot tip cultures of *Cymbidium. Lindleyana* 2(2):88-90

*Gu, Z.P. and Yan, T.J. 1989. Study on shoot tip culture and histological observations of *Cymbidium. grandiflorum. Acta Biologiae Experimentalis Sinica* 22(2):149-152

Harvais, G. 1982. An improved culture medium for growing the orchid *Cypripedium reginae* axenically. *Can. J. Bot.*, 60:2547-2555

Hasegawa, A. and Goi, M. 1987. Rhizome formation in *Cymbidium goeringii* Reichenbachfil and *Cymbidium kanran* Makinoin shoot tip culture. *J. Jap. Soc. Hort. Sci.* 56(1):70-78

Hasegawa, A., Ohashi, H. and Goi, M. 1985. Effects of BA, rhizome length, mechanical treatment and liquid shaking culture on the shoot formation from rhizome in *Cymbidium faberi* Rolfe. *Acta Horticulturae* 166:25-40

Hew, C.S. and Mah, T.C. 1989. Sugar uptake and invertase activity in *Dendrobium* tissues. *New Phytologist* 111(2):161-171

Hew C.S., Ting, S.K. and Chia, T.F. 1988. Substrate utilization by *Dendrobium* tissues. *Botanical Gazettee.* 149(2):153-157

Homma, Y. and Asahira, T. 1985. New means of *Phalaenopsis* propagation with internodal sections of flower stalk. *J. Jap. Soc. Hort. Sci.* 54(3):379-387

Ilahi, I. and Jabeen, M. 1987. Micropropagation of *Zingiber officinale* L. *Pak. J. Bot.* 19:61-66

Intuwong, O. and Sagawa, Y. 1973. Clonal propagation of Sarcanthine orchids by aseptic culture of infloresence. *Am. Orchid Soc. Bull.* 42:209-215

Intuwong, O. and Sagawa, Y. 1975. Clonal propagation of *Dendrobium* Goldenwave and other *nobile* types. *Am. Orchid Soc. Bull.* 44(4):319-322

Irawati, S.S., Suseno, H. and Idris, S. 1977. Tissue culture of *Aranthera* James Storie. *Orchid Rev.* 85:138-142

*Jambor-Benezer, E., Marta, K. and Mandy, A. 1986. *In vitro* pretreatment of *Phalaenopsis* buds. *Kerteszeki Egyetem Kozlemenyei.* 50(18):183-189

Johansen, M.B. 1967. Meristem propagation of orchids. *HortScience* 21:163-175

Kerbauy, G.B. 1984a. *In vitro* flowering of *Oncidium varicosum* mericlones (Orchidaceae). *Plant Sci. Lett.* 35(1):73-75

Kerbauy, G.B. 1984b. Plant regeneration of *Oncidium varicosum* (Orchidaceae) by means of root tip culture. *Plant Cell Rep.* 3:27-29

Kim, Y.J., Hong, Y.P., Park, Y.K., Cheung, S.K. and Kim, E.Y. 1988. *In vitro* propagation of *Cymbidium kanran. Rep. of the Rural Development administration, Horticulture.* 30(3):77-82

Kim, K.W. and Kako, S. 1982. Effect of plant growth regulators on organ formation in Cymbidium shoot apex cultured *in vitro. J. Jap. Soc. Hort. Sci.* 51(1):106-107

Kim, K.W. and Kako, S. 1984. Morphological and histological studies on the formation of protocorm - like bodies and explant development in *Cymbidium* shoot apex culture *in vitro. J. Kor. Soc. Hort. Sci.* 25(2):156-163

Kim, K.H., Ko, T.S., Kim, K.H., Jin, S.G., Song, C.H., Lee, W.H. and Lee, J.S. 1990. Studies on the aseptic multiplication *in vitro* by protocorm culture of *Aerides japonicum* and plantlet transplantation to the open field. *Res. Rep. of the Rural Development Administration, Horticulture.* 32(2):42-49

Kim, K.K., Kunisaki, J.T. and Sagawa, Y. 1970. Shoot tip culture of Dendrobium. *Am. Orchid Soc. Bull.* **39**:1077-1080

Knudson, L. 1946. A new nutrient solution for the germination of orchid seeds. *Am. Orchid Soc. Bull.* **15**:214-217

Kovoor, A. 1953. The biochemistry of action of Indole acetic acid on plant growth. *Compt. rend* **236**:309-311

Kukulczanka, K., Czastka, B. and Marczoner, A. 1987. The effect of the substratum on the *in vitro* culture of cymbidium. *Acta Agrobotanica* **40**(1-2):41-51

Kukulczanka, K., Kromer, K. and Roginska, B. 1989. Joint effect of growth regulators and peptone on regeneration of vuylstekeara 'cambira' orchid under tissue culture condition. *Acta Hort.* 251:161-166

Kukulczanka, K. and Wojciechowska, U. 1983. Propagation of two *Dendrobium* species by *in vitro* culture. *Acta Hort.* **131**:105-110

Kumar, P.K.S. 1991. Post-transplantation growth of *Dendrobium* hybrid seedlings in community pots. Paper presented for National Seminar on Biology, Improvement, Propagation and Commercialization of Indian Orchids at I.I.H.R., Bangalore.

Kusumoto, M. 1978. Effect of combination of growth regulating substances and of organic matter on the proliferation and organogenesis of *Cymbidium* protocorm cultured *in vitro*. *J. Jap. Soc. Hort. Sci.* **47**:391-400

Kusumoto, M. 1979. Effect of combination of growth regulators and of organic supplements on the proliferation and organogenesis of *Cattleya* PLBs cultured *in vitro*. *J. Jap. Soc. Hort. Sci.* 47(4):502-510

Kusumoto, M. 1980. Effects of coconut milk, agar and sucrose concentrations and medium pH on the proliferation of cymbidium protocorm like bodies cultured *in vitro*. *J. Jap. Soc. Hort. Sci.* 48(4):503-509

Lakshmidevi, S. 1992. *Standardisation of explant for in vitro propagation in Dendrobium* sp. M.Sc. thesis submitted to Kerala Agricultural University, Vellanikkara, Trichur.

Lam, T.W., Ernst, R., Arditti, J. and Ichihashi, S. 1991. The effects of complex additives and 6-(r-r-dimethyl allyl amino) purine on the proliferation of *Phalaenopsis* protocorms. *Lindleyana* 6(1):24-26

Lin, C.C. 1986. *In vitro* culture of flower stalk internodes of *Phalaenopsis* and *Doritaenopsis*. *Lindleyana* 1(3):158-163

Loh, C.S., Goh, C.J. and Rao, A.N. 1978. Some factors affecting morphogenesis of *Aranda* orchid tissue culture. *Proc. Symp. Orchidology*. Orchid Soc. South East Asia, Singapore. pp.43-55

Lundergan, C.A. and Janick, J. 1980. Regulation of apple shoot proliferation and growth *in vitro*. *Hort. Res.* 20:19-24

Madhurisharon and Vasundhara, G. 1990. Micro propagation of *Dendrobium* Joannie Ostenhault. *J. Orchid Soc. India.* 4(1-2):145-148

Maheshwari, J.K. 1980. Indian Orchids as an endangered plant group. In *Progress in Orchid Research* (Proceedings of National Symposium on Orchids). Chadha, K.L. and Singh, F. (ed.). I.I.H.R., Banglore. pp.40-49.

Matsui, T., Kawai, K. and Samata, Y. 1970. The effect of BA and NAA on organogenesis in *Cymbidium*. *Bull. Pac. Agr.* Tanagawa Univ. 10:99-106

Mikami, T., Kinoshita, T. and Saito, H. 1985. Clonal propagation of sugarbeet plants by apical meristem culture. *J. Fac. agric.* Hokkaido Univ. 62:325-331

Morel, G. 1965. Clonal propagation of orchids by meristem culture. *Cymbidium Soc. News.* 20(7):3-11

Mosich, S.K., Ball, E. and Arditti, J. 1974. Clonal propagation of *Dendrobium* by means of node cultures. *Am. Orchid Soc. Bull.* 43(12):1055-1061

Mowe, B.L. 1973. Germination and growth of *Dendrobium* (Inoglossum x Species) on several culture media. *Singapore J. Prim. industries* 1(1):20-30

Murashige, T. 1974. Plant propagation through tissue culture. *Ann. Rev. Plant Physiol.* 25:473-497

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol Plantarum* 15:473-497

Nimote, D.H. and Sagawa, Y. 1961. Ovule development in *Dendrobium. Am. Orchid Soc. Bull.* 30:813-819

Nitsch, J.P., Nitsch, C., Rossini, L.M.E. and Ha, B.P. 1967. The role of adenine in bud differentiation. *Phytomorph.* 17:446-453

Paek, K.Y. and Chun, C.K. 1985. Effect of NH_4^+, Ca^{2+} and Mg^{2+} concentrations on organogenesis and nutrient content in *Cymbidium* protocorm culture. *J. Kor. Soc. Hort. Sci.* 26(2):193-199

Paek, K.Y., Shim, G.B. and Kim, J.J. 1989. Exploitation of temperate Cymbidium and establishment of a micropropagation system. 1. Asymbiotic germination of temperate cymbidium and the effect of media and growth regulators on organogenesis. *J. Kor. Soc. Hort. Sci.* 30(3):234-247

Paek, K.Y. and Yeung, E.C. 1991. The effect of 1-Naphthalene Acetic Acid and N^6 Benzyl Adenine on the growth of *Cymbidium forrestii* rhizomes *in vitro*. *Plant Cell, Tissue Organ Cult.* 25(2):65-71

Panse, V.G. and Sukatme, P.V. 1985. *Statistical methods for agricultural workers.* 4th ed. I.C.A.R., New Delhi. pp.131-143

*Payawal, P.C. 1976. Histological response of Singapore Orchid to growth regulators. Kalikasan, *Philippine J. Biol.* 5(3):341-350

Rajmohan, K. 1985. *Standardisation of tissue/meristem culture techniques in important horticultural crops.* Ph.D. thesis, Kerala Agricultural University, Vellanikkara.

*Raskauskas, V., Kazlavskiere, R., Gudaviciene, N. and Sirvydyte, D. 1989. The selection of sterilizing material and nutrient medium for orchid propagation by meristem culture. *Lietuvos TSR Aukstuju Mokykiu Mokslo Darbai, Bidogia* 27:36-42

Reghunath, B.R. 1989. *In vitro studies on the propagation of cardamom (Elettaria cardamomum* Maton). Ph.D. thesis submitted to Kerala Agricultural University, Vellanikkara.

Rosa, M., Dalla, V. and Laneri, V. 1977. Modification of nutrient solution for germination and growth *in vitro* of some cultivated orchids and for the vegetative propagation of *Cymbidium* cultivars. *Am. Orchid Soc. Bull.* **46**(9):813-820

Sagawa, Y. 1961. Vegetative propagation of *Phalaenopsis* by stem cuttings. *Am. Orchid Soc. Bull.* **30**:808-809

Sagawa, Y. and Kunisaki, J.T. 1982. Clonal propagation of orchids by tissue culture. A.Fujiwara (Ed.). *Plant tissue culture.* Jpn. Assoc. Plant Tissue Cult., Tokyo. pp.683-684

Sagawa, Y. and Shoji, T. 1969. Clonal propagation of Dendrobiums through shoot meristem culture. *Am. Orchid Soc. Bull.* **36**:856-859

Sagawa, Y., Shoji, T. and Shoji, J. 1967. Clonal propagation of *Cymbidiums* through shoot meristem culture. *Am. Orchid Soc. Bull.* **35**:118-122

Sagawa, Y. and Valmayor, H.C. 1966. Embryo culture of Orchids. Dngarno, L.H. (Ed.). *Proc. Fifth World Orchid Conf.* Long Beach. pp.99-101

Sanguthai, S. and Sagawa, Y. 1973. Induction of polyploidy in *Vanda* by Colchicine treatment. *Hawaii Orchid J.* 2:17-19

Scully, R.M. 1965. Stem propagation of *Phalaenopsis*. *Bull. Pacific Orchid Soc.*. **23**:13-16

Scully, R.M. 1966. Stem propagation of *Phalaenopsis*. *Am. Orchid Soc. Bull.* **35**:40-42

Shantz, E.M. and Steward, F.C. 1952. Coconut milk factor: the growth promoting substances in coconut milk. *J. Am. Chem. Soc.* 74:393

Sharma, S.K. and Tandon, P. 1990. Asymbiotic germination and seedling growth of *Cymbidium elegans* Lindl. and *Coelogyne punctulata* Lindl. as influenced by different carbon sources. *J. Orchid Soc. India.* 4(1-2):149-159

Sheehan, T.J. 1983. Recent advance in botany, propagation and physiology of orchids. Janick, J. (Ed.). *Hort. Rev.* **5**:279-315

*Shimasaki, K. and Uemoto, S. 1987. Studies on the micropropagation of Japanese *Calanthe* species. *Sci. Bull. Faculty Agrl.* Kyushu University. 42(1-2):9-14

Shimasaki, K. and Uemoto, S. 1991. Rhizome induction and plantlet regeneration of *Cymbidium goeringii* from flower bud cultures *in. vitro*. *Plant Cell, Tissue and Organ Cult.* 25(1):49-52

*Singh, H. and Sagawa, Y. 1972. Vegetative propagation of *Dendrobium* by flower stalk cuttings. *Na Pusa Okika Hawaii Net.* 11:13

Soebijanto and Widiastoety, D. 1987. Stimulation of growth of orchid seedlings *Aranda* cv. Christine No.130. *Bull. Penelitan Hortikultura.* 15(3):25-31

Soediono, N. 1983. Use of coconut water, NAA, 2,4-D and Vitamins in shoot tip cultures of *Dendrobium* cv. Jacqueline Thomas 'White'. *Orchid Rev.* 91(1073):86-87

Start, N.D. and Cumming, B.G. 1976. *In vitro* propagation of *Saintpaulia ionantha*. *HortScience* 11:204-206

Stewart, J. and Button, J. 1978. Development of callus from *Epidendrum* root tips culture *in vitro*. *Am. Orchid Soc. Bull.* 47:607-612

Straus, J. and Rodney, R.E. 1960. Response of *Cupressus funebris* tissue culture to gibberellins. *Sci.* 131:1806-1807

Tanaka, M. and Sakanishi, Y. 1985. Regeneration capacity of *in vitro* cultured leaf segments excised from mature *Phalaenopsis* plants. *Bull. Univ. Osaka Prefect Ser B* 37:1-4

Thakur, R.N., Singh, P. and Khosla, M.K. 1989. *In vitro* studies on antifungal activities of some aromatic oils. *Indian Perfumer* 33(4):257-260

Thomas, W. and Thomas, B. 1992. Convenient combination. *Am. Orchid Soc. Bull.* 61:582-587

Vacin, E. and Went, F. 1949. Some pH changes in nutrient solution. *Bot. Gaz.* 110:605-613

Valmayor, H.L. 1974. Further investigation into nutrient media. Ospiha, H.H. (Ed.). *Proc. Seventh World Orchid conf.* Columbia. pp.211-229

Van der Pijl, L. and Dodson, C.H. 1966. *Orchid flowers, their pollination and evolution.* University of Miami Press, Miami.

Vij, S.P., Sood, A. and Sharma, M. 1991. Morphogenetic response of floral buds of *Dendrobium*: a study *in vitro*. Paper presented at the National Seminar on Biology, Improvement, Propagation and Commercialisation of Indian Orchids. IIHR, Bangalore.

Wang, X. 1988. Tissue culture of Cymbidium: plant and flower induction *in vitro*. *Lindleyana*. **3**(4):184-189

Weinright, H. 1988. Overcoming Problems in establishing micropropagules - guidelines for growers. *Prog. Hort.* 2(3):67-72

White, P.R. 1943. Nutrient deficiency studies and an improved nutrient for cultivation of excised tomato roots. *Growth* 7:53-65

* Widiastoety, D. 1986. Experiments on various kinds of media and buds from different sources in the tissue culture of an orchid (*Aranthera* James Storie). *Bulletin Penelitian Hortikultura* 13(3):1-8

Yadav, R.N. 1989. *in vitro* antimicrobial studies on the essential oil of *Ixora coccinea* Linn. *Indian Perfumer* **3**(2):128-129

Yam, T.W. and Weatherhead, M.A. 1991. Root tip cultures of several native orchids of Hong Kong. *Lindleyana* **6**(3):151-153

Yoneda, K., Sakamoto, T. and Sasaki, H. 1979. Studies on mericlones of orchids. 1. Propagation of cattleya protocorms by means of meristem culture. *Bull. of the College of Agric. and Veterinary Medicine*. **6**(36):64-73

* Zimmer, K., Seemann, P. and Holters, J. 1981. Previcur-N application after tissue culture. *Devstscher Gartenbav* **36**(29):1195-1197

Ziv, M. 1986. *In vitro* hardening and acclimatization of tissue culture plants. Winther, L.A. and Alderson, P.G. (Ed.). *Plant Tissue Culture and its Agricultural Applications*, Butterworths, London, pp.187-196

* Originals not seen

Plates

Bud expansion and bud initiation

Effect of CH (50 ppm) + NAA (2.0 ppm) + BA (5.0 ppm) on shoot proliferation in ½ MS medium

Effect of CH (100 ppm) + NAA (2.0 ppm) + BA (5.0 ppm) on shoot proliferation in ½ MS medium

Effect of CH (50 ppm) + NAA (2.0 ppm) + BA (5.0 ppm) on shoot proliferation in VW medium

BORO
Test

Effect of CH (100 ppm) + NAA (2.0 ppm) + BA (5.0 ppm) on shoot proliferation in VW medium

Effect of peptone (40 ppm) + NAA (2.0 ppm) + BA (5.0 ppm) on shoot proliferation in ½ MS medium

Effect of peptone (40 ppm) + NAA (2.0 ppm) + BA (5.0 ppm) on shoot proliferation in VW medium

—

Effect of CW (10 %) + NAA (2.0 ppm) + BA (5.0 ppm) on shoot proliferation in ½ MS medium

—

9 Effect of CW (5 %) + NAA (2.0 ppm) + BA (5.0 ppm) on shoot proliferation in ½ MS medium

10 Effect of CW (5 %) + NAA (2.0 ppm) + BA (5.0 ppm) and CW (10 %) + NAA (2.0 ppm) + BA (5.0 ppm) on shoot proliferation in VW medium

11 Effect of potato extract (10 %) + NAA (2.0 ppm) + BA (5.0 ppm) on shoot proliferation in ½ MS medium

12 Effect of potato extract (10 %) + NAA (2.0 ppm) + BA (5.0 ppm) on shoot proliferation in VW medium

13 Comparison between KC media and ½ MS media on *in vitro* rooting with glucose as carbon source

14 Planted out *in vitro* plantlets


BORO
TEST

15 *In vitro* plantlets planted out in different potting media

16 *In vitro* plantlets under controlled atmospheric conditions

APPENDIX-I

Chemical composition of the media

Chemical	Quantity (mg/l)		
	KC	MS	VW
1	2	3	4
Major elements			
$Ca(PO_4)_2$	-	-	200
$CaCl_2.2H_2O$	-	440	-
$Ca(NO_3)_2.4H_2O$	1000	-	-
Ferric citrate	-	-	28
$FeSO_4.H_2O$	25	27.8	-
KNO_3	-	1900	525
KH_2PO_4	250	170	250
$MgSO_4.7H_2O$	250	370	250
$(NH_4)_2SO_4$	250	-	500
NH_4NO_3	-	1650	-
Na_2.EDTA	-	37.3	-
Minor elements			
$CoCl_2.6H_2O$	-	0.025	-
$CuSo_4.5H_2O$	-	0.025	-
H_3BO_3	-	6.2	-
Kl	-	0.83	-
$MnSO_4$	7.5	22.3	7
$Na_2MoO_4.2H_2O$	-	0.25	-
$ZnSO_4$	-	8.6	-

Contd

Appendix-I. Continued

1	2	3	4
Organic constituents			
Glycine	-	2.0	-
Myo-inositol	-	100	-
Nicotinic acid	-	0.5	-
Pyridoxine HCl	-	0.5	-
Thiamine HCl	-	0.1	-
Sucrose	20.00 g	30.00 g	20.00 g
Agar	9.00 g	6.00 g	9.00 g

APPENDIX-II

Abstract of analysis of variance for the effect of different treatments

Sl. No.	Character	Treatment MSS	Error MSS	Level of significance %
1	Period (days for culture establishment)			
	a) ½ MS medium			
	i) Bud initiation	167.27	6.10	5
	ii) Bud expansion	109.88	6.27	5
	b) VW medium			
	i) Bud initiation	227.03	9.63	5
	ii) Bud expansion	323.99	6.40	5
2	Effect of cytokinin related substances on shoot proliferation			
	a) ½ MS medium			
	i) Shoots/culture	4.63	2.47	5
	ii) Length of shoot	1.07	0.28	5
	iii) Number of leaves	2.50	0.77	5
	b) VW medium			
	i) Shoots/culture	25.07	2.67	5
	ii) Length of shoot	0.98	0.72	5
	iii) Number of leaves	4.30	0.43	5
3	Effect of amino acid supplements on shoot proliferation			
	a) ½ MS medium			
	i) Shoots/culture	19.38	2.36	5
	ii) Length of shoot	1.92	0.22	5
	iii) Number of leaves	4.91	0.62	5
	b) VW Medium			
	i) Shoots/culture	74.39	39.21	5
	ii) Length of shoot	1.39	0.49	5
	iii) Number of leaves	3.83	0.60	5

Appendix-II. Continued

Sl. No.	Character	Treatment MSS	Error MSS	Level of significance %
4	Effect of palm milk on shoot proliferation			
	a) ½ MS medium			
	i) Shoots/culture	26.15	4.87	5
	ii) Length of shoot	1.38	0.59	5
	iii) Number of leaves	2.85	0.77	5
	b) VW medium			
	i) Shoots/culture	35.12	3.43	5
	ii) Length of shoot	0.96	0.27	5
	iii) Number of leaves	1.49	0.80	5
5	Effect of fruit juices on shoot proliferation			
	a) ½ MS medium			
	i) Shoots/culture	10.24	2.60	5
	ii) Length of shoot	1.26	0.21	5
	iii) Number of leaves	3.43	0.67	5
	b) VW medium			
	i) Shoots/culture	30.68	2.27	5
	ii) Length of shoot	4.77	0.33	5
	iii) Number of leaves	3.12	0.43	5
6	Effect of flower extracts on shoot proliferation			
	a) ½ MS medium			
	i) Shoots/culture	4.58	0.23	5
	ii) Length of shoot	0.62	0.16	5
	iii) Number of leaves	1.74	1.27	5
	b) VW Medium			
	i) Shoots/culture	24.26	1.53	5
	ii) Length of shoot	0.40	0.31	5
	iii) Number of leaves	1.24	0.73	5

contd.

Appendix-II. Continued

Sl. No.	Character	Treatment MSS	Error MSS	Level of significance %
7	Effect of banana pulp on shoot proliferation			
	a) ½ MS medium			
	i) Shoots/culture	0.46	0.28	5
	ii) Length of shoot	2.41	0.27	5
	iii) Number of leaves	3.69	1.11	5
	b) VW medium			
	i) Shoots/culture	43.66	2.28	5
	ii) Length of shoot	0.64	0.34	5
	iii) Number of leaves	5.92	0.33	5
8	Effect of potato extract on shoot proliferation			
	a) ½ MS medium			
	i) Shoots/culture	4.06	2.33	5
	ii) Length of shoot	1.59	0.24	5
	iii) Number of leaves	1.82	0.83	5
	b) VW medium			
	i) Shoots/culture	34.32	3.44	5
	ii) Length of shoot	0.75	0.23	5
	iii) Number of leaves	3.42	0.44	5
9	Effect of medicinal plant extracts on shoot proliferation			
	a) ½ MS medium			
	i) Shoots/culture	6.77	0.72	5
	ii) Length of shoot	2.41	0.27	5
	iii) Number of leaves	2.36	0.77	5
	b) VW Medium			
	i) Shoots/culture	46.76	2.11	5
	ii) Length of shoot	0.58	0.19	5
	iii) Number of leaves	1.02	0.17	5

contd.

Appendix-II. Continued

Sl. No.	Character	Treatment MSS	Error MSS	Level of significance %
10	Effect of carbon sources on shoot proliferation			
	a) ½ MS medium			
	i) Shoots/culture	7.62	1.72	5
	ii) Length of shoot	1.81	0.26	5
	iii) Number of leaves	1.81	0.78	5
	b) VW medium			
	i) Shoots/culture	16.75	2.46	5
	ii) Length of shoot	0.75	0.24	5
	iii) Number of leaves	2.22	0.83	5
11	Effect of carbon sources on in vitro rooting			
	Days for root initiation	285.75	1.59	1

STANDARDISATION OF MEDIUM SUPPLEMENTS FOR SHOOT PROLIFERATION IN *Dendrobium*

By

R. SUDEEP

ABSTRACT OF A THESIS

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requirement for the degree of

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ABSTRACT

Investigations were carried out at the Plant Tissue Culture Laboratory attached to the All India Co-ordinated Floriculture Improvement Project (AICFIP), College of Horticulture, Vellanikkara, during 1991-'93 to study the effect of various medium supplements on shoot proliferation in *Dendrobium*. Axillary buds of *Dendrobium nobile* were used as explants for inoculation. The explants were surface sterilized with 0.1% mercuric chloride for 10 minutes. Half strength MS medium and VW medium were employed for culture establishment and shoot proliferation.

The addition of cytokinin related substances, viz., adenine and adenine sulphate did not have any significant influence on shoot proliferation in either of the media. However, adenine influenced the production of leaves and length of shoots in MS medium, in combination with NAA 2.0 ppm and BA 5.0 ppm.

Among the amino acid supplements tried, casein hydrolysate influenced multiple shoot production and leaf production in half strength MS medium. In VW media, peptone enhanced the production of multiple shoots whereas shoot length and leaf production were promoted by casein hydrolysate.

Coconut water increased the number of shoots in both half strength MS medium and VW medium. In VW medium length of shoot and number of leaves also were influenced.

Tomato juice in combination with NAA and BA produced the maximum number of shoots and leaves in half strength MS medium. It also influenced the shoot production, shoot length and leaf production in VW medium.

Spathodea flower extract influenced shoot elongation in half strength MS medium. Spathodea bud liquid influenced shoot elongation and flower extract, the number of leaves, in VW media.

Banana pulp could significantly influence the shoot length and leaf number in VW medium alone.

Potato extract could not significantly influence shoot production in ½ MS medium, but in VW medium it significantly influenced shoot production, shoot length and leaf number. The shoots produced in media with potato extract was healthier when compared with others.

Oscimum leaf extract was found to have no significant influence in either of the media for any of the characters studied. In half strength MS medium, sucrose 3 per cent + NAA 2.0 ppm + BA 5.0 ppm and in VW media glucose 3 per cent + NAA 2.0 ppm + BA 5.0 ppm or sucrose 5 per cent was the best for producing multiple shoots. The shoots were the longest in ½ MS medium with sucrose but in VW medium, glucose was better. For *in vitro* rooting, KC medium with 3 per cent glucose was the best.

The maximum survival percentage of the *in vitro* plantlets occurred when coconut husk was used as the potting media and when the plantlets were kept in the open.

Among all the treatments tried, the best treatment for multiple shoot production was peptone 40 ppm + NAA 2.0 ppm + BA 5.0 ppm in VW medium which produced 16.67 shoots on an average. The longest shoot (5.53 cm) was produced by the combination of tomato juice 10 per cent + NAA 2.0 ppm + BA 5.0 ppm in VW

media. The treatment that produced the maximum leaves (6.33) was tomato juice 5 per cent in VW media.