MICROPROPAGATION STUDIES IN DRACAENA AND CORDYLINE

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1. INTRODUCTION

Dracaena, commonly known as Dragon tree belongs to family Dracaenaceae. The generic name is derived from the Greek word 'drakinia' meaning the dragons blood. It consists of about 40 species, some of them being grown as ornamentals. They have originated from tropical regions of Asia and Africa. Dashing dracaena is a high value commercial foliage ornamental plant in the national and international floricultural market. It occupies a position among the top 10 pot plants in the global floriculture trade.

Cordyline, originated from the tropical and sub-tropical regions of the world, East Himalayas, China, Malaysia and North Australia. Cordyline commonly called as cabbage palm and the generic name is derived from Greek word 'Kordyle' meaning club or cudgel referring to the shape of the roots (Pal, 2006). The cordyline genera belongs to family Laxmanniaceae (Liamas, 2003). Cordyline is very closely related to dracaena and differs from the latter only in the structure of ovary. Several species of cordyline are commonly known as dracaena (Randhawa and Mukhopadhyay, 2004). It is a long slender bush up to 3m in height and priced for its rich coloured foliage, including variegated forms. Cordylines and dracaenas are excellent pot plants in their juvenile state and some of them are also suitable for growing in borders and shrubberies under a mild climate, for shade gardens and greenhouse. At young stage of growth most of the species are widely used for table decorations.

The magnificent, delightful and charming foliages of dracaenas are used in floral designs, bouquets, wreaths, dried arrangements and indoor gardening. The plant commonly found in houses, offices, shops, banks, hotels, restaurants, clubs, hospitals and schools and sometimes several plants are grouped and grown in terrariums, bottles, bowls, dishes, troughs and aquarium cases as elegant table decorations (Beura *et al.*,2007).

Ornamental foliage plants from the world's tropical and subtropical regions provide the basis for today's foliage plant industry. The trade of ornamental foliage plants is comparatively a new venture in India and is gaining popularity. As foliage plants are produced primarily for interior decoration or plantscaping, a continued desire for multicolour foliage plants in interior designs has resulted in a dramatic increase in ornamental foliage plant production. At present, most of the foliage plants are exported to Germany, USA, Netherlands, U.K., Italy and Japan, constituting nearly 60 percent of India's floricultural exports (Ray *et al.*, 2006). Tissue culture technique is now used for propagation of numerous ornamental plants for commercial purposes and there has been a big market demand for exotic cultivars of dracaena and cordyline.

These foliage plants are propagated commercially by vegetative methods by division of suckers in species producing suckers or more frequently by air layering and from node or terminal cuttings of the stem. The old stems are cut into bits, each containing one node and then placed horizontally in moist sand in seed pans with the bud pointing upwards. The sand is kept just moist and the cuttings soon develop roots and start growing. Terminal cuttings also root, but the plants are not as good as those from node cuttings. The rate of multiplication is very slow as terminal cutting produces a single plant and a matured dracaena plant produces 3-4 plants through stem cuttings or 2-3 suckers at the base. The traditional method produces less number of plants from a single plant and also takes more time. Micro propagation technique overcomes these issues by giving more number of plants within a short period of time.

Micropropagation is a very economical means of multiplying a desirable plant species when time, space and personnel are often serious constraints. It is also possible to produce disease free, uniform propagules at a required quantity and at an appropriate time of the year. The use of tissue cultured planting material can rationalize foliage culture because direct planting into pots without special manipulation for a large harvest of propagule is possible. Moreover, the conventional mode of propagation enables us to produce only four daughter plants from one mother plant (Khan *et al.*, 2004). Therefore micro propagation by means of *in vitro* techniques is of great interest in order to speed up the propagation rate and to reduce the need for mother plants (George and Sherrington, 1993). The present study aims to optimize the micro propagation protocol for cordyline and dracaena in order to make the propagation feasible and economical under the following objectives,

1. To standardize the surface sterilization of explants.

2. To identify a suitable explant for shoot initiation.

3. To identify suitable growth regulators and their concentration for shoot multiplication and rooting.

4. To find out suitable media for hardening and *ex vitro* rooting of micropropagated plantlets.

2. REVIEW OF LITERATURE

It was Schwann who first drew attention to the fact that an individual cell has the ability to both grow and divide in a self regulatory manner and that an individual cell has the unique property of being totipotent, that is, whatever be its level of differentiation it will retain the capacity to regenerate into the whole organism of which it was once a part. These ideas paved way for the “cell theory”, the fundamental basis underlying plant cell tissue culture today (Collin and Edwards, 1998).

Propagation of ornamental plants by *in vitro* techniques has in recent years become a common place commercial practice. Haberlandt presented the fundamental principles of plant tissue culture which marked the commencement of the golden era in the field of plant tissue culture. The discovery and documentation of the role of plant hormones like auxins (Went and Thimann, 1937) and cytokinin (Skoog and Miller, 1957) in plant tissue culture served as major thrust for the advancements in this field of science. In addition to these discoveries, the invention of the culture medium by Murashige and Skoog (1962) laid the perfect foundation for a wide avenue for research on *in vitro* proliferation and multiplication of different plant species (Read *et al.,* 1998).

Work done on dracaena and cordyline is meager and as they are rhizomatous herbaceous plant literature of crops like banana, ginger and turmeric etc., have been reckoned as more related crops and literature on those crops also was reviewed and presented below along with the few available works on the species under study. The work done is reviewed under following headings.

2.1 Decontamination

Decontamination was reduced by shaking stempieces of dracaena in ethanol 70% for 10 seconds, followed by sterilization with commercial bleach 10% for 30 minutes and rinsing thrice with autoclaved water (Deberg, 1975). Vinterhalter (1989) also followed a similar method of surface sterilization for the micropropagation of Dracaena using commercial bleach 10% for 20 minutes. Chua *et al.* (1981) reported that the explants were disinfested by treating them with 0.5% sodium hypochlorite for 10 minutes along with several drops of wetting agent (Tween 20), after which they were rinsed several times in sterile water and placed on a sterile medium.

Vardja and Vardja (2001) sterilized the explants depending on their character and the initial material like leaves, buds, shoot segments, flower stalk, etc. by shaking in 70 to 96% ethanol for 10 to 60 seconds, followed by treating with freshly prepared and filtered chlorinated lime or chloramine solution at 6 to 9% for 10 to 30 minutes along with Tween-80 as a detergent. Thereafter, they were washed three times with sterilized distilled water. However, Khan *et al.* (2004) reported that the frequency of sterile Cordyline explants was increased when they were washed for 15-20 minutes in 20% NaOCl solution. This treatment was considered better than the one which involved the use of $HgCl_2$ due to its lethal toxicity to plants.

Badawy *et al.* (2005) observed the highest mean value of healthy, contamination free explants of *Dracaena fragrans* by using mercuric chloride 0.3 g/l. Considering all the viable characters such as survival percentage, aseptic culture, days to bud emergence, Beura *et al.* (2007) concluded that the sterilization of explant with 0.1 % $HgCl_2$ for 5minutes was found to be the best.

2.2 Explant

The type of explants, its size and the manner in which it is cultured can affect the initiation of the cultures and further morphogenetic response (Murashige, 1974). Often there is an optimum size of explants suited to initiate cultures. Very small shoot tips or fragments do not survive well while, it is difficult to decontaminate larger explants. The size of explant is also likely to influence the uptake of mineral salts irrespective of whether it is grown on liquid or solid medium (George and Sherrington, 1984).

Deberg (1975) reported that the dracaena stem pieces used for culture had 1 cm broad cut from 1 cm high disks with average thickness of 3 mm in the centre. In another experiment, stem explants 0.5 cm in length, including nodal and inter nodal tissues were used by Chua *et al.* (1981) in *Dracaena marginata.* The explants from the terminal portions of cordyline were cut into 5 cm long segments from which the leaves were removed, leaving the base intact. These stems were cut into transverse sections containing one nodal bud, each segment measuring approximately 2 cm in length (Khan *et al.,* 2004).

Sections of 3 mm length were taken out from shoot apices of *Cordyline terminalis* for callus culture (Mee*,* 1978) and placed on medium for callus culture. The shoot tips of 2 to 3 cm were cut from the mother plants of *Dracaena fragrans cv.* Massangeana used as explants (Badawy *et al.,* 2005) for similar experiments. Wong (1986) experimented with the shoot-tip explants from banana by removing the outer layers of tissue from suckers in *in vitro* propagation of banana.

Vinterhalter (1989) separated the upper young stem parts from potted *D. fragrans* plants and cut them into 2-4 mm thick segments to use as explants. Beura *et al.* (2007) used the single node explants of dracaena for bud response study. Shoot apices of about 1 cm long were excised from a 2–3 year old *ex vitro* grown *Cordyline terminalis* plant, and were cut into pieces 2–3 mm before placing on medium (Ray *et al.*, 2006).

2.3 Growth regulators

Selection of appropriate combinations of plant growth regulators is the most important aspect in developing a successful protocol for tissue culture. The importance of growth regulators in plant tissue culture is discussed below.

2.3.1 Shoot initiation and multiplication

The stimulation of axillary branching and/or adventitious bud formation was accomplished by placing an intact or fragmented shoot apex onto a basal medium supplemented with 0.2 to 10 mg/l of cytokinin. This treatment was reported to produce the best results with respect to the rate of proliferation (Vuylsteke and De Langhe, 1985).

Badawy *et al.* (2005) reported that in the tissue culture of dracaena, the highest shoot length was measured when using the media containing 1.0 mg/l IAA +1.0 mg/l Kinetin. The interaction between different concentrations of IAA, Kinetin and MS salt strength affected the shoot length significantly. The presence of 1.0 mg/l IAA +1.0 mg/l Kinetin supplemented to half strength MS produced the longest shoots during experiments.

The highest number of leaves was recorded as a result of the presence of 1.0 mg/l IAA + 1.0 mg/l Kin. At higher concentrations Kin inhibited the initiation of new leaves whereas IAA promoted the leaf initiation. The highest mean number of shoots was recorded by the use of 0.5 mg/l IAA + 5.0 mg/l Kinetin in the composition of the MS medium.

Paek *et al.* (1985) while working on *Cordyline terminalis* reported that using 1.0 mg/l IAA + 3.0 mg/l Kinetin was the most effective treatment for increasing shoot multiplication activity. Tian *et al.,* (1999) on *Dracaena sanderiana* cv. Virescens, found that using 3-3.5 mg/l BA + 0.02 mg/l NAA was the most effective treatment for increasing shoot multiplication. Also, on *Dracaena marginata cv. Tricolor*, exhibited the highest value of shoot proliferation on MS containing BA at 4.0 mg/l and NAA at 0.05 mg/l (Atta- Alla *et al.*, 1996 and El- Sawy *et al.*, 2000).

Beaura *et al.* (2007) studied shoot proliferation studies of dracaena and observed that in the 1st subculture the presence of BAP (2 mg/l) with NAA (0.5 mg/l) produced significantly longer shoots and more leaves as compared to the other treatments. Subsequently inclusion of GA_3 (1-9 mg/l) with BAP (2 mg/l) and NAA (0.5 mg/l) resulted in longer shoots and more leaves per plant.

Profuse adventitious shooting of Cordyline was observed in media containing a combination of kinetin at 4.0 mg/l and NAA at 0.5 mg/l. The maximum length of shoot was also obtained in the media having the same composition (98mm).These tiny shoots, when transferred to fresh medium continued to produce adventitious shoots. Therefore, a cyclic production of shoots was possible, leading to continuous cultures and generation of large number of shoots. In a comparative study made against full and half MS media, a clear picture was drawn exhibiting that shoots in full MS showed better results (Khan *et al.,* 2004).

Tissue culture of Dracaena, Cordyline, and Diffenbechia revealed a similar tendency for propagation. An increase in the content of cytokinins led to an increase in the multiplication rate and decrease in shoot length. It was also observed that the presence of 2 mg/l BA resulted in vitrification of shoots. Therefore, he opined that BA cannot be recommended for the tissue culture of these plants. A clump of relatively short shoots was formed on the multiplication medium. These shoots did not root well on rooting media. During prolonged (8 weeks) cultivation on the multiplication medium some of the shoots became elongated. However, new microshoots and buds kept on forming. In order to halt the multiplication and to promote elongation of the shoots already formed, the shoots in clumps (according to their length) were planted onto elongation media (Vardja and Vardja, 2001).

Kalimuthu *et al.* (2007) investigated different combinations with various concentrations of BAP and NAA to analyze the shoot initiation and shoot multiplication capacity of banana in the MS medium. The treatment which involved 3.0 mg/l of BAP and 0.2 mg/l of NAA of medium showed good results both for shoot initiation and multiplication. Rahman *et al*. (2004) reported that MS medium supplemented with 2.0 mg/l BAP is the best concentration for multiple shoot production in *Curcuma longa*. Similarly Panda *et al*. (2007) reported that, MS medium supplemented with 3.0 mg/l BAP is the best hormonal concentration for multiple shoot production in *Curcuma longa* (cv-Roma). Santilata *et al. (*2009) observed that the addition of NAA 0.5 mg/l with BAP 2.0 mg/l led to an improved response over BAP alone and also suggested that the combination of BAP and NAA were needed for producing more number of multiple shoots on *Zingiber officinale.*

2.3.2 Rooting

Badaway *et al.* (2005) studied about the micropropagation in *Dracaena fragrance* and found that using IBA at different concentrations significantly affected the number of roots formed. The highest mean value for the number of roots was obtained by using 5.0 mg/l IBA. Using NAA at different rates had significant effect on the number of roots. The highest number of roots was obtained from the control treatment as compared with that obtained from using 5.0 mg/l NAA, which gave no roots. Further, they studied the effect of the interaction between the concentrations of IBA and NAA on the number of roots. They found that the highest number of roots was obtained in the control explants, as compared with that obtained from using 1.0, 3.0 and 5.0 mg/l NAA combined with 1.0, 3.0 and 5.0 mg/l IBA. The results proved beyond doubt higher the concentration of NAA, lower the number of roots.

Paek *et al.* (1985) concluded that using 2.0 or 3.0 mg/l IBA was more successful in increasing rooting percentage in micropropagation of cordyline. Addition of 5mg/l NAA to MS medium improved the growth of *Cordyline terminalis* and adding 1mg/l of NAA to medium improved growth of *Dracaena marginata* as reported by Mee (1978) and Chua *et al.* (1981) respectively.

In a preliminary study on rooting of *Dracaena sanderiana*,done by Beura *et al.* (2007) 80% shoots rooted on half MS medium with IBA 3 mg/l and GA_3 2 mg/l. This treatment produced root primordia in 28 days. However, there was no rooting in the treatment having IBA without GA_3. Tian *et al.* (1999), observed that MS medium with IBA 2-3 mg/land GA_3 1.5 mg/l induced rooting in *Dracaena sanderiana*, where shoots were proliferated from axillary buds of the stem segment in *in vitro* condition.

Vinterhalter (1989) showed that there was a direct relationship between the concentration of IBA and root length in micropropagation of *Dracaena fragrance*. The mean number of roots per rooted shoot increased with increasing IBA concentration. Taking into account both the parameters, an IBA concentration of 0.5 mg/l was considered optimal for *in vitro* rooting. He observed that mean length of the longest root was 3.9 mm for shoots that rooted under darkness, compared to 46.45 mm for shoots that rooted at 16 h light/8 h darkness. Thus he concluded that root elongation require light.

The *in vitro* proliferated shoots produced roots with maximum frequency in half strength MS medium fortified with 0.5 mg/l IBA in case of banana (Uddin *et al.,* 2006). Santilata *et al*. (2009) observed that rooting of ginger profused in culture which had a combination of 1/2 MS+2.0 mg/l NAA. They also observed that the root primordia emerged from the shoot base starting from 6^{th} to 8^{th} days after shoot inoculation. Soon after 8^{th} day the root growth was rapid. With respect to induction of rooting, NAA has more effect than IBA as the number of days required for rooting was only 6-8 days as against 10 to 15 days required for similar response in case of IBA.

2.4 *Ex vitro* rooting and Hardening

2.4.1 *Ex vitro* rooting

By combining rooting *ex vitro* with acclimatization, the survival rate of transplanted plantlets can be raised. It has been widely used in micropropagation of *Gardenia jasminoides*, *Aloe vera, Cunninghamia lanceolata , Gerbera jamesonii, Gypsophila paniculata* and so on. The suitable temperature for *ex vitro* rooting is 18-30℃, the optimum is 25℃, and the relative humidity required is 80-90%. Under such conditions, the rooting plantlets can be cultured in perlite instead of common rooting medium (Qing and Quinglin, 2010).

The cut ends of anthurium shoots were dipped in IBA 2.5g/l and inserted in different rooting substrates and higher percentage of calli free roots were produced in vermiculite, peat moss and sphagnum moss when compared to agar or phytagel added media (Keat metha and Suska-Ard, 2004).

Rooting and acclamitization when done simultaneously can substantially reduce cost of micropropagation. But treatment with root inducing growth regulators may be required prior to transfer to a rooting medium (Sathyanarayana and Dalia, 2007).

The treated microshoots of *Stackhousia tryonii* were grown in a mist room for four weeks and assessed for survival, rooting percentage, number of roots and root length. The results showed that IBA at 2 g/l was most effective in inducing roots (Bhatia *et al.,* 2002). Xu *et al*. (2008) has done 2 hour-treatment of 120 mg/l IBA for the induction of roots in *Malus zumi* and was found to be the best method resulting with 86.3% of the treated shoots forming roots after 30 days and when given the reduced time for establishment, auxin dipped *ex vitro* rooting is the more favourable method than *in vitro* rooting.

In vitro regenerated microshoots were treated for *exvitro* rooting with 1000 ppm IBA for 10 minutes and planted in different media. 100% survival and rooting was observed in media that contained cocopeat along with biocontrol agent (Lavanya *et al*., 2009).

2.4.2 Hardening

One of the major obstacles in the application of tissue culture methods for plant propagation has been the difficulty in successful transfer of plantlets from laboratory to the field (Warde *et al.,* 1983). Reasons for such difficulty appear to be related to the dramatic change in the environmental conditions from culture vessels to field. Several workers have developed protocols to overcome some of these constraints.

The plantlets obtained through micropropagation should have roots that are capable of supporting further growth and development. They are usually transplanted into compost and kept in partial shade at a high ambient humidity for several days. A suitable environment is often created by covering the plantlets either with glass or clear polyethylene or by subjecting them into intermittent misting. Plants are hardened by gradually reducing the humidity and increasing the light.

The term media is sometimes used to describe the mixtures of materials such as peat, perlite, vermiculite, rockwool, sand and soil used for transferring the plantlets from *in vitro* conditions. Compost which is commonly used for rooting conventional cuttings is suitable for transferring these plantlets, but there may be marked differences in root growth and plantlet survival with different media (Rodriguez *et al.,* 1987). Peat may prove to be too acidic substrate for some species and some kinds of vermiculite are too alkaline in nature. In order to maximize the survival of *in vitro* derived plants, it is a routine practice to acclimatize them under high levels of relative humidity.

Rooted plantlets were replanted as explained by Vinterhalter (1989) in peat in which they easily adapted to greenhouse conditions without special hardening treatments. Chua *et al.* (1981) transferred the cultured plants of Dracaena to the green house in 5 cm plastic pots with medium consisting of sphagnum peat moss, perlite and vermiculite in the ratio of 1:1:1, hardened in the nursery and subsequently transplanted to the field.

Badaway *et al.,* (2005) subjected the plantlets that were obtained from *Dracaena fragrance* to the acclimatization procedures by transplanting the plantlets into hardening medium, i.e. peat moss and mixture of peat moss and sand at the ratio of 1:1 under coverage with polythene bags for three weeks and then the bags were removed. The acclimatization was successfully accomplished in the greenhouse under humid conditions, with a relative humidity of 80% and temperature of 25 ± 2℃.

Nazi *et al*. (2009), studied the *in vitro* grown turmeric plants, and brought about important findings which have facilitated the successful transfer of 70-80% of plants from *in vitro* to *ex vitro* conditions. After 30-50 days the potted plants were nurtured in glass house under complete sunlight for further acclimatization of plantlets. After another two weeks, these plants were transferred successfully into field. It was further observed that high humidity and moderate temperature greatly enhance the initial survival of potted and field grown plantlets. The different combination of compost also greatly affected the survival percentage of plantlets. Among the different combination tried, the best hardening response was observed in sand: soil: peat in 1:1:1 combination.

3. MATERIAL AND METHODS

The present studies on micropropagation of Dracaenas and Cordyline were conducted in the Tissue culture laboratory of the Department of Horticulture, College of Agriculture, University of Agricultural Sciences Dharwad.

The details of the materials used and the methods adopted in the experiments are described here under.

3.1 Plant material

The plant material for the experiments was collected from the plants maintained in the Floriculture unit of MARS, Department of Horticulture, University of Agricultural Sciences, Dharwad.

3.1.1 Explants and their preparation

Young shoots from the dracaena and cordyline plants maintained in the green house were collected and brought in to laboratory in clean polythene bags (Plate 1). These shoots were washed under running water. All leaves were removed using scalpel. Then shoot tips and individual nodes were excised with a scalpel and placed with the cut surface down on the medium after they were surface sterilized. The leaf was carefully removed along with base and was inoculated such that basal portion of the leaf was in contact with the media.

3.2 Growing media

According to the available literature on micro propagation of ornamental foliage plants, the Murashige and Skoog's medium (MS) (Murashige and Skoog, 1962) is the most commonly used growing medium for dracaenas and cordylines. Therefore, MS medium was used throughout the experiments and the term media used in the experiments refers to the MS media. The composition of MS medium is given in Appendix- 1.

3.2.1 Preparation of stock solutions

Stock solution of macronutrients, micronutrients and vitamins were prepared separately. Stock solutions of macronutrients (MS-A) was prepared at 10X strength while micronutrients and vitamins (MS-B, MS-C and MS-D) were prepared at a strength of 10 and 50 times the final concentration (10X and 50X) required to make a liter of the nutrient media. Each chemical was weighed using an electronic balance and dissolved separately in small quantities of double distilled water. The components of each stock were mixed and the final volume was made up using double distilled water. All the stock solutions after preparation were stored in proper plastic or glass bottles and stored at 4-5ºC. The iron stock, which was prepared separately, was stored in amber coloured bottle.

3.2.1 Growth regulator stocks

The stock solutions of all the growth regulators were prepared at a concentration of 100 mg/l. Stock solution of 6- Benzyl amino purine (BAP) was prepared by dissolving 10 mg of the growth regulator in a few drops of 1N NaOH and the volume was made up to 100 ml with double distilled water.

Stock solutions of Naphthalene acetic acid (NAA) and 2,4-Dicholorophenoxy acetic acid (2,4-D) were prepared by dissolving 10mg of the growth regulator in a few drops of absolute alcohol and volume was made up to 100 ml with double distilled water. The prepared stock solutions of growth regulators were stored in the refrigerator at 4-5ºC.

Dracaena

Cordyline

Plate.1. Plant materials used for the study

3.2.2 Quality of Chemicals

All chemicals used in the experiments were of analytical grade.

3.3 Preparation of culture media

The stock solutions were mixed in the required proportion along with growth regulators and sucrose (3%). Then the volume was adjusted by adding double distilled water. The pH of the medium was adjusted 5.6 to 5.8 using digital pH meter by adding either 0.1N HCl or NaOH drop wise while stirring with the help magnetic stirrer. Volume was finally made up to required level and agar (0.7%) was added to the medium. Agar in the medium was made to melt by gentle heating and the medium was poured into the baby jar bottles having 300 ml capacity.

3.4 Sterilization procedures

3.4.1 Media

Baby jars or culture tubes containing the media were autoclaved at a temperature of 121ºC at 15 lb per square inch pressure for 20 minutes.

3.4.2 Instruments

All the instruments used for sterile handling and transfer of cultures were sterilized by autoclaving at a temperature of 121ºC at 15 lb per square inch pressure for 20 minutes.

3.4.3 Sterile technique in laminar air flow cabinets

All the sterile transfer work was carried out in a laminar air flow cabinet. Before starting any sterile operation, the inner surface of the cabinet was swabbed with 70% ethyl alcohol. The UV lamp provided within the cabinet was then switched on for 15 to 20 minutes before use, followed by the airflow. During the course of transfer, between each transfer of explants to culture bottles or tubes, the instruments were dipped in alcohol and flame sterilized.

3.5 Culture establishments

3.5.1 Inoculation

Before inoculation of explants, the ultra violet light of laminar air flow was switched on for 20 minutes for sterilization of the area. Inoculation was carried out under aseptic condition under laminar air flow.

3.5.2 Culture condition

The culture tube or jams bottles were incubated in culture room having control over temperature and light. The temperature of culture room was maintained at 25 ± 2ºC with light intensity of 16 hours light and 8 hours dark.

3.5.3 Subculture

Microshoots formed in the bottles were taken out and were separated by dissecting them in the sterile environment of laminar airflow cabinet with sterile dissecting needle/scalpel and forceps. Then they were placed back in the test tubes/ bottles containing the fresh media.

3.5.4 Rooting

The micro shoots were taken out and placed back in the tubes containing media with different concentrations of IBA and NAA for rooting.

3.5.5 *Ex vitro* rooting and Hardening

Microshoots were taken out from the testtube, base of the shoot was moistened, treated with powder form IBA of different concentrations and planted in small pots containing different hardening media. These plants were maintained in polyhouse. They were watered once in two days initially, then once in a week. Later they were transferred to green house after 15 days for further acclimatization.

3.6 Experimental details

3.6.1 Experiment 1 - Standardization of surface sterilization

In this experiment, the efficacy of $HgCl_2$ with a treatment of a fungicide and a bactericide for disinfecting the explants was studied. Fungicide (carbendazim) and bactericide (streptomycin) were used for pretreatment in four treatments. Experiment was undertaken for dracaena and cordyline.

3.6.1.1 a Treatment details

T_1 - 0.05% $HgCl_2$ for 5 minutes

T_2 - 0.1 % $HgCl_2$ for 5 minutes

T_3 - 0.05% $HgCl_2$ for 10 minutes

T_4 - 0.1% $HgCl_2$ for 10 minutes

T_5 - 0.05% $HgCl_2$ for 5 minutes and explants pretreated with fungicide and bactericide

T_6 - 0.1 % $HgCl_2$ for 5 minutes and explants pretreated with fungicide and bactericide

T_7 - 0.05% $HgCl_2$ for 10 minutes and explants pretreated with fungicide and bactericide

T_8 - 0.1% $HgCl_2$ for 10 minutes and explants pretreated with fungicide and bactericide

T_9 – Control (without any chemicals and pretreatment)

Design	: CRD
Replication	: 3
Number of tubes per treatment	:10

3.6.1.1 b Observations recorded

a. Percent contamination: Out of total tubes inoculated, the Number of tubes contaminated were counted and expressed in percentage.

b. Per cent survival of explants: The number of plants survived out of total inoculated was counted and converted in to percentage.

c. Per cent response of explants: The number of plants responded out of survived was counted and converted in to percentage.

3.6.2 Experiment 2 – Standardizing explants for culture establishments

Different explants were used to study the response of different parts for shoot generation and multiplication for both the species dracaena and cordyline.

3.6.2.1a Treatment details

T_1 - Shoot tip

T_2 - Nodal cuttings

T_3 - Leaf base

Design	: CRD
Replication	: 7
Number of tubes per treatment	: 10

3.6.2.1b Observations recorded

a. Number of days taken for initiation of shoot: Number of days taken to show initial differentiation of shoot from the date of inoculation of different explants recorded and were expressed in mean number of days.

b. Percentage response: The number of plants responded out of inoculated was counted and converted in to percentage.

c. Number of shoots per explants: The number of shoots produced from single explants were noted.

3.6.3 Experiment 3 - Standardising different growth regulators and their concentrations for shoot multiplication and rooting

The effect of growth regulator's combinations on shoot proliferation and rooting from explants cultured on MS medium fortified with different levels of cyokinins and combinations with auxins were studied. First the effect of different concentrations of BAP on shoot multiplication studied, followed by BAP in combination with different concentrations of NAA. As the shoots formed were relatively shorter the effect of different concentrations of GA_3 was also evaluated to improve shoot length. Two auxins namely IBA and NAA were supplemented to the culture medium to study the effect of the same in rooting. As the dracaena didn't respond to the above experiments further studies were continued only with cordyline.

(I) Influence of BAP on shoot multiplication

3.6. 3. I a Treatment details

T_1 - 0.25 mg/l

T_2 - 0.5 mg/l

T_3 - 1.0 mg/l

T_4 - 2.0 mg/l

T_5 - 4.0 mg/l

T_6 - 8.0 mg/l

T_7 -10.0 mg/l

Design : CRD

Replications : 4

Number of tubes per treatment : 10

3.6.3. I b Observations recorded

a. Days taken for shoot initiation: Number of days taken for initiation of shoot after subculturing was noted.

b. Number of shoots formed per explants: The number of shoots formed by each explants after subculturing were noted.

(II) Influence of combinations of Cytokinin & Auxin

3.6.3. II a Treatment details

Best two concentrations of BAP from experiment I along with 0.1, 0.2, 0.5 mg/l of NAA.

T_1 -2 mg/l BAP + 0.1 mg/l NAA

T_2 -2 mg/l BAP + 0.2 mg/l NAA

T_3 -2 mg/l BAP + 0.5 mg/l NAA

T_4 -4 mg/l BAP + 0.1 mg/l NAA

T_5 -4 mg/l BAP + 0.2 mg/l NAA

T_6 -4 mg/l BAP + 0.5 mg/l NAA

Design : CRD

Replication : 3

Number of tubes per treatment : 10

3.6.3. II b Observations recorded

The observations were recorded as mentioned in 3.6.3.I b

(III) Influence of GA_3 in shoot proliferation

3.6.3. III a Treatment details

T_1 - 1 mg/l GA_3

T_2 - 2 mg/l GA_3

T_3 - 3 mg/l GA_3

T_4 - 4 mg/l GA_3

T_5 - 5 mg/l GA_3

Design : CRD

Replication : 4

Number of tubes per treatment : 10

3.6.3. III b Observations recorded

a) Number of shoots formed per explants: The number of shoots produced by each explants after sub culturing were noted.

b) Mean length of shoot : Length of all the multiple shoots formed per explants were recorded and their mean were calculated, which formed mean length of the shoots and the length of the longest shoot among multiple shoots was taken as longest shoot length, which were expressed in centimeters.

(IV) Influence of auxin on rooting

3.6.3. IV a Treatment details

T_1 - 0.5 mg/l of NAA

T_2 - 1.0 mg/l of NAA

T_3 - 2.0 mg/l of NAA

T_4 - 5.0 mg/l of NAA

T_5 - 0.5 mg/l of IBA

T_6 - 1.0 mg/l of IBA

T_7 - 2.0 mg/l of IBA

T_8 - 5.0 mg/l of IBA

Design : CRD

Replications : 3

Number of tubes per treatment : 10

3.6.3. IV b Observations recorded

a) Per cent of culture rooted: Number of tubes rooted out of total inoculated was counted and was converted into percentage.

b) Root characteristics

3.6.4 Experiment 4: Standardization *ex vitro* rooting and hardening media

Young shooted plantlets were taken out of the bottles, washed with distilled water, treated with PGR powder and planted in pots containing different hardening media and maintained in a polyhouse. Required quantity of IBA was taken, dissolved in alcohol and mixed with talc powder. The media used for hardening were peat and potting mixture. Enriched coir pith peat was used after sterilization. The potting mixture media was prepared by mixing the sieved soil, farmyard manure and sand in 1: 1: 1 ratio after sterilization.

3.6.4.1a Treatment details

Factor A

A_1 - IBA 10 ppm

A_2 - IBA 15 ppm

A_3 - IBA 20 ppm

A_4 - IBA 25 ppm

Factor B

B_1 - Peat media

B_2 - Potting mixture

Treatment combinations

$T_1 – A_1B_1$

$T_2 – A_1B_2$

$T_3 – A_2B_1$

$T_4 – A_2B_2$

$T_5 – A_3B_1$

$T_6 – A_3B_2$

$T_7 – A_4B_1$

$T_8 –A_4B_2$

Design	: Factorial CRD
Replications	: 3
Number of plants per treatment	: 5

3.6.4.1b Observations recorded

a) Percent rooting of plantlets: Number of rooted plants were counted and was converted into percentage.

b) Number of roots: Number of roots formed per microshoot were recorded and average was worked out.

c) Root length: Length of the primary root was measured from base to tip of the root and expressed in centimeters.

3.6.5 Statistical analysis

The experimental data relating to rooting percentage, percent survival, percent contamination and percent response were transformed into arcsine values and analysed under CRD. The data pertaining to *exvitro* rooting and hardening was analysed by two factorial CRD. The data was subjected to ANOVA test as suggested by Panse and Sukhatme (1967). Critical difference values were tabulated at one percent probability where ever F test was found significant. 't' test was also done for the comparison in case of explants and also for BAP treatments.

4. EXPERIMENTAL RESULTS

The results obtained in the present investigation on “Micropropagation studies in Dracaena and Cordyline” are presented under the following headings.

4.1 Standardization of surface sterilization

The explants treated with mercuric chloride in combination with fungicides and bactericides for varying time periods at different concentrations in order to establish maximum contaminant free cultures. The results are presented in Table 1a and 1b.

Among the treatments, the highest contamination was recorded in control (100%) for both dracaena and cordyline. The least contamination with highest percentage of contaminant free explants were established when they were exposed to 0.1 % $HgCl_2$ for 10 minutes (90 % in cordyline and 86.7 % in dracaena)followed by 0.1 % $HgCl_2$ for 5 minutes (86.7 % in cordyline and 83.3 % in dracaena) which were pretreated with fungicide and bactericides. But highest percentage of explant response without any toxicity was obtained when they were exposed to 0.1 % $HgCl_2$ for 5 minutes (87.8 %) which proved to be the best treatment and was significantly superior to other treatments in case of cordyline explants (Plate 2a). But for dracaena the response of explants was nil (0.0 %).

4.2 Standardising explants for culture establishments

The data on response of explants to number of days taken for initiation of growth, number of shoots produced and percentage response are presented in Table 2.

4.2.1 Number of days taken for initiation of growth

There was no significant difference among the explants on number of days taken for initiation of growth. But minimum time for growth was taken by shoot tip explants which elongated after 7.6 days followed by nodal cuttings (15.6).

4.2.2 Percent response of explants

There was significant difference between the treatments for percent response of explants of cordyline. Highest response was for shoot tip (97.1%). However, all the three explants of dracaena didn’t show any response (Plate 3c).

4.2.3 Mean number of shoots produced per explants

In cordyline, there was no significant difference between the different types of explants for number of shoots developed. The nodal cuttings produced the highest number of shoots (1.57) followed by shoot tip explants (1).

Leaf base explants of cordyline didn’t respond to any of the observations.

As the dracaena did not show any response, the rest of the experiments as per the programme were continued with cordyline without a change in the stated objectives.

4.3 Standardising different growth regulators and their concentrations for shoot multiplication and rooting

4.3.1 Influence of BAP on shoot multiplication

a. Number of days taken for initiation

There was significant difference with respect to time taken for initiation of shoots. As the concentration of BAP increased, the days taken for shoot initiation decreased. Explant inoculated in media with 2 mg/l of BAP had taken more days (43.25) and with 10 mg/l BAP took less days (18.75) for shoot initiation (Table 3.1).

b. Number of shoots produced per explants

Treatments with 2 mg/l and 4 mg/l BAP only responded to the shoot multiplication which produced 1.78 and 4.33 shoots respectively (Table 3.2). But there was no significant difference between these two treatments (Plate 4). Surprisingly all the other treatments for

Table 1a: Effect of surface sterilization on percent contamination and survival of explants of Cordyline

Sl. No	Treatment	%contamination	% survival	% response
1	0.05% $HgCl_2$ for 5 minutes	80.0 (63.4)	20.0 (26.9)	50.0 (45.3)
2	0.1 % $HgCl_2$ for 5 minutes	80.0 (63.4)	20.0 (26.9)	50.0 (45.23
3	0.05% $HgCl_2$ for 10 minutes	80.0 (63.4)	20.0 (26.91)	50.0 (45.3)
4	0.1% $HgCl_2$ for 10 minutes	76.7 (61.2)	23.3 (29.1)	55.3 (48.4)
5	0.05% $HgCl_2$ - pretreated with Fungicides & Bactericides for 5 minutes	66.7 (54.8)	33.3 (35.5)	49.7 (45.1)
6	0.1 % $HgCl_2$ - pretreated with Fungicides & Bactericides for 5 minutes	13.3 (21.1)	86.7 (69.3)	87.8 (69.6)
7	.05% $HgCl_2$ - pretreated with Fungicides & Bactericides for 10 minutes	43.3 (41.1)	56.7 (49.1)	64.0 (53.4)
8	0.1% $HgCl_2$ - pretreated with Fungicides & Bactericides for 10 minutes	10.0 (18.4)	90.0 (72.0)	14.7 (22.6)
9	Control	100.0 (90.0)	0.0 (4.1)	0.0 (4.1)
	S.Em±	1.5	1.5	2.4
	CD at 1%	6.0	6.0	9.6

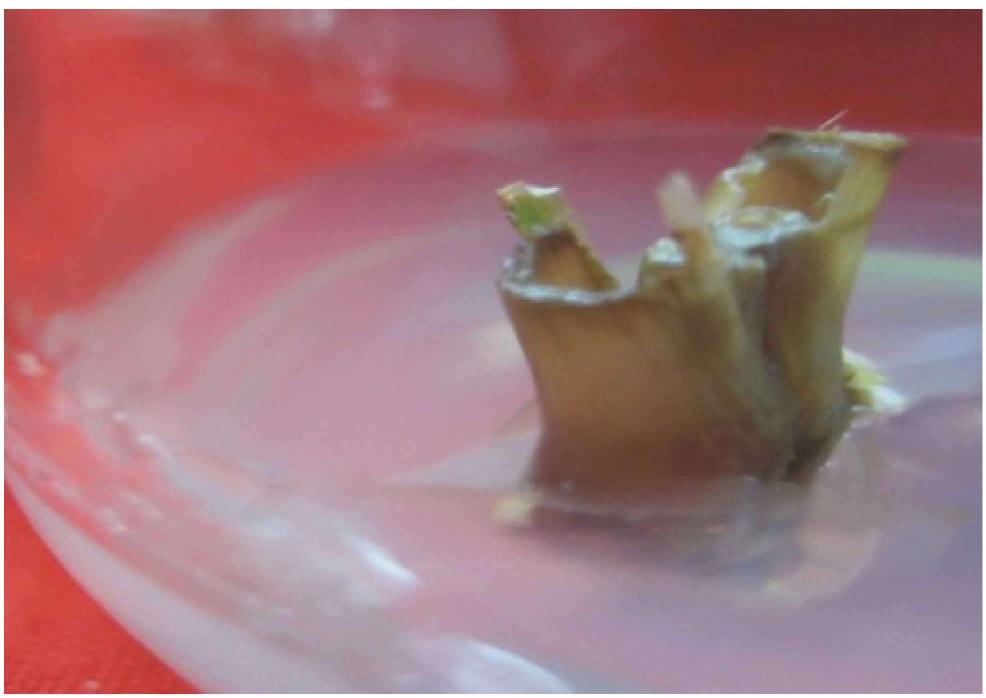
Figures in parenthesis indicate arcsine transformed values

Table 1b: Effect of surface sterilization on percent contamination and survival of explants of Dracaena

Sl. No	Treatment	%contamination	% survival	% response
1	0.05% $HgCl_2$ for 5 minutes	80.0 (63.4)	20.0 (26.9)	-
2	0.1 % $HgCl_2$ for 5 minutes	83.3 (66.1)	16.7 (24.2)	-
3	0.05% $HgCl_2$ for 10 minutes	80.0 (63.4)	20.0 (26.91)	-
4	0.1% $HgCl_2$ for 10 minutes	76.7 (61.2)	23.3 (29.1)	-
5	0.05% $HgCl_2$ - pretreated with Fungicides & Bactericides for 5 minutes	70.0 (56.2)	30.0 (33.5)	-
6	0.1 % $HgCl_2$ - pretreated with Fungicides & Bactericides for 5 minutes	16.7 (23.8)	83.3(66.5)	-
7	.05% $HgCl_2$ - pretreated with Fungicides & Bactericides for 10 minutes	46.7 (31.1)	53.3 (47.2)	-
8	0.1% $HgCl_2$ - pretreated with Fungicides & Bactericides for 10 minutes	13.3 (21.1)	86.7 (69.3)	-
9	Control	100.0 (90.0)	0.0 (4.1)	-
	S.Em±	1.9	1.9	
	CD at 1%	7.5	7.5	

Figures in parenthesis indicate arcsine transformed values

a) Explant sterilized with 0.1% Mercuric chloride for 5 minutes showing shoot initiation



b) Explant sterilized with 0.1% Nercuric chloride for 10 minutes showing toxicity

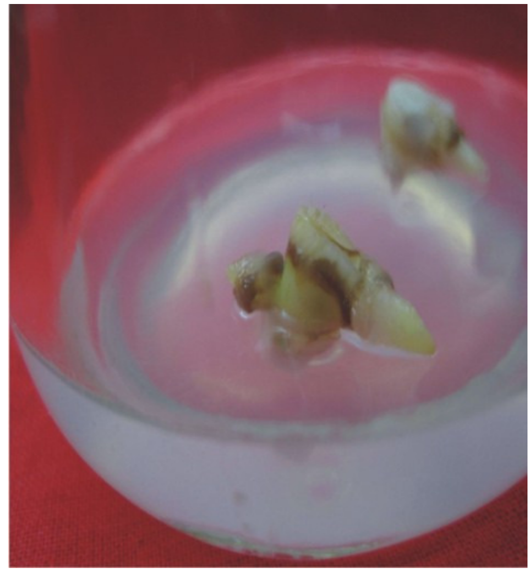
Plate.2. Response of cordyline explants to surface sterilization

Table 2: Effect of explants types on culture establishment and number of shoots formed in Cordyline and Dracaena

Sl. No.	Explants type	No of days taken for initiation of growth		Percentage response		No of shoots produced/explant	
		Cordyline	Dracaena	Cordyline	Dracaena	Cordyline	Dracaena
1	Shoot tip	7.6	-	97.1 (84.7)	0	1.0	0
2	Nodal cuttings	15.6	-	78.3 (61.7)	0	1.6	0
3	Leaf base	-	-	-	0	-	0
	T test (1%)	NS		S		NS	

Figures in parenthesis indicate arcsine transformed values
Leaf base explants did not show any response

a) Shoot tip of Cordyline



b) Nodal cutting of Cordyline

c) Drying of Dracaena nodal cutting

Plate.3. Culture establishment as observed in different explants

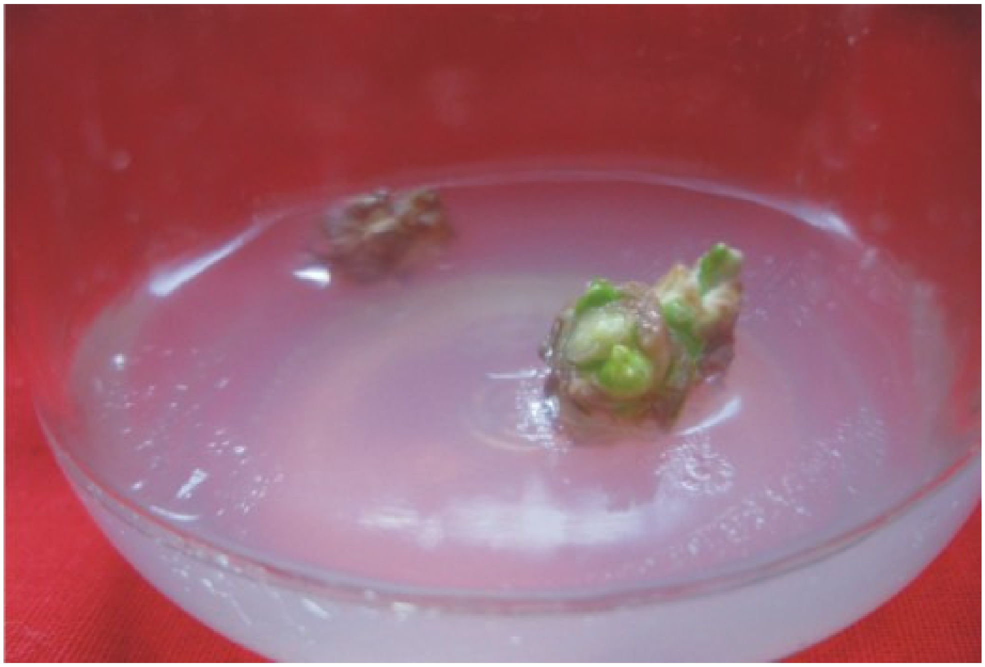
Table 3.1: Effect of BAP on number of days taken for shoot initiation in Cordyline

Sl.No	Concentration of BAP	No.of days taken for shoot initiation
1	2.0 mg/l	43.25
2	4.0 mg/l	32.75
3	8.0 mg/l	24.75
4	10.0 mg/l	18.75
	S.Em±	0.95
	CD at 1%	4.08

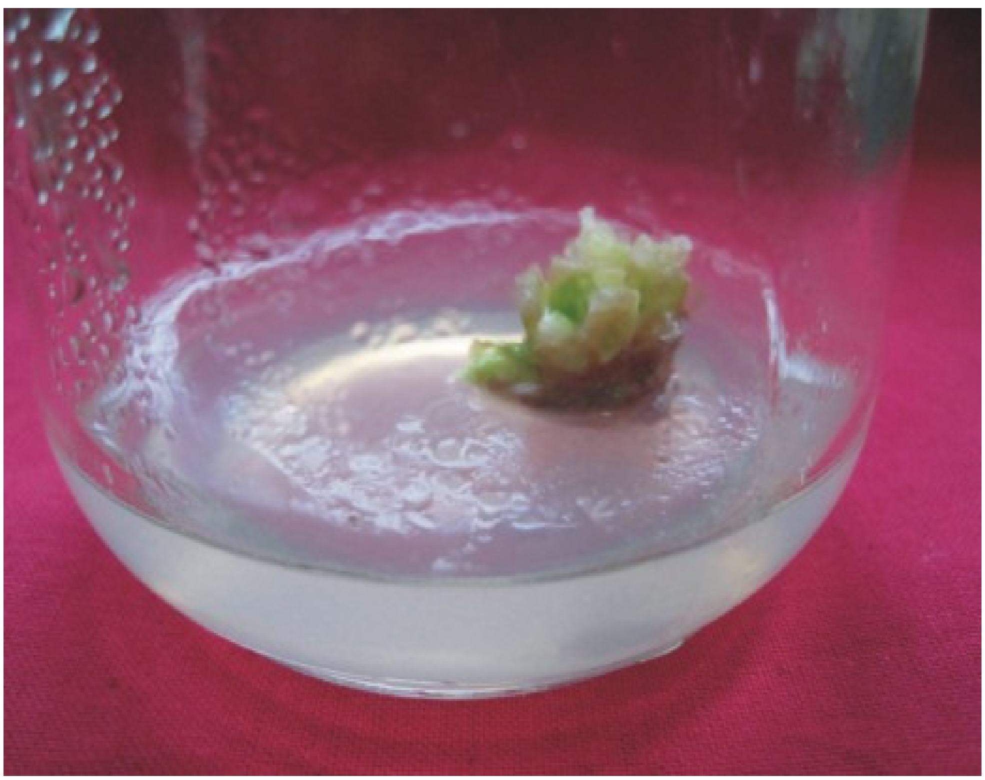
Table 3.2: Effect of BAP on number of shoots in Cordyline

Sl. No.	Concentration of BAP	No. of shoots
1	2.0 mg/l	1.78
2	4.0 mg/l	4.33
	T test (1%)	NS

*Treatments with 8 mg/l and 10 mg/l BAP produced only clumps (Plate 4).



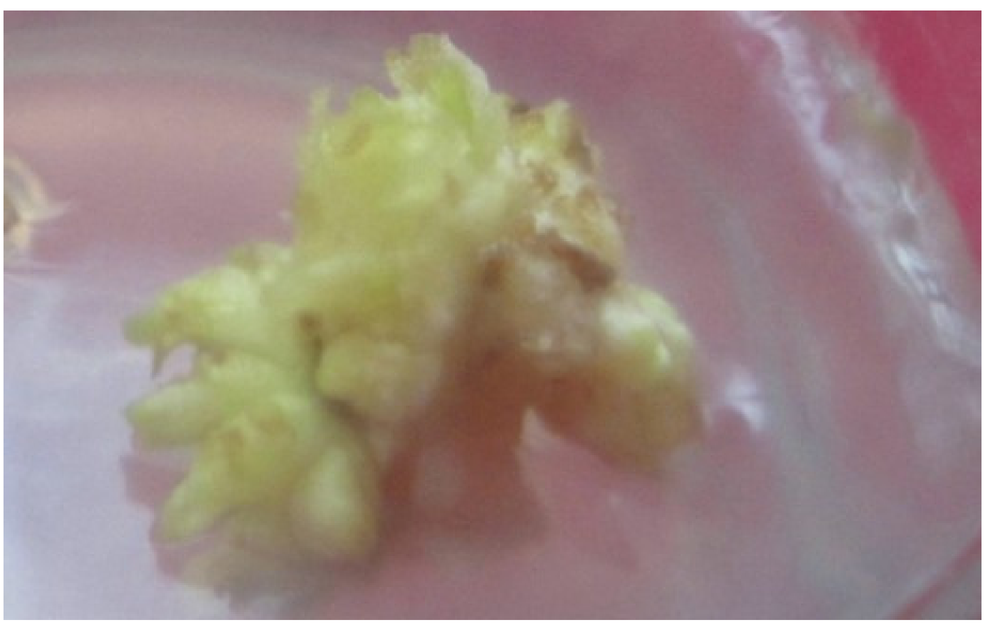
a) 2 mg/l BAP



b) 4 mg/l BAP

Plate.4. Effect of BAP on shoot multiplication at lower concentrations

a) 8 mg/l BAP



b) 10 mg/l BAP

Plate.5 Formation of clumps instead of multiple shoots in medium supplemented with BAP at higher concentrations

Table 4: Influence of cytokinin and auxin on shoot multiplication in Cordyline

Sl. No.	Concentrations (mg/l)	No.of days taken for shoot initiation	No. of shoots formed
1	2 mg/l BAP + 0.1 mg/l NAA	22.00	0.73
2	2 mg/l BAP + 0.2 mg/l NAA	22.67	0.67
3	2 mg/l BAP + 0.5 mg/l NAA	18.67	1.13
4	4 mg/l BAP + 0.1 mg/l NAA	17.67	1.30
5	4 mg/l BAP + 0.2 mg/l NAA	17.67	2.03
6	4 mg/l BAP + 0.5 mg/l NAA	14.33	5.67
	S.Em±	0.73	0.20
	CD at 1%	3.16	0.86

Plate.6. Shoot multiplication of cordyline in MS medium containing 4 mg/l BAP +0.5 mg/l NAA

shoot multiplication produced only clumps of cultures which were not measurable as shown in Plate 5.

4.3.2 Influence of combinations of cytokinin and auxin

The results of the experiment on the effect of BAP and NAA on days taken for shoot initiation and number of shoots formed are presented in Table 4.

a. Days taken for shoot initiation

There was significant difference among the treatments. Media with 4 mg/l BAP + 0.5 mg/l NAA took significantly less number of days for shoot initiation followed by media with 4 mg/l BAP + 0.1 mg/l NAA, 4 mg/l BAP + 0.2 mg/l NAA, 2 mg/l BAP + 0.5 mg/l NAA which were on par with each other. Media containing 2 mg/l BAP + 0.1 mg/l NAA and 2 mg/l BAP + 0.2 mg/l NAA took maximum days as compared to other treatments.

b. Number of shoots formed

There was significant difference between the six treatments with respect to the number of shoots formed. Media with 4 mg/l BAP + 0.5 mg/l NAA produced significantly more number of shoots (5.67) as shown in Plate 6, followed by media having 4 mg/l BAP + 0.2 mg/l NAA (2.03) Media with 2 mg/l BAP + 0.1 mg/l NAA (0.73) and 2 mg/l BAP + 0.2 mg/l NAA (0.67) produced lower number of shoots compared to other treatments.

4.3.3 Effect of GA_3 on shoot proliferation

The results of the experiment on effect of GA_3 on mean length and number of shoots are presented in Table 5 and Plate 7.

a. Number of shoots

There were significant differences among the growth regulator treatments with respect to the number of shoots. Highest number of shoots (5.9) produced by media fortified with 5 mg/l GA_3 along with BAP and NAA. The rest of treatments were on par with each other in case of number of shoots. The lowest number of shoots (3.4) was recorded by media with 2 mg/l GA_3, which was on par with rest of the treatments.

b. Mean length of the shoots

The treatment in this experiment differed significantly. Media fortified with GA_3 5 mg/l showed significantly higher shoot length (3.18) followed by 4 mg/l of GA_3 (2.48). The lowest length (1.38) was found in the media fortified with 1 mg/l of GA_3.

4.3.4 Effect of growth regulators on *invitro* rooting

There was no *in vitro* rooting in micropropagation of Cordyline as the cultures didn’t respond to the growth regulators positively. The occurrence of callus on the bases of *in vitro* shooted plantlets during rooting stage was seen which hampered root formation (Plate 8).

4.4 Standardisation of *ex vitro* rooting and hardening

Microcuttings were taken outside the culture bottle for *ex vitro* rooting and hardening. They were treated with different concentrations of IBA and planted in different media. The results on effect of IBA and media on percent rooting, number of roots and root length are presented in Table 7.

4.4.1 Percent rooting

There were no significant difference among the different concentrations of IBA and media. But the highest percentage of rooting was obtained by treating with IBA 25 ppm in peat media.

4.4.2 Number of roots

Both IBA treatments and the media differed significantly with respect to number of roots (Plate 9).The highest number of roots was produced when the micro shoots were grown in peat media (3.21) as compared to those grown in soil mixture (2.41). Likewise IBA 25 ppm

Table 5: Effect of GA_3 on number of shoots and mean length of shoots in Cordyline

Sl. No.	Treatments(GA_3)	Mean length of shoots(cm)	No of shoots
1	1 mg/l	1.38	3.6
2	2 mg/l	1.93	3.4
3	3 mg/l	2.08	3.6
4	4 mg/l	2.48	4.1
5	5 mg/l	3.18	5.9
	S.Em±	0.09	0.16
	CD at 1%	0.38	0.66

* Media fortified with 4 mg/l BAP + 0.5 mg/l NAA

Plate.7. Influence of GA_3 (5 mg/l) along with BAP 4 mg/l and NAA 0.5 mg/l on shoot elongation

treated shoots produced significantly higher number of roots (3.55) which was on par with IBA 20 ppm (2.98).

4.4.3 Length of roots

The influence of different concentrations of IBA was found to be significant in length of roots which were produced by microshoots. But there was no significant difference between the media in root length. The plants treated with 25 ppm of IBA produced significantly longer roots (5.55 cm) followed by the plants treated with 20 ppm IBA. Among the media the length of roots was found higher (4.78 cm) in peat media compared to potting mixture media (4.57 cm).

Plate.8. Callus formation in cordyline instead of rooting in media containing auxins

Table 6: Effect of different IBA concentrations and media on percent rooting, number of roots and root length of Cordyline plants

IBA concentrations	% rooting		Mean	Number of roots		Mean	Root length (cm)		Mean
	Peat	Potting M		Peat	Potting M		Peat	Potting M	
IBA 10 ppm	86.6 (72.3)	73.3 (59.2)	80.0 (65.7)	2.67	2.07	2.37	4.03	3.83	3.93
IBA 15 ppm	80.0 (63.4)	80.0 (63.4)	80.0 (63.4)	2.67	2.00	2.33	4.23	4.23	4.23
IBA 20 ppm	93.3 (81.1)	86.7 (72.3)	90.0 (76.7)	3.47	2.50	2.98	5.10	4.90	5.00
IBA 25 ppm	93.3 (81.1)	86.7 (72.3)	90.0 (76.7)	4.03	3.07	3.55	5.77	5.33	5.55
Mean	88.3 (74.5)	81.7 (66.7)	85	3.21	2.41	2.81	4.78	4.57	4.66
Factors	SEm±	CD at 1%		SEm±	CD at 1%		SEm±	CD at 1%	
Media (M)	3.57	NS		0.11	0.46		0.09	NS	
Concentrations (C)	5.06	NS		0.16	0.65		0.13	0.52	
C × M	7.15	NS		0.22	NS		0.18	NS	

Figures in parenthesis indicate arcsine transformed values

a) peat

b) Potting mixture

Plate.9. Microshoots rooted ex vitro in different rooting media

5. DISCUSSION

The present investigation was undertaken to standardize the protocols for the *in vitro* culture establishment, multiplication and rooting of dracaena and cordyline, also hardening of propagated plants for better field establishment. During the course of investigation studies were made to find suitable explants, standardize surface sterilization method, and evaluate growth regulators on shoot multiplication and to find the best hardening medium. The results of the study are discussed in this chapter.

5.1 Culture establishment

The function of culture establishment is to disinfect the explants, establish them in culture media and to stabilize them for multiple shoot production.

5.1.1 Standardization of surface sterilization

Micropropagation involves culturing different explants under aseptic conditions in which surface sterilization or disinfection is one of the important prerequisites for successful micropropagation. Removing contaminants from the surface of the organ/ explants is of prime concern (Hartmann *et al.*, 2002). The contamination of explants may be due to the presence of fungi, bacteria, moulds etc., on the surface or in the cracks, scales etc. Disinfestation requires the use of chemicals that are toxic to microorganisms but non-toxic to plant materials. Tissue culture became possible with the use of convenient and effective disinfectants such as ethanol, sodium hypochlorite, calcium hypochlorite etc., (Krikorian, 1982).

In current investigation the effect of merucuric chloride was evaluated on reducing contamination rate and increasing the percentage healthy cultured plants. It was observed that $HgCl_2$ at 0.1% is good for surface sterilization. The efficacy of mercuric chloride might be due to its extreme poisonous nature due to high bleaching action of two chloride atoms and tendency of mercuric ions to combine strongly with protein causing death of organism (Pauling, 1955).

Badawy *et al.* (2005) obtained the highest mean value of healthy, contamination free explants using $HgCl_2$ 0.3 g/l. Beura *et al.* (2007) concluded that the sterilization of explants with 0.1 % $HgCl_2$ for 5minutes was found to be the best by considering all the viable characters such as survival percentage, aseptic culture, and days to bud emergence.

The highest percentage of contaminant free explants was obtained when they were exposed to 0.1 % $HgCl_2$ for 10 minutes (90%) pretreated with fungicides and bactericides. But the survival was reduced to 14.7% in case of cordyline (Plate 2b). This might be due to the high bleaching activity of the chemical due to more exposure of explants to the sterilant. The highest response of explants and less contamination without any toxicity is obtained when they were exposed to 0.1 % $HgCl_2$ for 5 minutes (87.8%) along with fungicides and bactericides (Plate 2a). Same trend was seen in dracaena also but the percent response of explants was nil.

This is because, the major contaminants were removed by carbendazim and streptomycin pretreatment and probably the chloride in $HgCl_2$ commonly present as bichloride of mercury. Hence it can be inferred that application of fungicides and bactericides showed significant effect in reducing the contamination rather than use of chemical singly, which produced less amount of contaminant free cultures. Mubarak and Choudhary (1992) used streptomycin sulphate plus mercuric chloride 0.1 percent for sterilization of flower buds of carnation.

5.1.2 Standardising explants for culture establishments

The type of organs or explants chosen influences the success in establishment of the cultures and their subsequent growth. Not all the tissues or organs of a plant are equally capable of exhibiting morphogenesis (Hartmann *et al.*, 2002).

In the present study, attempts were done to identify a suitable explant for micropropagation of dracaena and cordyline. Among the various explants, shoot tips gave the quickest response for initial growth. On the other hand nodal cuttings took more time for growth. These differences among the explants might be due to the difference in physiological state of explants (Sreelatha *et al.,* 1998). The maximum response of shoot tips can be due to the fact that shoot tip has meristamatic region where cell division and differentiation occurs (Hartmann *et al.*, 2002). In case of dracaena younger the parent material from where the explants were taken the sooner was development of sleeping buds (Deberg 1976).

The results on multiple shoot production revealed that there was no significant difference between nodal cuttings and shoot tips. Hence it was felt that both were suitable as explants in the micropropagation of cordyline. Similar observations were also recorded by Badawy *et al.* (2005) and Ray *et al.* (2006) using shoot tips as explants and Beura *et al.* (2007), Debergh, 1975, and Chua *et al.* (1981) using nodal and stem pieces for micropropagation of dracaena and cordyline.

When leaf base explants were used, it was unable to establish *in vitro*. On the contrary stem explants appeared to be very good material and this was probably due to the presence of rudimentary cambiums seen in several monocots like dracaena (Debergh 1975). The cambial tissue could have benefitted stem pieces in recording better multiple shoot production, on account of better dedifferentiation whereas the leaf base explants could not register appreciable multiple shoot production.

5.1.3 Standardising different growth regulators and their concentrations for shoot production and rooting

Ratio of auxins to cytokinins was critical in the regulation of *in vitro* morphogenesis of callus (Skoog and Miller, 1957). Auxin control cell elongation, apical dominance and adventitious root formation. Cytokinin support cell division and are vital for the induction of multiple shoot formation in clonal propagation. However gibberellins support cell division and cell elongation (Rajmohan et al., 2005).

5.1.3.1 Shoot multiplication

The data obtained on the Influence of BAP alone and along with NAA revealed that as the concentration of BAP increased the days taken for shoot initiation decreased and formation of clumps became more prominent. This might be due to the fact that suppression of apical dominance at higher BAP concentration led to the production of more number of shoots with reduced length, since the cytokinins were known to reduce apical dominance and promote lateral growth.

Similar observations of cordyline and dracaena, an increase in the content of cytokinins in tissue culture media led to an increase in the multiplication rate and decrease in shoot length (Vardja and Vardja, 2001). Only two treatments with 2 mg/l and 4 mg/l BAP showed better response in terms of shoot production out of which media with 4 mg/l BAP produced more number of shoots. Rest of the treatments produced only clumps of buds which were not measurable (Plate 5).

The combination of BAP and NAA improved shoot multiplication as against BAP alone resulting in more number of multiple shoots by the use of these growth regulators in combination. A lower level of auxin and higher level of cytoikinin will increase bud formation by hormonal control of organ formation (Razdan, 2002). This was in confirmity with the results of Atta- Alla *et al.* (1996) and El-Sawy *et al*. (2000) on *Dracaena marginata cv. Tricolor*, who demonstrated that the highest shoot proliferation produced on MS medium containing BA at 4.0 mg/l and NAA at 0.05 mg/l. Beaura *et al.* (2007) showed that the presence of BAP (2 mg/l) with NAA (0.5 mg/l) produced significantly longer shoots and more leaves in dracaena. Khan *et al.* (2004) reported profuse adventitious shooting of cordyline in media containing a combination of kinetin at 4.0 mg/l and NAA at 0.5 mg/l.

Results revealed that the least number of days taken for shoot initiation together with more number of shoots was recorded by the media with the combination growth regulator i.e., BAP at 4 mg/l+ NAA at 0.5 mg/l (Plate 6).

As the shoots formed were relatively shorter the effect of different concentrations of GA_3 was evaluated to improve shoot length. Media fortified with 4 mg/l BAP + 0.5 mg/l NAA +5 mg/l GA_3 produced more number of shoots with highest mean length of shoots (Plate 7). This was in confirmation with Beaura *et al.* (2007) who reported inclusion of GA_3 (5 mg/l) with BAP (2 mg/l) and NAA (0.5 mg/l) resulted in longer shoots. Vardja and Vardja, (2001) also mentioned the importance of elongation media to break multiplication and to promote elongation of the already formed shoots in Draceana, Cordyline and Diffenbachia.

5.1.3.2 Rooting

Shoots obtained in the present study were transferred to rooting media but the multiplied shoots did not repond to *invitro* rooting rather callus tissues were formed instead of roots (Plate 8). The occurrence of callus on bases of *in vitro* plantlets during rooting stage hampers and slows root formation (Alkhateeb, 2008). Atkinson *et al.* (1991) also reported failure in rooting due to callus production in antirrhinum. The reason attributed may be genetic background, physiological state of mother plant and the explants taken for rooting which interacts with hormones and environmental conditions (Nemeth, 1986). Hartmann *et al.* (2002) also reported that external application of auxin gives little or no rooting response in certain plants.

When culture passes through numerous multiplication cycles or subdivisions such cultures may not root readily (Sathyanarayana and Dalia, 2007). Shoot cultures may become habituated to cytokinin and continue to proliferate even after culture has been transferred to medium without any growth regulator leading to habituation of cultures (Hartmann *et al.,* 2002).

Vardja and Vardja, (2001) concluded that in such cases shoots should be either transferred onto a sterile rooting medium, or the fresh liquid rooting medium should poured onto the previous elongation medium, or rooting should be performed *ex vitro.*

5.2 Standardization of *ex vitro* rooting and hardening

As microshoots failed to repond positively to the *in vitro* rooting, *ex vitro* rooting was undertaken in different hardening media. Rooting and acclamitization when done simultaneously can substantially reduce cost of micropropagation .Treatment with root inducing growth regulators may be required prior to a rooting medium (Sathyanarayana and Dalia, 2007).

In present study percent rooting of plants were non-significant among the treatments. But both IBA concentrations and different media were found to significantly affect the number of roots (Plate 9). Different concentrations of IBA found to differ significantly in root length.

Keat Metha and Suska-Ard (2004) treated IBA 2.5 g/l on cut ends of anthurium shoots and inserted in different rooting substrates. The higher percentage of calli free roots was produced by vermiculite, peatmoss and sphagnum moss when compared to agar or phytagel added media. However Kwapata *et al*. (2010) were able to resolve the rooting problem by the dipping of the in vitro-produced shoots in IBA followed by their cultures in media containing IBA, IAA or NAA.

The survival rate of transplanted plantlets can be improved by combining rooting *ex vitro* with acclimatization and has been widely used in micropropagation of plants like *Gardenia jasminoides, Aloe vera*, *Cunninghamia lanceolata, Gerbera jamesonii,* and *Gypsophila paniculata*. The suitable temperature for *ex vitro* rooting is 18-30℃, optimum being 25℃ with relative humidity of 80-90% in which the rooting plantlets can be cultured in perlite instead of common rooting medium (Qing and Quinglin, 2010).

5.3 Prtocol for micropropagation of cordyline

Based on results, a protocol for micropropagation of Cordyline is given below.

1. Shoot tips or nodal cuttings of 1-2 cm should be isolated.

2. The isolated plantlets are to be washed with detergent (Tween 20) followed by three rinses in distilled water and these are to be treated with bactericide and fungicide for 5 minutes and then washed with distilled water. They are to be treated with 0.1% $HgCl_2$ for 5 minutes and then are to be washed 3-4 times with sterile distilled water in laminar air flow cabinet.

3. Explants may be cultured on basal MS medium with 4 mg/l BAP + 0.5 mg/l NAA for three weeks.

4. These shoots may be sub-cultured on basal MS medium with 4 mg/l BAP + 0.5 mg/l NAA + 5 mg/l GA_3

5. After two – three subcultures microshoots may be treated with 25 ppm IBA in powder form and rooted *ex vitro* in peat medium in tunnels at a relative humidity of > 90%

5.4 Future line of work

1. Micropropagation studies on Dracaena by taking different explants of different age level or by taking different media other than MS medium is to be studied.

2. Effect of different adjuvants like coconut water on shoot multiplication is to be studied

3. Studies to induce *in vitro* rooting are needed by reducing the callus growth through addition charcoal or by using different combinations of auxins.

4. Since callus production was profuse in all explants propagation of Cordyline through somatic embryogenesis can be explored.

6. SUMMARY

The present study on micropropagation of Dracaena and Cordyline was conducted in the Tissue culture laboratory at the Department of Horticulture, College of Agriculture, University of Agricultural Sciences Dharwad during, 2008 -2010

Both dracaena and cordyline are high value commercial foliage ornamental plants in floricultural market at national and international levels. These foliage plants are propagated commercially by vegetative methods either by division of suckers or by air layering. These traditional methods produce less number of plants from a single plant and also take more time. Micropropagation technique overcomes these issues by giving more number of plants within a shorter period of time.

The present investigation were carried out in order to find out suitable explants for culture establishment, to standardize method for their surface sterilization, to evaluate different concentrations of growth regulators for shoot multiplication and rooting, and to establish rooting *ex vitro* along with hardening. All explants of dracaena failed to show any response under *in vitro* condition. Hence the studies were continued with only cordyline.

Among the various methods of surface sterilization $HgCl_2$ at 0.1% concentration for 5minutes pretreated with fungicides and bactericides emerged as best treatment giving maximum number of contaminated free cultures with better response.

Shoot tip, nodal cuttings were found to be suitable for micropropagation of cordyline out of different explants evaluated. Whereas the leaf base did not show any response.

Among different concentrations, shoot multiplication was significantly promoted on the media containing BAP 4 mg/l along with 0.5 mg/l of NAA. While shoot length was better, when GA_3 5 mg/l was added to the same media. However there was no *in vitro* rooting by the growth regulators in the present study.

Microshoots exhibited maximum *ex vitro* rooting when treated with 25 ppm IBA and planted in peat media.

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APPENDIX

Appendix I: Composition of stock solutions of Murashige and Skoog's medium

Stock solution 1: MS-A 1000ml (10X)	
NH_4NO_3	16.5 g
KNO_3	19.0g
$CaCl_2$. $2H_2O$	4.4g
$mgSO_4.7H_2O$	3.7g
KH_2PO_4	1.7g
Stock solution 2: MS-B 1000ml (10X) The following chemicals were dissolved in 1L of distilled water	
$MnSO_4$ $.4H_2O$	223 mg
$ZnSO_4.4H_2O$	86 mg
H_3BO_3	62 mg
KI	8.3 mg
$Na_2MoO_4.2H_2O$	2.5 mg
$CuSO_4.5H_2O$	0.25 mg
$CoCl_2.6H_2O$	0.25 mg
Stock solution 3: MS-C 1000ml (10X)	
$CaCl_2.2H_2O$	4.4 mg
Stock solution 4: MS-D1000ml (10X) The following chemicals were dissolved in 100ml of distilled water	
$FeSO_4.7H_2O$	278 mg
$Na_2EDTA.2H_2O$	373 mg
Stock solution 5: MS-E 100ml (50X) The following chemicals were dissolved in 100ml of distilled water	
Glycine	10 mg
Nicotinic acid	2.5 mg
Pyridoxine	2.5 mg
Myo-Inositol	500 mg
Thimine	0.5 mg

MICROPROPAGATION STUDIES IN DRACAENA AND CORDYLINE

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ABSTRACT

An investigation on micropropagation in Dracaena and Cordyline was conducted during 2009-2010 in the tissue culture lab of the Department of Horticulture, College of Agriculture, University of Agricultural Sciences, Dharwad. The study on surface sterilization of explants pretreated with a fungicide (bavistin) and a bactericide (streptomycin) revealed that 0.1% $HgCl_2$ for 5 minutes showed minimum contamination without any toxicity to both the plants.

As regards suitability of explants, shoot tip and nodal cuttings were the best for culture establishment in case of Cordyline. Shoot tips gave the quickest response for initial growth and nodal cuttings took more time for growth. In case of multiple shoot production, there was no significant difference between nodal cuttings and shoot tips. The explants of dracaena did not show any response.

In vitro proliferated explants were sub cultured on MS medium with BAP at different concentrations. Increase in the concentration of BAP in the media led to an increase in the multiplication rate. However there was a decrease in shoot length which resulted in formation of clumps. Media with 4 mg/l BAP showed better response in terms of shoot production. The combination of BAP and NAA improved shoot multiplication as against BAP alone resulting in more number of multiple shoots (4 mg/l BAP + 0.5 mg/l NAA). As the shoots formed were relatively shorter, GA_3 was added to improve shoot length. Media fortified with 4 mg/l BAP + 0.5 mg/l NAA +5 mg/l GA_3 produced more number of shoots of more length.

Shoots obtained in the present study were transferred to rooting media but the multiplied shoots did not respond to *in vitro* rooting rather callus tissues were formed instead of roots, so *ex vitro* rooting was undertaken. Treating the microshoots with 25 ppm IBA and hardening the shoots in peat media found to be effective with respect to percent rooting, number of roots and root length.