MICROPROPAGATION AND CROP IMPROVEMENT OF CORDYLINE (*Cordyline terminalis* (L.) KUNTH)

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis entitled " Micropropagation and crop improvement of cordyline (*Cordyline terminalis* (L.) Kunth) " is a bonafied 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.

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Certified that the thesis entitled " **Micropropagation and crop improvement of cordyline (*Cordyline terminalis* (L.) Kunth)** " is a record of research work done independently by **Mrs. R. Lavanya**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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EXTERNAL EXAMINER

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R. Lavanya

R. Lavanya

Dedicated to

my loving parents and sister

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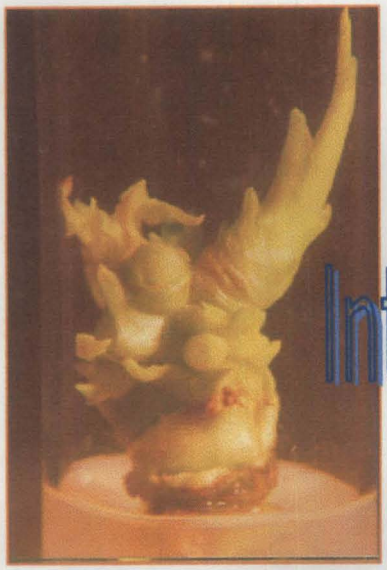
ABBREVIATIONS

BA	-	6-Benzyl amino purine
BAP	-	6-Benzyl amino-9-(2-tetrahydropyranyl)-9H-purine
2,4-D	-	2,4-Dichlorophenoxy acetic acid
EDTA	-	Ethylene diamine tetra acetate
KIN	-	N^6-Furfuryl amino purine (kinetin)
Gy	-	Gray
IAA	-	Indole acetic acid
IBA	-	Indole butyric acid
2ip	-	N^6-(2-Isopentenyl) adenine
MS	-	Murashige and Skoog's (1962) medium
NAA	-	Napthalene acetic acid
SH	-	Shenk and Hilderbrandt (1972) medium
TDZ	-	Thidiazuron
WPM	-	Woody Plant Medium (1980)



Introduction

INTRODUCTION

With the population increasing, lack of open space and the development of multistoreyed housing system, houses with garden space are becoming a rarity. Under this situation people have to depend largely on indoor plants for decorating their surroundings. Besides, urbanization has fostered the trend of indoor gardening all over the world resulting in increased demand for attractive indoor plants. The excellent ability of most of the foliage plants to adapt to low light intensities has enabled their use for interior decoration.

India is bestowed with rich land resources and wide range of agroclimatic zones and offers a great potential to produce a wide variety of high value crops, including foliage plants, especially those which are popularly called as 'tropical exotics'. However, the potential of foliage plant production on a commercial scale has not been exploited fully. The market for most of the foliage plants is year round and the increased demand for foliage plants, both in the international and domestic market, calls for considerable augmentation of local production (Swarup, 1993).

Cordyline is an important foliage plant of the genus *Cordyline,* belonging to the family Agavaceae. It consists of about 20 species of evergreen trees and shrubs. Most of the species are native to tropical and sub tropical regions of Asia and Australia (Joiner, 1981). The genus name *Cordyline* derives from 'cordyle' a club, probably alluding to the swollen stem bases of some species which give a rather club like appearance. Important ornamental species of cordyline are *Cordyline terminalis, C. australis, C. rubra* and *C. indivisa.* However, *C. terminalis* is the most important and commercially exploited species. Some of the important varieties in cultivation are Baby Doll, Lily Put, Kiwi, Hawaiian flag and Mahatma.

Cordylines are very useful ornamental plants of great beauty with richly coloured foliage and make attractive foliage plants for large pots, tubs and for the

conservatory. They can be used as floor plants, desk or tabletop plants or as focal points in gardens. The attractive colour of the foliage, hardiness, ease of cultivation, suitability to interior conditions and freedom from serious pests and diseases contribute much to the popularity of this ornamental plant.

Cordyline is propagated by seeds, stem sections and suckers (Beckett, 1987). Though plant propagation is easy through stem sections and suckers, there are insufficient stock plants of most cultivars to meet the current demand. To overcome this bottleneck in the propagation procedure, an alternative method is essential.

In recent years micropropgation has become one of the important methods for clonal multiplication of ornamental plants, but the reported works in *Cordyline* are scanty. Hence, it would be desirable to develop a proper protocol for mass multiplication of cordyline so that good quality plants can be made available to the grower at a reasonable rate in sufficient number. The present study was thus undertaken to study the response of various explants of cordyline and to identify the most suitable explant and media combination for *in vitro* propagation. Attempts were also made to find out the optimum dose of γ - irradiation, for inducing morphological variation in this ornamental plant of commercial importance.

Review of literature

REVIEW OF LITERATURE

Literature on aspects pertaining to the *in vitro* propagation of cordyline is briefly reviewed hereunder. In aspects where not much information is available in cordyline, relevant reports on other members of the family Agavaceae and other foliage ornamentals are also included.

2.1 Routes of *in vitro* propagation

Micropropagation is attracting considerable attention for obtaining large number of genetically pure elite propagules (Anand and Bir, 1984). According to Murashige (1974) there are three possible routes available for *in vitro* propagule multiplication.

a) Enhanced release of axillary buds (direct organogenesis from the meristematic region)
b) Production of adventitious buds and through organogenesis (direct or callus mediated organogenesis, from the meristematic or non meristematic region)
c) Somatic embryogenesis

For the enhanced release of axillary buds, primary meristems, such as, shoot tips and axillary buds are mainly used as explants. Shoot tip cultures ensure genetic uniformity while organogenesis through a callus phase may be useful for recovery of useful variant lines. Somatic embryogenesis, though limited to a few plant species, is the most rapid mode of plant regeneration (Evans *et al.*, 1981)

2.2 *In vitro* studies in cordyline

2.2.1 Explants

Success of *in vitro* propagation always depends on the proper selection of explants. The response varies in consonance with the type, stage and physiological age of the explants.

Route	Explant	Reference
Enhanced release of axillary buds	Stem segments	Kunisaki (1975)
Enhanced release of axillary buds	Axillary bud	Beruto *et al.* (1985)
Enhanced release of axillary buds	Apical and axillary buds	Welander (1988)
Enhanced release of axillary buds	Axillary buds	Hvoslef-Eide (1990)
Enhanced release of axillary buds	Axillary buds	Hvoslef-Eide (1993)
Enhanced release of axillary buds	Shoot tips and stem cutting	Alla *et al.* (1996a)
Direct organogenesis	Shoot tips	Paek *et al.* (1985)
Direct organogenesis	Shoot segments	Podwyszynska and Olszewski (1995)
Callus mediated organogenesis	Shoot apices	Mee (1978)
Callus mediated organogenesis	Polar shoots	Leffring *et al.* (1985)

2.2.2 Surface sterilants.

The explants collected from the field harbour numerous microorganisms which when inoculated into the nutrient medium contaminate the entire *in vitro* system. Hence, surface sterilization is resorted prior to inoculation of explants. While sterilizing the explant minimum damage should occur to the plant or part to be cultured. The efficiency of the sterilants depends upon the type of chemical, concentration, duration of exposure etc.

In one study stem tips of cordyline were surface sterilized for 10 min in 0.50 per cent sodium hypochlorite, sectioned into 0.5 cm length, soaked 5 min in 0.25 per cent sodium hypochlorite, rinsed in sterile water and placed on the media (Kunisaki, 1975). Paola *et al.* (1986) disinfected the apical buds of *Diffenbachia* cv. Tropic White, measuring 8-10 mm for 20 minutes in 12 Vol hydrogen peroxide followed by 12 per cent sodium hypochlorite for 20 minutes.

According to Kobza and Vachunova (1989) chlorinated lime at 10 per cent concentration and 0.2 per cent $HgCl_2$ were the best sterilants for *Dracaena*. Binh *et al.* (1990) rinsed agave explants in 70 per cent ethyl alcohol for 30 s, then sterilized in 0.05 per cent mercuric chloride for 10 min and finally rinsed 5 times in autoclaved distilled water.

In a subsequent study Kobza and Vachunova (1991) used $HgCl_2$ at 0.2 per cent or Savo at 3 per cent successfully for disinfecting stem explants of *Dracaena concina*. Maity *et al.* (1994) surface sterilized cordyline explants by treating with 0.1 per cent mercuric chloride containing 1-2 drops of Tween-20, for 4-10 min. The material was then thoroughly washed with sterile distilled water prior to inoculation in a Murashige and Skoog (MS) medium.

2.2.3 Culture media

Composition of media influences the growth and morphogenesis of plant tissues. The principal components of plant tissue culture media are inorganic nutrients (macronutrients and micronutrients), carbon source(s) or organic supplements, growth regulators and gelling agent. Murashige and Skoog's (1962) medium (MS) developed for tobacco has been used for *in vitro* culture of plant species. Because of the desired salt composition MS medium is widely used (Razdan, 1993).

According to Mee (1978) MS medium was ideal for the induction of callus on cut surface of shoot apices of *Cordyline terminalis*. Evans *et al.* (1983) opined that MS medium was ideal for *in vitro* propagation of most of the members of the family Liliaceae and Agavaceae, if supplemented with growth factors.

Full strength MS medium with sucrose at the rate of 20-30 g l^{-1} resulted in the greatest shoot numbers than glucose (Evaldsson and Welander, 1985) in *Cordyline terminalis* cv. Atom. Vinterhalter and Vinterhalter (1992) could

overcome the inhibitory effect of MS macronutrient salts on lateral root formation in *Dracaena fragrans* by decreasing the concentration of 5 macronutrient salts.

Podwyszynska and Olszewski (1995) reported modified MS medium as ideal basal medium for *Cordyline fruticosa* cv. Atom, and also stated that different gelling agents used did not influence the multiplication rate, but the uptake of macroelements differed significantly. Samyn (1995) tried different sucrose concentrations in MS medium and could obtain more true to type shoots with sucrose 30-40 g l^{-1} though the multiplication rate was low.

MS medium as the ideal medium for *in vitro* propagation of cordyline was also reported by various other workers (Henny *et al.*, 1981, Evans *et al.*, 1983, Razdan, 1993)

2.2.4 Medium supplements

Medium supplements are certain complex organic additives which influence the establishment and growth of *in vitro* cultures. Apart from the in organic constituents of the media, which give consistent results, the organic medium supplements often do not give any definite results. Adenine, adenine sulphate, casein hydrolysate, activated charcoal, yeast extract, peptones, coconut water, tomato juice, banana homogenate etc are some of the complex substances added to the media.

In cordyline, use of activated charcoal for *in vitro* rooting (Maene and Debergh, 1985), coconut water for indirect organogenesis (Mee, 1978 and Joiner, 1981) and adenine sulphate for multiple shoot production (Joiner, 1981 and Paek *et al.*, 1985) have been reported.

2.2.5 Culture environment.

Environmental factors play an important role in the success of tissue culture. The physical form of medium, pH, light, temperature and relative humidity greatly influence the process of growth and differentiation of tissues.

Murashige (1974) observed that light requirement for differentiation involves a combination of several components, namely, intensity, quality and duration. He found that the optimum light intensity for shoot formation in a large number of herbaceous species to be around 1000 lux. In low light intensities the shoots are greener and taller. For some plants, high light intensity from the beginning of elongation stage is not advised. On *Dracaena* spp. and *Cordyline* spp., for example, it induces bud clumps comparable to apical dominance; one bud of the cluster elongates and suppresses the development of the other buds. This can be avoided by giving a few weeks of low light intensity until the buds start to elongate, followed by higher light intensity (Debergh and Maene, 1981). Paek *et al.* (1985) could develop multiple shoots in cordyline with a light intensity of 500-1500 lux. The optimum daylight period was considered to be 16 hours for a wide range of plants. (Murashige, 1977). The quality of light also influenced organogenic differentiation (Weis and Jaffe, 1969). Blue light promoted shoot-bud differentiation, where as red light stimulated rooting in tobacco (Letouze and Beauchesne, 1969).

Kunisaki (1975) incubated successfully the cordyline cultures at a constant temperature of 20°C and under continuous light of about 2.1 klx. Mee (1978) reported 28° to 30°C room temperature and a continuous light intensity of 1,000 lux (Gro lux) for incubating cultures intended for callus production. Yeoma (1986) reported that the environmental temperature at the original habitat of a particular species should be taken into consideration while fixing the culture temperature under *in vitro* conditions. However, most tissue cultures are grown successfully at temperature around 25±2°C (Razdan, 1993).

Relative humidity is rarely a problem except in arid climates, where rapid drying occurs. Hu and Wang (1983) reported that air humidity is not frequently controlled and when it is controlled, 70 per cent has been found to be the most frequent setting.

2.2.6 Routes

2.2.6.1 Enhanced release of axillary buds.

The enhanced axillary branching method of shoot multiplication may be initially slower than the other two methods but with each passage the number of shoots increases logarithmically and within a year astronomical figures can be arrived at (Bhojwani and Razdan, 1983). The greatest success using this technique has been achieved, in herbaceous horticultural plants. The success may be due to the weak apical dominance and strong root regenerating capacity of the herbaceous plants (Hu and wang, 1983).

Welander (1988) tried enhanced release of axillary buds from apical and axillary bud explants of *Cordyline terminalis* cv. Atom. Shoot proliferation from axillay buds was obtained in MS medium when supplemented with 0.7 mg l^{-1} NAA and 0.5 mg l^{-1} BA. Multiple shoots were obtained by transferring into MS medium with similar additives, but reduced NAA (0.5 mg l^{-1}) and full or half strength of macro nutrients.

Kunisaki (1975) achieved *in vitro* propagation of *Cordyline terminalis* through the induction and proliferation of shoots of stem explants using a modified Murashige and Skoog medium supplemented with 0.5 ppm of 6-benzyl amino purine. Pierik and Steegmans (1983) reported enhanced release of axillary buds on MS nutrient agar medium containing 25 mg l^{-1} Fe EDTA, 3 per cent sucrose, 5 mg l^{-1} nicotinic acid, 1 mg l^{-1} BA and 0.1 mg l^{-1} NAA in yucca.

Binh *et al.* (1990) reported the extensive proliferation of multiple shoot primodia in *Agave cantala, A. fourcroydes* and *A. sisalana* stolon explants when the basal MS medium was supplemented with 0.07 mg l^{-1} NAA + 0.1 mg l^{-1} IBA + 0.5 mg l^{-1} Kinetin. Alla *et al.* (1996a) used MS medium in combination with

2.0 mg l^{-1} BA + 0.1 mg l^{-1} NAA for the production of multiple shoots in *Cordyline terminalis*. The number of shoots and leaves per stem cutting explant were significantly greater with 0.5-4.0 mg l^{-1} BA than with the same concentration of kinetin or 2ip. Highest rate of shoot proliferation was recorded on MS medium containing BA at 4 mg l^{-1} and NAA at 0.05 mg l^{-1} in *Dracaena marginata* var. Tricolor (Alla *et al.*, 1996b).

Alla and Staden (1997) excised the shoot tips of *Yucca aloifolia* and cultured on half strength MS medium supplemented with 3 per cent sucrose and 0.2 per cent gelrite. The highest number of proliferated shoots was obtained with 4.5 µM TDZ plus 1.1 µM NAA. The best shoot growth of green and variegated cultivars of *Yucca elephantipes* was obtained on medium supplemented with 5 mg l^{-1} KIN 0.125 mg NAA l^{-1} and for variegated cultivars on medium supplemented with 5 mg l^{-1} KIN and 0.5 mg l^{-1} NAA (Sakr *et al.*, 1999)

Proliferation of good quality shoots in agave was achieved on agar solidified basal MS medium supplemented with L2 vitamins and 13.3 µM benzyl adenine (Ruvalcaba *et al.*, 1999).

2.2.6.2 Organogenesis

Organogenesis may be direct or callus mediated (Evans *et al.*, 1981). In general, the most desirable method of multiplication would be adventitious organogenesis, because it enables a substantially faster increase in propagules (Debergh and Maene, 1981). Induction of callus has been made possible only in some plants. The extended proliferation by repeated subculture may result in reduction of regenerative capacity of callus.

Paek *et al.* (1985) reported successful multiplication of shoot tips of *Cordyline terminalis* on a solid MS medium + IAA at 1.0 mg l^{-1} + KIN at 3.0 mg l^{-1} + adenine sulphate at 100 mg l^{-1} + 150 mg l^{-1} $Na_2H_2PO_4$. For rapid multiplication sub culturing was recommended every six weeks. Robert *et al.* (1987) used high cytokinin concentrations for inducing adventitious shoot

formation on stem explants of *Agave fourcroydes.* When these shoots were excised and sub cultured, new callus formed at their base from which new shoots were produced. *In vitro* multiplication of shoot buds directly from the rhizome of *Agave sisalana* is possible on SH medium supplemented with 4.4 µM BA. MS medium supplemented with auxins and cytokinin was found to be unsuitable for regeneration (Das, 1992).

Mee (1978) induced callus on cut surface of shoot apices of *Cordyline terminalis* in MS media supplemented with 10 per cent by volume coconut water and 2,4-D 3.0 mg l^{-1}. The callus growth was maintained by transferring at monthly intervals two to three times on basal medium, and then allowing to differentiate on the same medium minus 2,4-D. MS medium enriched with BA + IAA or BA + NAA was found to be the best medium for *Dracaena.* Callus explants did not transmit leaf colour striation (Kobza and Vachunova, 1989).

Agave arizonica was successfully propagated *in vitro* using modified MS medium by Powers and Backhaus (1989). Adventitious shoots developed from callus which formed on bubil explants grown on medium supplemented with 1.4 µM 2,4-D. The shoots proliferated by subculture on medium supplemented with 44.4 µM 6-benzyl amino purine (benzyl adenine), and either 0.50 or 5.4 µM NAA. The development of calli from seed fragments of an *Agave* species and subsequent regeneration of shoots from callus was reported by Groenewald *et al.* (1977).

Rapid multiplication of *Dracaena* plants through callus mediated organogenesis was reported by Kobza and Vachunova (1991). They could proliferate callus from stem explants on MS medium supplemented with 2,4-D (0.5 mg l^{-1}). Regeneration of plants were obtained by transferring the callus cultures into medium containing KIN 1 mg l^{-1} + IAA 0.8 mg l^{-1} with out loss in leaf coloration. Stem segments of *Cordyline terminalis* showed high growth of soft and creamy white callus on MS basal nutrient agar medium, supplemented with 2,4-D (0.1 mg l^{-1} – 0.5 mg l^{-1}). Multiple shoot development was enhanced

when the callus was transferred into MS basal nutrient liquid medium containing 2,4-D or NAA (0.01 mg l^{-1} – 1.0 mg l^{-1}) and BAP (10 mg l^{-1} – 20 mg l^{-1}) (Maity *et al.* 1994).

Nikam (1997) initiated callus from rhizome and stem explants of *Agave sisalana* on MS, SH, Gamborg and White's medium, supplemented with different concentrations of BA, KIN, NAA, IAA and 2,4-D, either in combination or singly. Optimum number of shoots was obtained from stem and rhizome explants, either directly or from callus. The optimum concentrations of growth regulators were 0.5 mg l^{-1} BA + 0.25 mg l^{-1} 2,4-D for callus initiation, 0.1 mg l^{-1} BA + 2 mg l^{-1} NAA for shoot induction and proliferation and 1.5 mg l^{-1} BA + 0.1 mg l^{-1} IBA or 1 mg l^{-1} BA + 0.1 mg l^{-1} for lateral bud induction in *Cordyline* (Meshkova *et al.*, 1999).

2.2.6.3 Somatic embryogenesis.

Somatic embryogenesis is the process by which haploid or diploid somatic cells develop into differentiated plants through characteristic embryological stages without fusion of gametes. The first report of somatic embryogenesis was given by Reinert (1959) in carrot cultures. General pattern of *in vitro* embryogenesis includes direct initiation from differentiated tissue and indirect initiation via callus intermediary.

Indirect somatic embryogenesis has been observed in leaf explants of *Yucca* on MS medium containing 2.0 mg 2,4-D + 1.0 mg BA per litre. Direct somatic embryogenesis was also observed, but to a lesser extent than that of in direct somatic embryogenesis (Alla and Van, 1996). Somatic embryogenesis was also reported for the endangered ornamental species *Agave victoria-reginae* (Rodriguez – Garay *et al.*, 1996). Bach *et al.* (1998) developed somatic embryos on the medium with 0.5 µM 2,4-D and 5 µM BA. They were characterized by well developed hypocotyls and roots, and inconspicuously formed cotyledons.

2.2.7 Root induction.

The plantlets produced *in vitro* should have a strong and functional root system. To obtain full plants the shoots must be transferred to a rooting medium which is different from the shoot multiplication medium, especially in its growth regulator composition.

Generally auxin favours rhizogenesis. Among the auxins, NAA and IBA have been the most effective for induction of rooting (Ancora *et al.*, 1981). Mee (1978) observed that in *Cordyline terminalis*, roots were not induced by 2,4-D, whereas addition of NAA improved root growth. Beruto *et al.* (1985) also reported that the presence of IBA in the medium improved *in vitro* rooting in cordyline.

MS macronutrient salts inhibited lateral root formation in *Dracaena* and this could be overcome by decreasing the concentration of all 5 macronutrient salts (Vinterhalter and Vinterhalter, 1992).

Rooting was promoted by treatment with IBA at 3 mg l^{-1} for cordyline and rooting was more successful if the shoots were allowed to grow to 3 cm (Paek *et al.*, 1985). According to Maene and Debergh (1985) presence of BA inhibited rooting in cordyline, but addition of charcoal to liquid stage IIIa (bud elongation and preparation for rooting) medium eliminated the residual effect of BA applied during stage II (bud induction and multiplication).

Proliferated shoots of *Cordyline* readily rooted on MS medium with or with out IAA, IBA or NAA but the best rooting was obtained with 3 mg NAA per litre (Alla *et al.*, 1996a). In the case of *Dracaena,* rooting of proliferated shoots occurred on MS medium with IBA or NAA. Addition of benzyladenine increased the number of roots per plantlet (Alla *et al.*, 1996b).

Meshkova *et al.* (1999) could induce rooting in *Cordyline* on MS medium containing 0.75 mg l^{-1} BA + 0.05 mg l^{-1} IBA or 0.5 mg l^{-1} BA + 0.25 mg l^{-1} 2,4-D. MS medium supplemented with IAA 1 mg l^{-1} induced root initiation (4-5 roots/shoot) in cordyline (Murali *et al.*, 1999).

2.3 Hardening and planting out

In vitro leaves are the only source to cover metabolic demands and to sustain the plants adaptation and regrowth, during the first few days after transplanting micropropagated plants to green house condition (Huylenbroeck *et al.*, 1998).

Wainwright (1988) observed that the environment in a tissue culture container is that of very high humidity, low light levels and usually a constant temperature. Leaves or shoots or plantlets leaving the environment are, as a result, very poorly adapted to resist the low relative humidity, high light levels, and more variable temperature found *in vivo*. Light, temperature and relative humidity are the major factors to be controlled during acclimatization. Hu and Wang (1983) suggested a period of humidity acclimatization for newly transferred plantlets. The methods of controlling relative humidity are by using polythene tent, misting and fogging. In polythene tent, as the aerial environment is closed, it is also possible to take advantage of CO_2 enrichment during hardening (Lakso *et al.*, 1986).

Maene and Debergh (1987) reported that, in cordyline, vitrification or similar physiological disorders were overcome by incubating stage IIIa cultures under high illumination and/or under low relative humidity in the container. Gas mixtures of desired composition (O_2, CO_2, ethylene, air etc) can be used to alter humidity (Weathers *et al.*, 1988).

Oliphant (1990) reported that application of paclobutrazol to the rooting medium can eliminate the need for a period of acclimatization before transfer.

The absence of growth retardants in liquid culture resulted in the formation of leafy, vitreous shoots in the case of *Philodendron*. When transferred to the green house, survival of plantlets that had been treated with growth retardants (ancimidol or paclobutrazol) during proliferation was 100 per cent, compared to 78 per cent for control. Growth retardants had a dwarfing effect even after 5-6 weeks, in the green house (Ziv, 1991). Elevated CO_2 levels (800 ml/m^3) during acclimatization increased survival rate and plant height of rooted and non-rooted plantlets (Telgen *et al.*, 1992).

Hagiladi and Watad (1992) opined that drenching of paclobutrazol is more effective than foliar spray. It effectively reduced the shoot length in cordyline, but the number of leaves was not affected, except for the highest drench concentration, which reduced it by 10 per cent.

The rooted plantlets of *Cordyline* were transferred successfully to a peat based compost (Welander, 1988). Kobza and Vachunova (1989) used perlite:peat (1:1) mixture for successful establishment of *Dracaena* plantlets.

In the case of *Yucca*, plant height and number of leaves per plant in the green cultivar were the highest in peat moss + sand (1:1 v/v), and in the variegated cultivars in peat moss + sand (2:1 v/v) (Sakr *et al.*, 1999). In another study pine bark + garden soil + sand (1:1:1) was found to be the best media for cordyline (Ziaullah *et al.* 1999).

2.4 Nutritional requirement

Plants have varying fertilizer needs. Growth parameters of the acclimatized plants are mostly dependent on the availability of nutrients. Apples Diaz (1984) reported that application of slow release fertilizers considerably increased shoot production in *Cordyline terminalis*. Weathers *et al.* (1988) employed a nutrient mist for growth of plant tissues of cordyline. In this case

plant height was increased by 4-6 times, and shoot quantity was increased by 3-20 times compared to the controls.

Alla *et al.* (1996a) obtained tallest plants of *Cordyline* with a 15:15:15 NPK formulation. According to Ding *et al.* (1997) 16:16:16 NPK fertilizer was best for *C. fruticosa,* giving a 42 per cent increase in plant height, compared with the control. The optimum requirement of fertilizer was 12 g per pot.

2.5 Induced mutagenesis

The possibility of producing adequate quantity of plants of known superior types with uniform quantity can be assured through *in vitro* propagation. In addition, standardization of this technique offers possibilities of genetic manipulations of the species through induced mutagenesis.

Mutation breeding through ionizing radiation may induce new variants, which are otherwise non-existent or restricted in nature. In ornamentals any variant with difference in colour or shape will be of great value.

Jasrai *et al.* (1994) isolated a mutant of *Kalanchoe* showing altered phyllotaxy of whorled nature. Normally *Kalanchoe* plants exhibit an opposite, deccusate phyllotaxy. In the case of *Cordyline* the effect of irradiance was tested by Hvoslef-Eide (1990). The lowest irradiance of 75 μmol m^{-2} s^{-1} was significantly inferior to the other irradiances (15, 30 and 45 μmol m^{-2} s^{-1}) with respect to bud sprouting. According to Fereol *et al.* (1996), gamma irradiation on *in vitro* plantlets increased genetic variation in *Alpinia.*

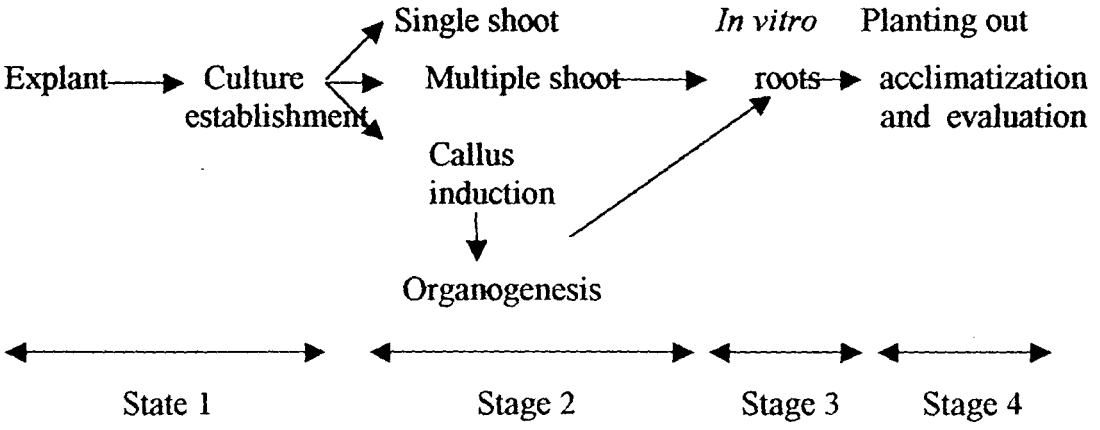
Materials and Methods

MATERIALS AND METHODS

The investigations on micropropagation and crop improvement of cordyline [*Cordyline terminalis* (L.) Kunth.] were carried out in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during 1998-2000. The details regarding the experimental material used, methodology adopted and analytical techniques followed are described in this chapter.

In the present study, the response of various explants to *in vitro* cloning was attempted through enhanced release of axillary buds and organogenesis (direct and callus mediated). The stages involved are illustrated in Fig.1.

Fig.1. *In vitro* propagation technique in *Cordyline terminalis*



Stage 1 - Physiological preconditioning of the explant and explant establishment

2 - Induction of axillary shoots/callus mediated organogenesis and rapid multiplication

3 - *In vitro* rooting and acclimatization

4 - Planting out and evaluation

3.1 Explants

Details of different types of explants used are given in Table 1.

Table 1. Explants used for *in vitro* culture studies of *Cordyline terminalis*

Route	Explant
1. Enhanced release of axillary buds	Shoot tips and nodal segments
2. Organogenesis	
1) Direct	Shoot tips, nodal segments and axillary buds
2) Callus mediated	Shoot tips, nodal segments, axillary buds and leaf segments

Table 2. Different chemicals used for surface sterilisation of explants in *Cordyline terminalis*

Sl. No.	Sterilant	Concentration (%)	Duration of treatment (min)	Explant
1	Ethyl alcochol	70	2	Nodal segments, Shoot tips, axillary buds
2	Mercuric chloride	0.05	10, 12, 15	Leaf segments
3	Mercuric chloride	0.10	5, 10, 15	,,
4	Calcium hypochlorite	5.0, 7.5, 10.0	5, 10, 20, 30	Nodal segments, Shoot tips, axillary buds
5	Sodium hypochlorite	1.0, 1.5, 2.0	5, 10, 20, 30	,,
6	Ethyl alcohol wiping + mercuric chloride	70 0.1	12, 15, 18, 20	,,
7	Ethyl alcohol wiping + calcium hypochlorite	70 5.0, 7.5, 10.0	5, 10, 20, 30	,,
8	Ethyl alcohol wiping + sodium hypochlorite	70 1.0, 1.5, 2.0	5, 10, 20, 30	,,

3.1.1 Collection and preparation of explants

The explants were collected from one year old cordyline plants maintained in the shade house attached to tissue culture laboratory. The plants were given prophylactic sprays (0.2% carbendazim) against microbial contamination.

Mature and immature stems were used for taking nodal segments, shoot tips and axillary buds. Leaf segments were collected at different stages of plant growth. Immature leaves, young leaves and mature leaves were used. The method of preparation of explant varied with the type of explant.

After collecting the stem segments, the leaves were removed along with petioles and washed with tap water. Stem segments were cut into pieces of 5 cm and washed with tap water containing few drops of teepol and then treated with 0.2 per cent carbendazim (50% WP) for 10 minutes. Then they were washed with distilled water, blotted and wiped with cotton dipped in 70 per cent alcohol.

Leaves collected were first washed with tap water and cut into 5 cm bits and then washed with tap water containing few drops of teepol. They were then washed with distilled water, blotted and wiped with cotton dipped in 70 per cent alcohol. Further sterilization procedures were carried out in laminar air flow chamber.

3.1.2 Standardisation of surface sterilization methods

Surface sterilization of explants were done in order to make the explants free of contaminations and micro organisms.

Details regarding the chemicals used for the surface sterilization of the explants are given in Table 2. Observations were made on the percentage of contamination and survival of ten cultures, each, after every week. MS medium with BAP 2.0 mg l^{-1} was used for this purpose.

After sterilization the explants were rinsed four times with sterile distilled water and dried by carefully transferring then onto sterile filter paper pieces on a sterile petriplate. Nodal segments were then reduced to sizes of 1.0 cm to 1.5 cm. Axillary buds and shoot tips were reduced to size of 1.0 cm. The leaf pieces along with midrib were reduced to size of 0.8 to 1.0 cm^2.

3.2 Culture media

MS (Murashige and Skoog, 1962), SH (Shenk and Hilderbrandt, 1972) and WPM (Lloyd and Mc Cown, 1980) media were used for the study. The chemical composition of the media is given in Appendix-I

The chemicals used for preparation of the culture media were of analytical grade from British Drug House (BDH), Sisco Research Laboratories (SRL), Merck or Sigma. Standard procedures were followed for the preparation of the media. Stock solutions of major and minor nutrients were prepared first by dissolving the required quantity of chemicals in double distilled water and were stored under refrigerated conditions in amber coloured bottles. The stock solutions of nutrients were prepared at two month interval and those of growth regulators, vitamins and amino acids were prepared at six week interval.

Required quantities of the stock solutions were pipetted out into a clean vessel which was rinsed with distilled water. Sucrose and inositol were added fresh and dissolved. The solution was made up to the required volume by adding double glass distilled water. The pH of the solution was adjusted between 5.5 and 5.8. Agar was then added to the medium and stirred thoroughly.

The agar was then melted by keeping the solution in a water bath, maintained at a temperature of 90-95°C, until the media became clear. About 15 ml of the medium was poured hot to oven sterilized culture vessels which were previously rinsed twice with double distilled water and dried. The

containers with the medium were then tightly plugged with non absorbant cotton wool plugs. Borosilicate test tubes of size 100 x 25 mm and 150 x 25 mm were used as the containers.

The test tubes with medium were autoclaved for 20 minutes at 15 psi pressure and 121°C temperature. After sterilization the culture tubes were immediately transferred to air conditioned culture room for further use.

3.3 Explant inoculation

Inoculation was carried under strict aseptic conditions, in a laminar air flow cabinet. Sterilized forceps, surgical blades, petridishes and blotting papers were used. The explants, after cutting to sufficient size with a sterile knife, were transferred into the test tubes near the flame over a gas burner. In the case of axillary bud explants, axillary buds were scooped out from the stem segments after sterilisation and used for inoculation.

The culture vessels were then transferred to a culture room, where they were incubated at a temperature of 27 ± 2°C. Artificial illumination of intensity 2400 lux was provided using cool white fluorescent lamps. Photoperiod was maintained as 16 h per day.

3.4 Season of explant collection

A preliminary trial was conducted to standardize the best season for inoculation, which favoured maximum culture establishment and minimum contamination rate. For this purpose explants were collected and inoculated from January to December. Data pertaining to the meteorological parameters during this period are presented in Appendix II. The medium used was MS, containing optimum amounts of nutrients and growth regulators. Observations on the percentage of cultures survived were recorded at weekly intervals.

3.5 The routes of micropropagation

3.5.1 Enhanced release of axillary buds

3.5.1.1 Explant choice

The explants used for the enhanced release of axillary buds were shoot tips and nodal segments. Observations were recorded on the response from 10 cultures.

3.5.1.2 Culture establishment

The culture establishment trials were carried out using shoot tips and nodal segments in MS medium, supplemented with cytokinins, BAP and kinetin (0.5 to 3.0 mg l^{-1}) alone and in combination with NAA (0.5 mg l^{-1}). Observations on the number of days taken for shoot bud emergence, number of shoots and number of days taken for shoot elongation were recorded up to three weeks of culturing.

3.5.1.2.1 Effect of media on culture establishment

In addition to MS medium, WPM and SH medium were also tried in culture establishment, shoot proliferation and rooting stages.

3.5.1.3 Shoot proliferation

The elongated buds in the Stage 1 from shoot tips and nodal segments were separated carefully under aseptic condition, and cultured to induce shoot proliferation. Trials were conducted in MS medium supplemented with cytokinins (BAP, kinetin and 2ip, each at 0.5, 1.0, 2.0 and 3.0 mg l^{-1}), alone and in combination with NAA (0.5 and 1.0 mg l^{-1}). Nature of response of the elongated buds from shoot tips and nodal segments as influenced by cytokinins

were recorded on the formation of multiple axillary buds, callus growth, shoot formation and root formation.

3.5.1.4 Elongation of multiple axillary bud aggregates

Elongation of multiple axillary buds were tried on MS medium, both with full concentration of inorganic salts (MSa) and half the concentration of inorganic salts (MSb), supplemented with IAA (0.5, 1.0, 2.0 and 3.0 mg l^{-1}) and responses were recorded. Observations on the number of days taken for shoot elongation, length of shoot etc. were recorded.

3.5.1.4.1 Standardisation of media supplements

Effect of media supplements, viz., coconut water (0, 5, 10 and 15%), activated charcoal (0.0, 0.1, 0.5, 1.0%), adenine sulphate (0, 60, 80 and 100 mg l^{-1}) and casein hydrolysate (0, 80, 100 and 120 mg l^{-1}) were tried for the culture establishment, shoot proliferation and elongation of shoots in MS basal medium. Observations on the number of days taken for sprouting or callusing, shoot proliferation, shoot elongation and root initiation, number of shoots and roots produced were recorded.

3.5.1.5 *In vitro* rooting (Stage 3)

Studies on *in vitro* rooting were carried on elongated shoots from Stage 2 in MS basal medium. Effect of different media (MS, SH and WPM) were tried. Effect of different auxins (IAA, NAA and IBA each at 1.0, 2.0 and 3.0 mg l^{-1}) were also studied. Observations on days taken for root initiation, number and length of root and nature of roots were recorded.

3.5.1.5.1 Effect of activated charcoal on rooting

Trials were also conducted to study the effect of different levels of activated charcoal on rooting of elongated shoots of *Cordyline*. MS medium was

supplemented with activated charcoal at different levels (0.0, 0.1, 0.5 and 1.0%) and observations were recorded.

3.5.1.6 Planting out (Stage 4)

Sterile water was added to the culture vessels after removing the cotton plugs and kept as such for 5 to 10 minutes. The rooted plantlets were taken with the help of forceps and the media adhering to the roots were completely removed by thorough washing with running tap water. The observations regarding plant height, number of leaves per plantlet, average length of the roots and number of roots were recorded.

In order to study the effect of media on the growth of plantlets, the following media were tried.

1. Fine sand
2. Coconut fibre
3. Peat moss
4. Vermiculite

The plantlets were treated with 0.2 per cent carbendazim (50% WP) solution for 30 minutes before planting out. After planting out the plants and media were drenched with 0.2 per cent carbendazim (50% WP) solution.

3.5.1.6.1 Standardisation of hardening treatments

The *in vitro* produced plantlets were subjected to both pre-transfer and post- transfer hardening treatments for acclimatization.

Pre-transfer treatment

(*In vitro* treatments)

1. Growing plantlets in media containing triadimefon (1.0, 2.0, 3.0, 4.0 mg l^{-1}) for one month at rooting stage

Post-transfer treatment

1. Keeping the plantlets in open
2. Covering the plantlets with polythene cover with holes for 2 weeks
3. Keeping the plantlets in net house
4. Spraying 20.0 mg l^{-1} triadimefon solution once in a week on plantlets kept in net house
5. Drenching with 20.0 mg l^{-1} triadimefon solution once in a week on plantlets kept in net house

Water was sprayed frequently to prevent the plantlets from desiccation. Observations were made on percentage of plantlet survival, at weekly intervals, up to 8 weeks.

3.5.1.6.2 Field evaluation

Effect of nutrient solution

To study the requirement of nutrients the micropropagated plantlets were supplied with a solution of 17:17:17 NPK complex. The treatments given were as follows.

Method of application*	Quantity applied / plant / week (g)
1. Soil application (alternate day)	0.00, 0.50, 0.75 and 1.00
2. Foliar spray (daily)	0.00, 0.25, 0.50 and 0.75

* 1 per cent solution of the respective treatment

Observations were made on plant height, number of leaves, leaf length and leaf breadth.

3.5.2 Organogenesis

3.5.2.1 Explant choice

Details of the explants used for both direct organogenesis and callus mediated organogenesis are given in Table 1.

3.5.2.2 Direct organogenesis

Shoot tips, axillary buds and nodal segments, inoculated to the MS medium supplemented with auxins (IAA, 2,4-D at concentrations 0.5, 1.0, 2.0, 3.0 and 4.0 mg l^{-1} and cytokinins (BAP, kinetin and 2ip at 1.0, 2.0 and 3.0 mg l^{-1}). The observations like number of cultures showing direct organogenesis, days taken for organogenesis and number of shoots and roots produced etc. were recorded.

3.5.2.3 Callus mediated organogenesis

3.5.2.3.1 Callus initiation

Explants, such as, shoot tips, nodal segments and axillary buds were placed in MS medium supplemented with 2,4-D (0.5 mg l^{-1}), alone and in combination with cytokinins (BAP and kinetin at 0.5, 1.0, 2.0 and 3.0 mg l^{-1}) for the induction of callus.

MS medium supplemented with coconut water (5,10 and 15%) alone and in combination with 2,4-D (0.5, 1.0 and 2.0 mg l^{-1}) were also used for callus induction, and incubated at 25°C ± 2 with 12 h photoperiod provided by white fluroscent tubes.

Leaf segments were inoculated to MS medium supplemented with various concentration of 2,4-D (0.5, 1.0, 2.0 and 3 mg l^{-1}) NAA (6.0, 8.0 10.0 and

12.0mg l^{-1}) and 2,4-D (0.5 and 1.0 mg l^{-1}) in combination with NAA (0.5, 1.0 and 5.0 mg l^{-1}) for the production of callus and incubated at 25°C ± 2, under continuous darkness.

The observations like percentage of cultures callusing, days taken for callusing, callus intensity and nature of callus were recorded. Callus index was worked out as below.

$$CI = P \times I$$

where P is the percentage of cultures

I is the callus intensity

3.5.2.3.2 Callus differentiation studies

The callus derived from nodal segments and shoot tips were then transferred to MS medium supplemented with various cytokinins (BAP, kinetin, 2ip at concentrations 0.5, 1.0 and 2.0 mg l^{-1} alone and BAP (0.5, 1.0 and 2.0 mg l^{-1}) in combination with kinetin and 2ip (0.5, 1.0 and 2.0 mg l^{-1}) and also MS medium supplemented with coconut water (5, 10 and 15%) to study its influence on organogenesis.

Callus derived from leaf segments were transferred to MS basal, half strength MS basal, MS medium supplemented with various cytokinins BAP (0.0, 2.5, 5.0, 7.5, 10, 12.5 and 15.0 mg l^{-1}) kinetin (0.0, 2.5, 5.0, 7.5, 10.0 mg l^{-1}) alone, and in combination with NAA (0.50 and 1.0 mg l^{-1}), MS medium supplemented with activated charcoal (0.1, 0.5, 1.0%) and also liquid medium supplemented with BAP and 2ip (1.0, 2.0, 3.0 and 4.0 mg l^{-1}).

Observations like number of days taken for differentiation, number of shoots and nature of shoots were recorded.

3.6 *In vitro* mutagenesis

In order to find out the optimum dose of gamma irradiation, callus and nodal segments cultured on basal MS media were exposed to gamma rays at 25, 50, 75, 100, 150 or 200 Gy. The irradiated samples were transferred to media for evaluation. From the results (Table 3) obtained the LD 50 (the dose which gave 50 per cent or more mortality) was found out. Since the doses above 25 Gy were lethal for all the explants, the doses 1, 5, 10, 20 and 25 Gy were fixed for further studies. After irradiation, calli were subcultured on to shoot regeneration media, nodal segments were cultured on to shoot multiplication media and proliferated shoots were cultured on to elongation media standardized from the present study. Observations were made on the following aspects.

1. Days taken for shoot regeneration from callus/ shoot multiplication from nodal segments
2. Percentage callus showing shoot regeneration
3. Number of shoots/culture
4. Number of leaves/shoot
5. Length of the largest shoot
6. Variation if any

Table 3: Response of various stages of explants of cordyline to γ-irradiation

Treatment (Gy)	% Survival		
	Culture establishment stage	Callus stage	Shoot proliferation stage
25	60.0	50.0	50.0
50	0.0	0.0	0.0
75	0.0	0.0	0.0
100	0.0	0.0	0.0
150	0.0	0.0	0.0
200	0.0	0.0	0.0

All the observations were recorded daily on 10 tubes under each treatment.

3.7 Statistical analysis

The data collected on different characters were analysed by applying the technique of analysis of variance (ANOVA) for completely randomized design (CRD) following Panse and Sukhatme (1985).

Results

RESULTS

The results of the studies on the micropropagation and crop improvement of cordyline conducted in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara are presented below

4.1 Standardisation of surface sterilization methods

The results of surface sterilization of cordyline explants using different chemicals are presented in Table 4.

Among the different sterilants, mercuric chloride (0.10%) was found to be better than calcium hypochlorite and sodium hypochlorite solution. A survival percentage of 100.00 was recorded when the nodal segments and axillary bud explants were surface sterilized with ethyl alcohol wiping, followed by mercuric chloride (0.10 %) for 18 minutes. Ethyl alcohol (70.0 %) wiping, followed by mercuric chloride (0.10%) for 20 minutes resulted in a survival percentage of 80.00 for nodal segment and axillary bud explants.

Treatment of nodal segment and axillary bud explants with ethyl alcohol wiping, followed by 5.0 per cent calcium hypocholorite solution for 5, 10, 20 and 30 minutes resulted in 60.00, 60.00, 80.00 and 60.00 per cent survival, respectively. When the nodal segments and axillary buds were treated with 2.0 per cent sodium hypochlorite solution for 5, 10, and 20 minutes survival levels to the extent of 60.00, 60.00 and 60.00 per cent, respectively, were obtained.

Maximum survival percentage (100.00) for shoot tip explants was observed in the treatment with ethyl alcohol wiping + mercuric chloride (0.10 %) for 18 minutes. The shoot tip explants recorded 80.00 per cent survival, when treated with ethyl alcohol wiping + 5.0 per cent calcium hypochlorite for 20 minutes, ethyl alcohol wiping + 7.5 per cent calcium hypochlorite for 30 minutes or ethyl alcohol wiping + 1.0 per cent sodium hypochlorite for 20 minutes.

Table 4: Effect of surface sterilants on the survival of cordyline explants

Culture period – 3 weeks
Medium – MS medium with BAP 2.0 mg l^{-1}

Treatments	Concentration (%)	Duration (Minutes)	Survival (%)			
			Shoot tips	Nodal Segments	Axillary buds	Leaf bits
1. Ethyl alcohol	70.00	2	0.00	0.00	0.00	–
2. Mercuric chloride	0.05	10	–	–	–	0.00
		12	–	–	–	20.00
		15	–	–	–	30.00
3. Mercuric chloride	0.10	5	–	–	–	20.00
		10	0.00	0.00	0.00	100.00
4. Calcium hypochlorite	10.00	10	0.00	0.00	0.00	–
5. Sodium hypochlorite	2.00	10	0.00	0.00	0.00	–
6. Ethyl alcolhol wiping +	70.00					
mercuric chloride	0.10	12	10.00	0.00	0.00	–
		15	20.00	20.00	20.00	50.00
		18	100.00	100.00	100.00	–
		20	60.00	80.00	80.00	–
7. Ethyl alcohol wiping +	70.00					
calcium hypochlorite	5.00	5	60.00	60.00	60.00	–
		10	60.00	60.00	60.00	–
		20	80.00	80.00	80.00	–
		30	70.00	60.00	60.00	–
	7.50	5	30.00	40.00	40.00	–
		10	50.00	40.00	40.00	–
		20	50.00	40.00	40.00	–
		30	80.00	80.00	80.00	–
	10.00	5	0.00	0.00	0.00	–
		10	10.00	0.00	0.00	–
		20	60.00	60.00	60.00	–
		30	40.00	40.00	40.00	–
8. Ethyl alcohol wiping +	70.00					
sodium hypochlorite	0.50	5	10.00	0.00	0.00	–
		10	10.00	0.00	0.00	–
		15	60.00	60.00	60.00	–
		20	10.00	20.00	20.00	–
	1.00	5	0.00	0.00	0.00	–
		10	20.00	0.00	0.00	–
		15	60.00	60.00	60.00	–
		20	80.00	60.00	60.00	–
	1.50	5	0.00	0.00	0.00	–
		10	30.00	0.00	0.00	–
		15	70.00	60.00	60.00	–
		20	60.00	60.00	60.00	–
	2.00	5	50.00	60.00	60.00	–
		10	20.00	60.00	60.00	–
		15	20.00	60.00	60.00	–
		20	20.00	60.00	60.00	–

Low rate of survival was observed in ethyl alcohol wiping + 0.5 per cent sodium hypochlorite for 5, 10 and 20 minutes (10.00 %, each), ethyl alcohol wiping + 1.0 per cent sodium hypochlorite for 10 minutes (20.00 %), ethyl alcohol wiping + 2.0 per cent sodium hypochlorite for 10, 15 and 20 minutes (20.00%, each) and ethyl alcohol wiping + 0.1 per cent mercuric chloride for 15 minutes (20.00%).

Mercuric chloride (0.10 %) treatment of leaf explants for 10 minutes resulted in 100.00 per cent contamination free cultures. The treatments using the same sterilant for 5 minutes resulted in 20.00 per cent survival.

4.1.2 Seasonal influence on the *in vitro* establishment of explants of cordyline.

Data on the influence of season of explant collection on culture establishment of explants of cordyline (nodal segments, axillary buds and shoot tips) are presented in Table 5, Fig. 2. Maximum survival percentage (100.00, each) of the nodal segments was obtained during the months of November and December and minimum rate of survival during the months of June and July (40.00%, each).

In the case of axillary bud explants maximum survival percentage (100.00) was observed during March. Lowest rate of explant survival (40.00%, each) and highest rate of contamination (60.00%, each) was recorded during the periods of June and July. The trend represented a gradual increase in the rate of survival as the time proceeded from July to March, except during February and then the steady decline from April to July.

Better survival of cultures and least contamination were noticed with shoot tip explants collected during the period from August to April. During May, June and July the survival percentage reduced drastically, the minimum being during the months of June and July (30.00, each)

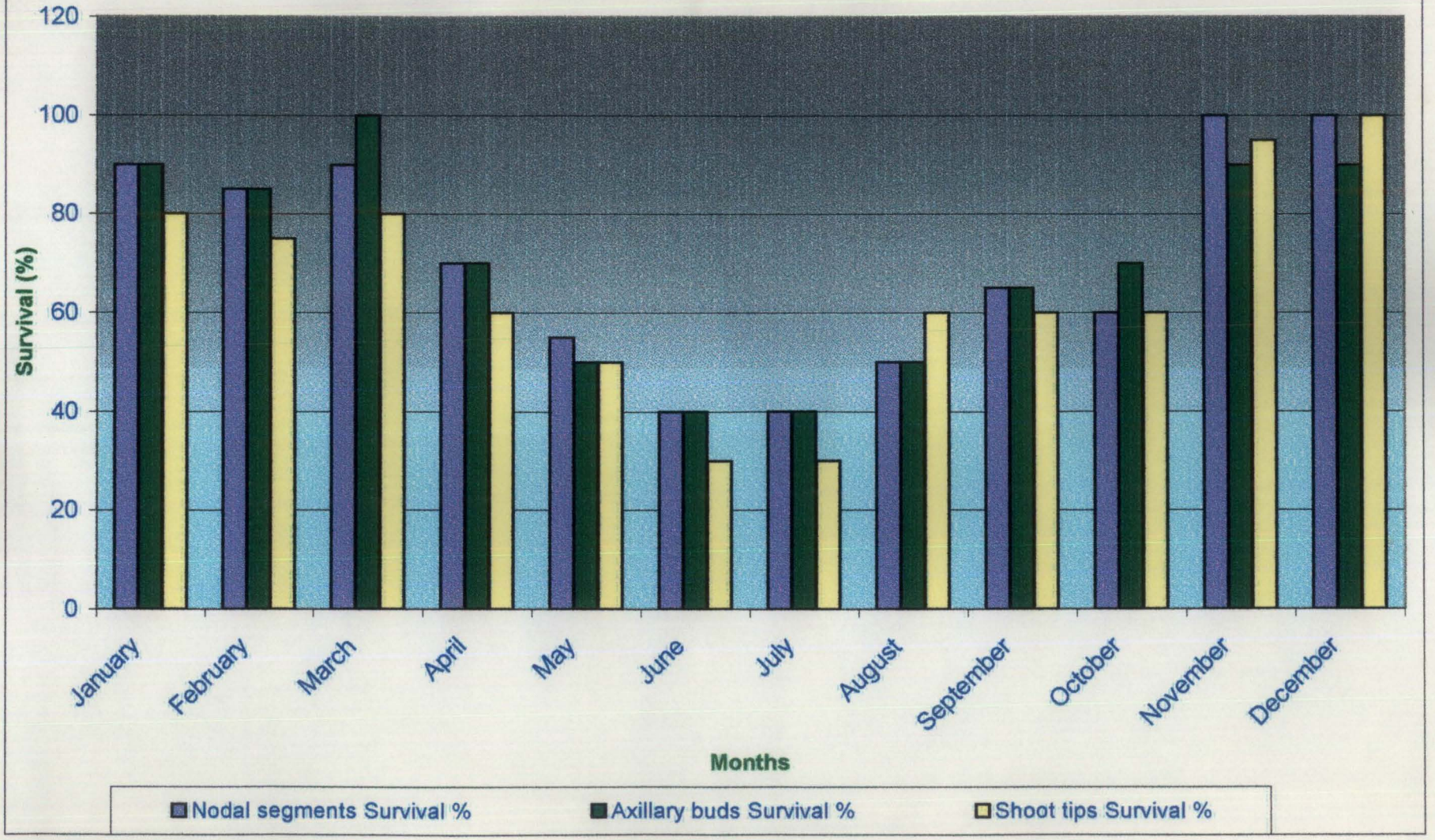
Table 5: Seasonal influence on the *in vitro* establishment of explants of cordyline

Culture period : 2 weeks
Basal medium : MS medium with BAP 2.0 mg l^{-1}

Month	Nodal segments		Axillary buds		Shoot tips	
	Contamina -tion (%)	Survival (%)	Contamina -tion (%)	Survival (%)	Contamina -tion (%)	Survival (%)
January	0.00	90.00*	0.00	90.00*	20.00	80.00
February	15.00	85.00	15.00	85.00	20.00	75.00*
March	0.00	90.00	0.00	100.00	10.00	80.00*
April	30.00	70.00	30.00	70.00	40.00	60.00
May	45.00	55.00	50.00	50.00	40.00	50.00*
June	60.00	40.00	60.00	40.00	70.00	30.00
July	60.00	40.00	60.00	40.00	70.00	30.00
August	50.00	50.00	50.00	50.00	40.00	60.00
September	35.00	65.00	35.00	65.00	40.00	60.00
October	35.00	60.00*	30.00	70.00	30.00	60.00*
November	0.00	100.00	0.00	90.00*	0.00	95.00*
December	0.00	100.00	0.00	90.00*	0.00	100.00

* The remaining explants dried inside the tube

Fig. 2. Seasonal influence on the *in vitro* establishment of explants of cordyline



4.2 The routes

4.2.1 Enhanced release of axillary buds

4.2.1.1 Culture establishment

The culture establishment trials were carried out using nodal segments and shoot tips in MS medium supplemented with cytokinins (BAP and KIN) alone, and in combination with NAA. The results are presented in Tables 6 to 8.

4.2.1.1.1 Effect of cytokinins on nodal segments

Trials were conducted with BAP and KIN at different levels (0.5, 1.0, 2.0 and 3.0) on culture establishment of nodal segments and the results are presented in Table 6.

Number of days for bud emergence

The average number of days taken for bud emergence varied from 4.4 to 7.0. The minimum period was taken by the treatment BAP 3.0 mg l^{-1} (4.4 days) and was significantly different from all other treatments. Maximum days (7.0) for bud emergence was taken by the treatment KIN 0.5 mg l^{-1}.

Number of shoots

The mean number of shoots ranged from 1.0 to 3.1 under various treatments. Maximum number of shoots (3.1) was produced by the treatment BAP 2.0 mg l^{-1} (Plate 1), followed by the treatment KIN 3.0 mg l^{-1} (2.0). Minimum number of shoots (1.0, each) was produced by the treatments BAP 1.0 mg l^{-1}, KIN 1.0 mg l^{-1} and KIN 2.0 mg l^{-1}.

Table 6: Effect of BAP and KIN on culture establishment of nodal segments of cordyline

Basal medium : MS
Culture period : 4 weeks
Average of 10 cultures

Treatments ($mg\ l^{-1}$)		Time taken for bud emergence (days)	Number of shoots per explants	Time taken for shoot elongation (days)	Nature
BAP	0.5	5.9	1.1	19.3	Bulging at the bottom
"	1.0	5.6	1.0	18.8	Slight callusing
"	2.0	5.1	3.1	17.6	"
"	3.0	4.4	1.2	17.4	60 per cent cultures showed callusing
KIN	0.5	7.0	NC	NC	Bulging at the bottom
"	1.0	6.2	1.0	20.5	"
"	2.0	5.2	1.0	18.5	Slight callusing
"	3.0	5.1	2.0	17.5	"

NC : No change

Number of days for shoot elongation

The average number of days taken for shoot elongation varied from 17.4 to 20.5 and differed significantly. Minimum days (17.4) was taken by the treatment BAP 3.0 mg l^{-1}. Maximum number of days was taken by the treatment KIN 1.0 mg l^{-1} (20.5) followed by BAP 0.5 mg l^{-1} (19.3 days).

Effect of cytokinins on shoot tips

Data pertaining to the effect of cytokinins (BAP and KIN) on culture establishment of shoot tips of cordyline are presented in Table 7.

Number of days for bud emergence

The average time taken for bud emergence ranged from 5.5 days to 7.8 days and differed significantly. The treatment KIN 3.0 mg l^{-1} has taken the least time for bud emergence (5.5) (Plate 2) and was found similar with the treatments BAP 2.0 mg l^{-1} (5.6 days), BAP 1.0 mg l^{-1} (5.7 days) and KIN 2.0 mg l^{-1} (5.8 days). The longest time for bud emergence was taken by the treatment KIN 0.5 mg l^{-1} (7.8 days).

Number of shoots

The average number of shoots per culture varied from 1.0 to 2.5. Maximum number of shoots (2.5) was observed in the medium supplemented with KIN 3.0 mg l^{-1}, followed by BAP 2.0 mg l^{-1} (2.0). Shoot number was minimum (1.0) in the medium supplemented with BAP 1.0 mg l^{-1} and was on par with KIN 0.5 mg l^{-1}(1.0) and KIN 1.0 mg l^{-1} (1.0).

Number of days for shoot elongation

The time taken for the elongation of buds ranged from 17.2 to 21.0 days. The least time (17.2 days) was taken in the medium supplemented with 2.0 mg l^{-1}

Table 7: Effect of BAP and KIN on culture establishment of shoot tips of cordyline

Basal medium : MS
Culture period : 4 weeks
Average of 10 cultures

Treatments ($mg\ l^{-1}$)		Time taken for bud emergence (days)	Number of shoots	Time taken for shoot elongation (days)	Nature
BAP	0.5	6.3	1.1	19.3	Bulging at the bottom
"	1.0	5.7	1.0	18.5	"
"	2.0	5.6	2.0	17.2	Slight callusing
"	3.0	6.2	1.2	17.6	Callusing
KIN	0.5	7.8	1.0	NC	Bulging at the bottom
"	1.0	6.2	1.0	21.0	Slight callusing
"	2.0	5.8	1.2	18.6	"
"	3.0	5.5	2.5	17.6	"

NC: No change

BAP. Maximum days for the elongation of shoots (21.0) was taken by the treatment having 1.0 mg l^{-1} KIN followed by the treatments BAP 0.5 mg l^{-1} (19.3 days) KIN 2.0 mg l^{-1} (18.6 days) BAP 1.0 mg l^{-1} (18.5 days), BAP 3.0 mg l^{-1} (17.6 days) and KIN 3.0 mg l^{-1} (17.6 days).

4.2.1.1.2 Effect of BAP and KIN in combination with NAA on nodal segments (Table 8, Fig. 3)

Number of days for bud emergence

The number of days taken for bud emergence from nodal segments ranged from 6.6 days to 11.2 days in various treatments and were significantly different. Results of the preliminary trials showed that NAA at higher concentration induced callus, so NAA was tried at 0.5 mg l^{-1} concentration only.

Minimum days for bud emergence (6.6) was taken by the treatments KIN 0.5 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 1.0 mg l^{-1} + NAA 0.5 mg l^{-1} and KIN 3.0 mg l^{-1}, + NAA 0.5 mg l^{-1} and were significantly superior to the treatments BAP 3.0 mg l^{-1} + NAA 0.5 mg l^{-1} (7.0 days), BAP 1.0 mg l^{-1} + NAA 0.5 mg l^{-1} (7.8 days), KIN 2.0 mg l^{-1} + NAA 0.5 mg l^{-1} (7.8 days); all these treatments being statistically on par.

Maximum days (11.2) for bud emergence was taken by the medium supplemented with BAP 0.5 mg l^{-1} + NAA 0.5 mg l^{-1}. This was found to be on par with the medium supplemented with BAP 2.0 mg l^{-1} + NAA 0.5 mg l^{-1} (11.0 days).

Number of shoots

Average number of shoots produced in various treatments differed significantly and varied from 1.0 to 2.6. The maximum number of shoots was

Table 8: Effect of BAP and KIN in combination with NAA on culture establishment of nodal segments and shoot tips in cordyline

Basal medium : MS
Culture period : 4 weeks

Treatments ($mg\ l^{-1}$)	Nodal segments			Shoot tips		
	Time taken for bud emergence (days)	No. of shoots (Mean)	Time taken for shoot elongation (days)	Time taken for bud emergence (days)	No. of shoots (Mean)	Time taken for shoot elongation (days)
BAP 0.5 + NAA 0.5	11.2	1.0	20.8	12.0	1.0	21.6
BAP 1.0 + NAA 0.5	7.8	2.6	18.8	7.6	2.4	19.0
BAP 2.0 + NAA 0.5	11.0	1.2	20.4	10.8	1.2	20.4
BAP 3.0 + NAA 0.5	7.0	1.8	18.0	8.0	1.6	18.4
KIN 0.5 + NAA 0.5	6.6	1.0	17.6	7.0	1.0	18.4
KIN 1.0 + NAA 0.5	6.6	1.0	16.8	6.8	1.0	17.4
KIN 2.0 + NAA 0.5	7.8	1.0	16.6	7.8	1.0	17.2
KIN 3.0 + NAA 0.5	6.6	1.0	16.8	6.6	1.0	16.4
CD (0.05)	1.21	0.50	1.30	1.18	0.41	1.67
SEm ±	0.42	0.17	0.45	0.41	0.14	0.58

produced by the treatment BAP 1.0 mg l^{-1} + NAA 0.5 mg l^{-1} (2.6), followed by BAP 3.0 mg l^{-1} + NAA 0.5 mg l^{-1} (1.8).

Treatment BAP 2.0 mg l^{-1} + NAA 0.5 mg l^{-1} produced 1.2 shoots and was on par with BAP 0.5 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 0.5 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 2.0 mg l^{-1} + NAA 0.5 mg l^{-1} and KIN 3.0 mg l^{-1} + NAA 0.5 mg l^{-1} , which produced 1.0 shoot, each.

Number of days for shoot elongation

The average time taken for shoot elongation ranged from 16.6 to 20.8 days and the differences were significant.

The treatment KIN 2.0 mg l^{-1} + NAA 0.5 mg l^{-1} recorded the shortest period (16.6 days) for shoot elongation and was statistically homogeneous with treatments having KIN 1.0 mg l^{-1} + NAA 0.5 mg l^{-1} (16.8 days), KIN 3.0 mg l^{-1} + NAA 0.5 mg l^{-1} (16.8 days), KIN 0.5 mg l^{-1} + NAA 0.5 mg l^{-1} (17.6 days), BAP 3.0 mg l^{-1} + NAA 0.5 mg l^{-1} (18.0 days) and BAP 1.0 mg l^{-1} + NAA 0.5 mg l^{-1} (18.8 days).

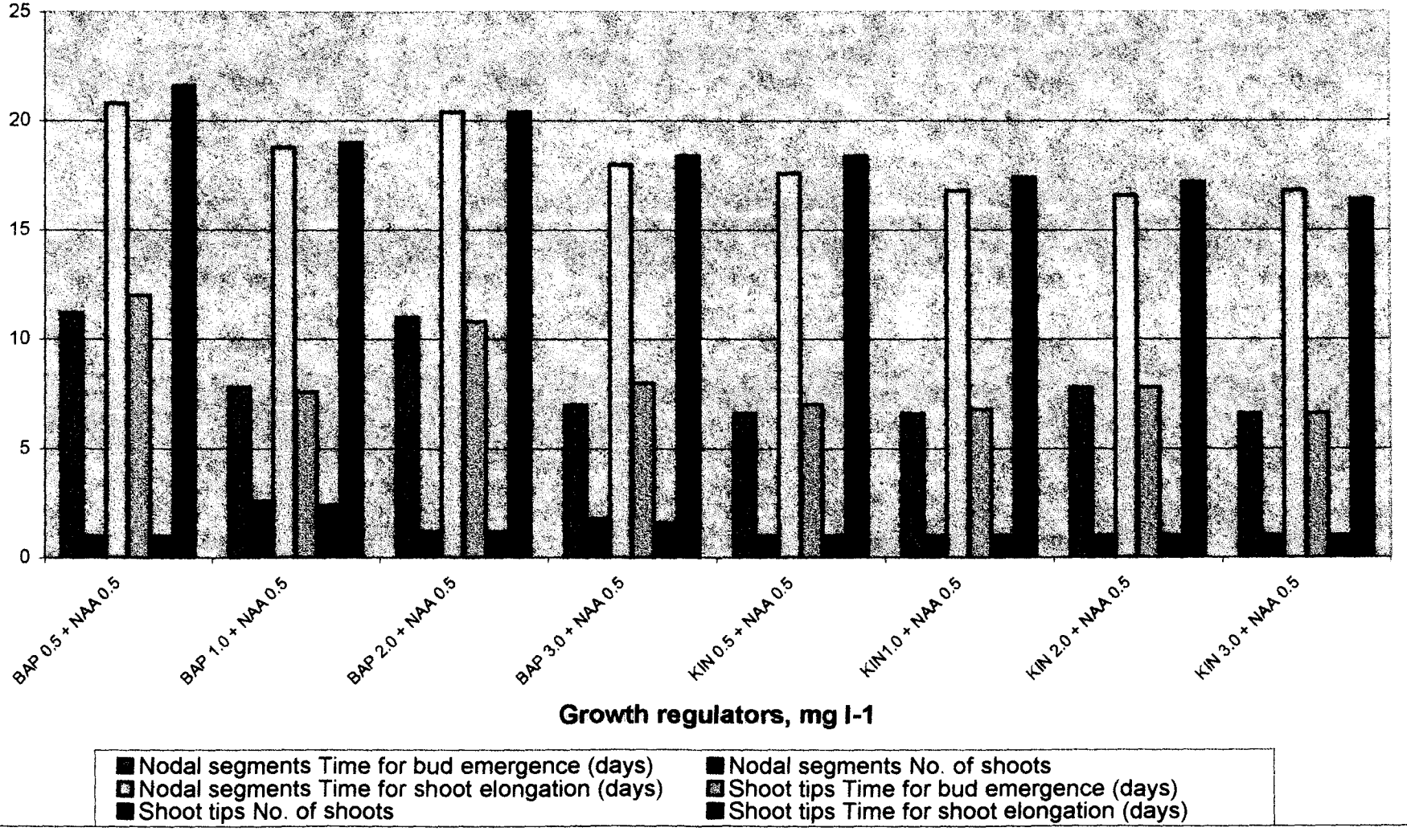
Maximum days for shoot elongation was taken when the medium was supplemented with BAP 0.5 mg l^{-1} + NAA 0.5 mg l^{-1} (20.8 days). This was on par with the treatment having BAP 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}, which took 20.4 days for shoot elongation.

4.2.1.1.3 Effect of BAP and KIN in combination with NAA on shoot tips
(Table 8, Fig. 3)

Number of days for bud emergence

The time taken for bud emergence revealed significant differences and varied from 6.6 days to 12.0 days under various treatments. The shortest time

Fig. 3. Effect of BAP and KIN in combination with NAA on culture establishment of nodal segments and shoot tips



(6.6 days) for bud emergence was recorded in MS medium supplemented with KIN 3.0 mg l^{-1} + NAA 0.5 mg l^{-1} and was found to be homogeneous with the time taken for bud emergence in treatments like KIN 1.0 mg l^{-1} + NAA 0.5 mg l^{-1} (6.8 days), KIN 0.5 mg l^{-1} + NAA 0.5 mg l^{-1} (7.0 days), BAP 1.0 mg l^{-1} + NAA 0.5 mg l^{-1} (7.6 days) and KIN 2.0 mg l^{-1} + NAA 0.5 mg l^{-1} (7.8 days).

The longest time for bud emergence was observed in MS medium supplemented with BAP 0.5 mg l^{-1} + NAA 0.5 mg l^{-1} (12.0 days) followed by the treatment having BAP 2.0 mg l^{-1} + NAA 0.5 mg l^{-1} (10.8 days).

Number of shoots

The average number of shoots produced by various treatments recorded significant differences and ranged from 1.0 to 2.4. The maximum number of shoots (2.4) was produced in MS medium supplemented with BAP 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}, followed by BAP 3.0 mg l^{-1} + NAA 0.5 mg l^{-1} (1.6).

Minimum number of shoots (1.0, each) was observed in treatments KIN 3.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 0.5 mg l^{-1} + NAA 0.5 mg l^{-1}, BAP 0.5 mg l^{-1} + NAA 0.5 mg l^{-1}.

Number of days for shoot elongation

The time taken for shoot elongation differed significantly and ranged from 16.4 days to 21.6 days. The minimum number of days (16.4) was taken by the medium supplemented with KIN 3.0 mg l^{-1} + NAA 0.5 mg l^{-1} and was on par with those produced by the treatments KIN 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 0.5 mg l^{-1} + NAA 0.5 mg l^{-1} and BAP 3.0 mg l^{-1} + NAA 0.5 mg l^{-1}. These treatments took 17.2, 17.4, 18.4 and 18.4 days, respectively.

The longest time (21.6, days) for elongation of shoots was taken by the medium supplemented with BAP 0.5 mg l^{-1} + NAA mg l^{-1}, which was followed by BAP 2.0 mg l^{-1} + NAA 0.5 mg l^{-1} (20.4 days) and BAP 1.0 mg l^{-1} + NAA 0.5 mg l^{-1} (19.0 days).

4.2.1.1.4 Effect of basal media on culture establishment

Of the different media (MS, SH and WPM) tried to screen out the best basal medium for the culture establishment of cordyline explants, the following results were obtained (Table 9).

Number of days for bud emergence

The shortest time (4.9 days) for bud emergence in case of nodal segments was taken by MS medium supplemented with 3.0 mg l^{-1} IAA which was significantly superior to WPM medium supplemented with 3.0 mg l^{-1} IAA. Maximum days (7.6) for bud emergence was noticed in SH medium supplemented with 3.0 mg l^{-1} IAA.

In case of shoot tip explants minimum number of days (11.4) for bud emergence was taken by the WPM medium supplemented with 3.0 mg l^{-1} IAA and was on par with SH medium supplemented with 3.0 mg l^{-1} IAA (11.5 days). Maximum days (12.3) for bud emergence was recorded in MS medium supplemented with 3.0 mg l^{-1} IAA.

Number of shoots

The number of shoots produced did not differ significantly among the media.

Table 9: Effect of media on the culture establishment of nodal segments and shoot tips in cordyline

Basal medium + IAA 3mg l^{-1}
Culture period : 4 weeks

Treatments (mg l^{-1})	Nodal segments			Shoot tips		
	Time taken for bud emergence (days)	No. of shoots (Mean)	Time taken for shoot elongation (days)	Time taken for bud emergence (days)	No. of shoots (Mean)	Time taken for shoot elongation (days)
MS	4.9	1.2	11.8	12.3	1.0	24.0
SH	7.6	1.0	13.9	11.5	1.2	24.0
WPM	6.6	1.1	13.2	11.4	1.0	23.7
CD (0.05)	0.65	0.28	1.20	0.81	0.22	1.55
SEm ±	0.23	0.10	0.41	0.28	0.08	0.53

Number of days for bud elongation

The number of days taken for bud elongation in nodal segments varied from 11.8 days to 13.9 days and differed significantly. Minimum days for elongation (11.8) was recorded in MS medium supplemented with 3.0 mg l^{-1} IAA. Maximum days (13.9) for bud elongation was taken by SH medium having 3.0 mg l^{-1} IAA and was on par with WPM medium supplemented with 3.0 mg l^{-1} IAA (13.2 days). In the case of shoot tips there was no significant difference in bud elongation among the three media.

4.2.1.2 Shoot proliferation

The elongated buds derived from nodal segments and shoot tips of cordyline in Stage 1 were cultured in MS medium containing various levels of cytokinins (BAP, KIN and 2ip), alone and in combination with NAA. The responses observed were the production of multiple axillary buds and callus, data pertaining to which are presented in Tables 10 to 12.

4.2.1.2.1 Effect of BAP

Data on the organogenic responses of elongated shoots as influenced by BAP (0.5, 1.0, 2.0 and 3.0 mg l^{-1}), alone and in combination with NAA (0.5 and 1.0 mg l^{-1}) in MS medium are given in Table 10.

A very high rate of axillary bud production was observed when MS medium was supplemented with BAP 1.0 mg l^{-1} + NAA 0.5 mg l^{-1} (Plate 3) and BAP 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}. High rates of axillary bud production was observed in the treatment BAP 3.0 mg l^{-1} + NAA 0.5 mg l^{-1}.

Medium rate of axillary bud production could be noticed in treatments when MS medium was supplemented with BAP 0.5 mg l^{-1} + NAA 0.5 mg l^{-1}, BAP 0.5 mg l^{-1} + NAA 1.0 mg l^{-1} and BAP 2.0 mg l^{-1}. Low rate of axillary bud

Table 10: Response of elongated shoots derived from nodal segments and shoot tips as influenced by BAP and NAA in cordyline

Basal medium : MS
Culture period : 3 weeks
Average of 10 cultures

Treatments		Nature of response	
BAP (mg l^{-1})	NAA (mg l^{-1})	Multiple axillary buds	Callus growth
0.5	0.0	-	-
1.0	0.0	-	+
2.0	0.0	++	++
3.0	0.0	+	++
0.5	0.5	++	++
1.0	0.5	++++	-
2.0	0.5	++++	-
3.0	0.5	+++	-
0.5	1.0	++	++
1.0	1.0	-	+++
2.0	1.0	-	+++
3.0	1.0	-	-

- No response
+ Low rate of production (upto 10 buds)
++ Medium rate of production (11 - 20 buds)
+++ High rate of production (21 - 30 buds)
++++ Very high rate of production (≥ 31 buds)

production was observed when MS medium was supplemented with BAP 3.0 mg l^{-1}. The treatments BAP 0.5 mg l^{-1}, BAP 1.0 mg l^{-1}, BAP 1.0 mg l^{-1} + NAA 1.0 mg l^{-1}, BAP 2.0 mg l^{-1} + NAA 1.0 mg l^{-1} and BAP 3.0 mg l^{-1} + NAA 1.0 mg l^{-1} could not produce multiple axillary buds.

Callus production was high in treatments involving BAP 1.0 mg l^{-1} + NAA 1.0 mg l^{-1}, BAP 2.0 mg l^{-1} + NAA 1.0 mg l^{-1}. Medium rate of callus production was observed in treatments with BAP 2.0 mg l^{-1}, BAP 3.0 mg l^{-1}, BAP 0.5 mg l^{-1} + NAA 0.5 mg l^{-1} and BAP 0.5 mg l^{-1} + NAA 1.0 mg l^{-1}. Low rate of callus production was observed in treatments having BAP 1.0 mg l^{-1}.

Callus production was not observed in treatments involving BAP 0.5 mg l^{-1}, BAP 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}, BAP 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}, BAP 3.0 mg l^{-1} + NAA 0.5 mg l^{-1} and BAP 3.0 mg l^{-1} + NAA 1.0 mg l^{-1}.

4.2.1.2.2 Effect of KIN

Data showing the effect of KIN (0.5 mg l^{-1}, 1.0 mg l^{-1}, 2.0 mg l^{-1} and 3.0 mg l^{-1}), alone and in combination with NAA (0.5 mg l^{-1} and 1.0 mg l^{-1}) on elongated shoots of cordyline in MS medium are presented in Table 11.

Medium rate of multiple axillary bud production was observed in MS medium supplemented with KIN 3.0 mg l^{-1} (Plate 4). The rate of production of multiple axillary buds was low in treatments KIN 2.0 mg l^{-1} and KIN 2.0 mg l^{-1} + NAA 1.0 mg l^{-1}.

Treatments like KIN 0.5 mg l^{-1}, KIN 1.0 mg l^{-1}, KIN 0.5 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 3.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 0.5 mg l^{-1} + NAA 1.0 mg l^{-1}, KIN 1.0 mg l^{-1} + NAA 1.0 mg l^{-1} and KIN 3.0 mg l^{-1} + NAA 1.0 mg l^{-1} could not induce axillary buds.

Table 11: Response of elongated shoots derived from nodal segments and shoot tips as influenced by KIN and NAA in cordyline

Basal medium : MS
Culture period : 3 weeks
Average of 10 cultures

Treatments		Nature of response		
KIN ($mg\ l^{-1}$)	NAA ($mg\ l^{-1}$)	Multiple axillary buds	Callus growth	Others
0.5	0.0	-	-	Bulging of the base
1.0	0.0	-	-	"
2.0	0.0	+	+	-
3.0	0.0	++	+	-
0.5	0.5	-	-	Rhizogenesis & shoot base bulging
1.0	0.5	-	-	"
2.0	0.5	-	-	"
3.0	0.5	-	-	-
0.5	1.0	-	++	Rhizogenesis
1.0	1.0	-	++	"
2.0	1.0	+	+	Rhizogenesis, base bulging
3.0	1.0	-	-	-

+ Low rate of production (upto 10 buds)
++ Medium rate of production (11 - 20 buds)
- No response

Plate. 1. Multiple axillary bud production from nodal segments

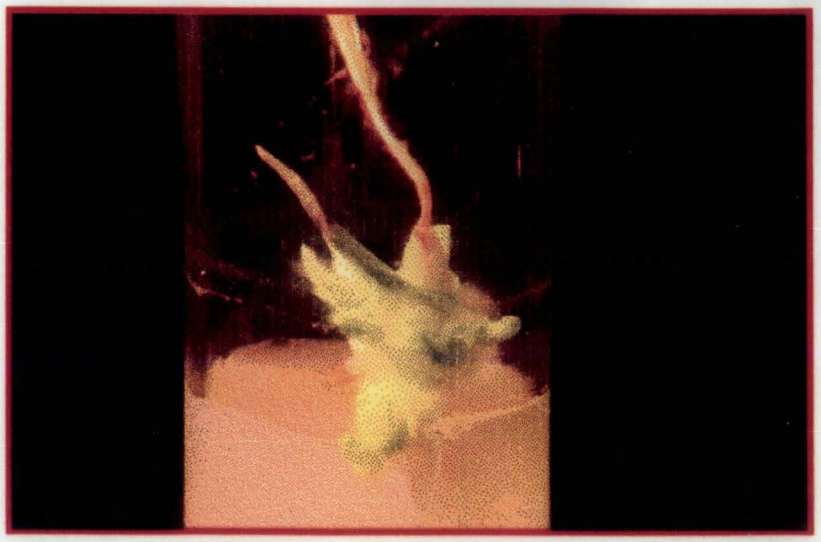


Plate. 2. Multiple axillary bud production from shoot tips

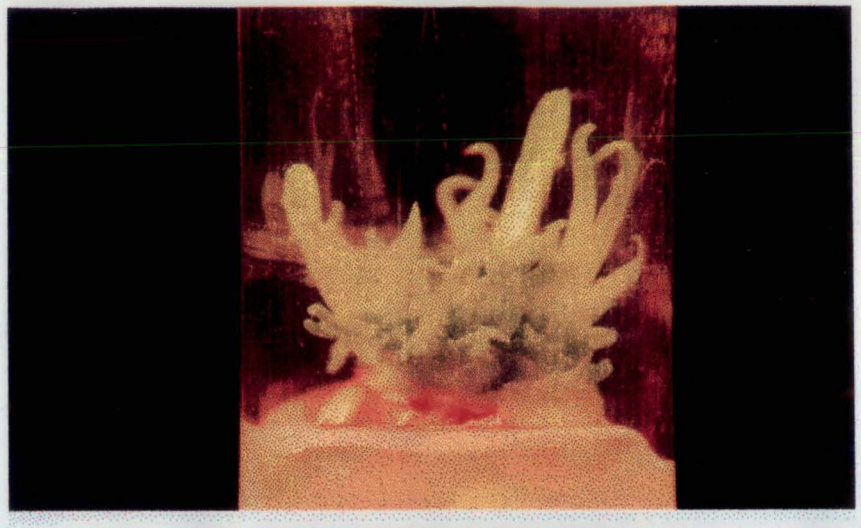


Plate. 3. Shoot proliferation in MS+BAP1.0mg l^{-1}+NAA 1.0mg l^{-1}

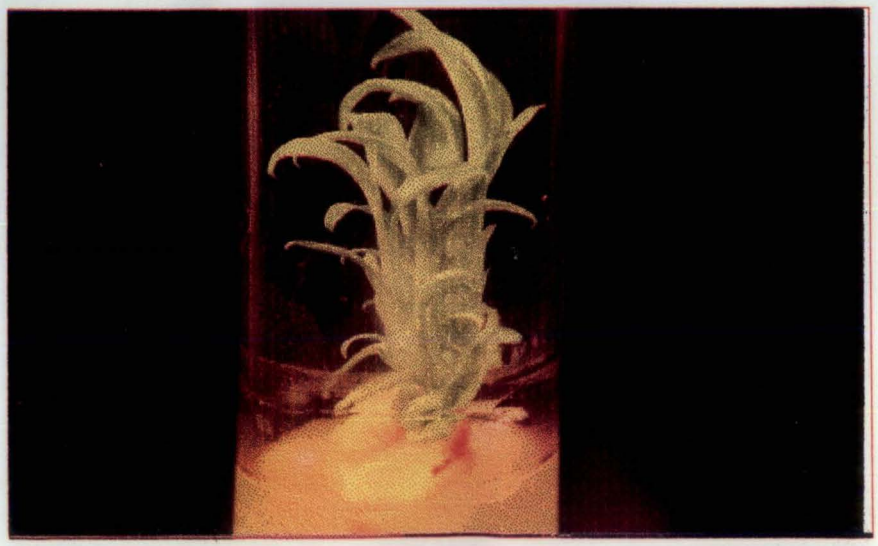


Plate. 4. Shoot proliferation in MS + KIN 3.0 mg l^{-1}

Callus production was medium in the treatments KIN 0.5 mg l^{-1} + NAA 1.0 mg l^{-1} and KIN 1.0 mg l^{-1} + NAA 1.0 mg l^{-1}. The rate of production of callus was low when KIN 2.0 mg l^{-1}, KIN 3.0 mg l^{-1} and KIN 2.0 mg l^{-1} + NAA 1.0 mg l^{-1} were used.

Callus production was not observed in treatments involving KIN 0.5 mg l^{-1}, KIN 1.0 mg l^{-1}, KIN 0.5 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 3.0 mg l^{-1} + NAA 0.5 mg l^{-1} and KIN 3.0 mg l^{-1} + NAA 1.0 mg l^{-1}.

Of the different treatments, those involving KIN 0.5 mg l^{-1}, KIN 1.0 mg l^{-1}, KIN 0.5 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 3.0 mg l^{-1} + NAA 0.5 mg l^{-1} and KIN 3.0 mg l^{-1} + NAA 1.0 mg l^{-1} could produce neither multiple axillary buds nor callus. They showed bulging of the shoot base. Rhizogenesis was noticed when MS medium was supplemented with KIN 0.5 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}, KIN 0.5 mg l^{-1} + NAA 1.0 mg l^{-1}, KIN 1.0 mg l^{-1} + NAA 1.0 and KIN 2.0 mg l^{-1} + NAA 1.0 mg l^{-1}.

4.2.1.2.3 Effect of 2ip

Data pertaining to the organogenic response of elongated shoots derived from nodal segments and shoot tips of cordyline as influenced by 2ip (0.5 mg l^{-1}, 1.0 mg l^{-1}, 2.0 mg l^{-1} and 3.0 mg l^{-1}), either alone or in combination with NAA (0.5 mg l^{-1} and 1.0 mg l^{-1}) in MS medium are given in Table 12.

High rate of multiple axillary bud production was observed at 2ip 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}. Medium rate of multiple axillary bud production was observed at 2ip 2.0 mg l^{-1}, 2ip 3.0 mg l^{-1}, 2ip 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}, 2ip 3.0 mg l^{-1} + NAA 0.5 mg l^{-1}. In treatments with 2ip 2.0 mg l^{-1}, 2ip 3.0 mg l^{-1}, 2ip 1.0 mg l^{-1} + NAA 1.0 mg l^{-1}, 2ip 2.0 mg l^{-1} + NAA 1.0 mg l^{-1} callus production was low. Rhizogenesis was observed in all treatments having 2ip and NAA.

Table 12: Response of elongated shoots derived from nodal segments and shoot tips as influenced by 2ip and NAA in cordyline

Basal medium : MS
Culture period : 3 weeks
Average of 10 cultures

Treatments		Nature of response		
2ip ($mg\ l^{-1}$)	NAA ($mg\ l^{-1}$)	Multiple axillary buds	Callus growth	Others
0.5	0.0	-	-	-
1.0	0.0	-	-	-
2.0	0.0	++	+	-
3.0	0.0	++	+	-
0.5	0.5	-	-	Rhizogenesis
1.0	0.5	++	-	"
2.0	0.5	+++	-	"
3.0	0.5	++	-	"
0.5	1.0	-	-	"
1.0	1.0	+	+	"
2.0	1.0	+	+	"
3.0	1.0	-	-	"

- No response
+ Low rate of production (upto 10 buds)
++ Medium rate of production (11 - 20 buds)
+++ High rate of production (21 - 30 buds)

4.2.1.2.4 Effect of media

Data on the effect of different media on the cultures originating from nodal segments and shoot tips with respect to multiple axillary bud aggregate formation are given in Table 13, Plate 5.

Days for initiation of multiple axillary buds.

Minimum time for multiple axillary bud production was noticed in MS medium (12.2 days) followed by WPM medium (18.5 days). Multiple axillary bud production was not noticed in SH medium.

MS medium produced very high rate of multiple axillary buds where as WPM medium showed medium rate of multiple axillary bud production.

4.2.1.3 Elongation of bud aggregates

4.2.1.3.1 Effect of media and IAA

The bud aggregates taken from the stage 2 were subjected to elongation studies in full strength MS salt medium (MSa), half strength MS salt medium (MSb); and MSa + IAA at 0.5 mg l^{-1}, 1.0 mg l^{-1}, 2.0 mg l^{-1} and 3.0 mg l^{-1}. The observations are presented in Table 14.

Days taken for shoot elongation varied significantly in different treatments. It ranged from 7.0 days (MSb) to 13.0 days (MSa + IAA 3.0 mg l^{-1}). Days taken for shoot elongation in full strength MS medium (MSa), MSa + IAA 1.0 mg l^{-1}, MSa + IAA 0.5 mg l^{-1} and MSa + IAA 2.0 mg l^{-1} were 8.60, 9.0, 10.80 and 11.50, respectively.

Table 13: Effect of media on multiple axillary bud aggregate formation and elongation in cordyline

Basal medium + BAP2.0 mg l^{-1} + NAA 0.5 mg l^{-1}
Culture period : 3 weeks
Average of 10 cultures

Medium	Time taken for initiation of multiple axillary buds (days)	Multiple axillary buds	Nature of response
MS	12.2	++++	-
SH	-	-	Shoot elongation
WPM	18.5	++	-

- No response
++ Medium rate of production (11-20 buds)
++++ Very high rate of production (≥ 31 buds)

Table 14: Effect of IAA on elongation of bud aggregates in cordyline

Basal medium : MS
Culture period : 3 weeks
Average of 10 cultures

Treatments (mg l^{-1})	Time taken for shoot elongation (days)	Time taken for root initiation (days)	No. of shoots	Length of shoots (cm)	No. of roots	Nature of roots
MSa	8.60	8.40	9.4	10.62	16.6	Long, creamy white roots with root hairs
MSb	7.00	9.30	3.7	9.52	13.7	"
MSa+IAA 0.5	10.80	14.90	3.3	6.40	2.3	Slender and white roots
MSa+IAA 1.0	9.00	13.40	10.0	9.26	3.6	"
MSa+IAA 2.0	11.50	14.20	9.4	9.09	8.0	"
MSa+IAA 3.0	13.00	17.00	4.4	5.80	2.6	"
CD (0.05)	1.36	2.00	1.87	1.16	2.98	
SEm ±	0.48	0.70	0.66	0.41	1.05	

MSa : Full strength MS medium
MSb : Half strength MS medium

Days taken for root initiation

The number of days taken for root initiation varied significantly ranging from 8.4 days (MSa) to 17.0 days (MSa + IAA 3.0 mg l^{-1}). The minimum time taken for root initiation (8.4 days) in full strength MS medium was on par with that of half strength MS medium (9.3 days). Days taken for root initiation in MSa + IAA 0.5 mg l^{-1}, MSa + IAA 2.0 mg l^{-1} and MSa + IAA 1.0 mg l^{-1} were 14.90, 14.20 and 13.40, respectively.

Number of shoots

The number of shoots produced in different treatments also varied significantly, ranging from 3.3 (MSa + IAA 0.5 mg l^{-1}) to 10.0 (MSa + IAA 1.0 mg l^{-1}). Full strength MS medium (9.4) and MSa + IAA 2.0 mg l^{-1} (9.4) were on par with the best treatment.

The minimum number of shoots produced in MSa + IAA 0.5 mg l^{-1} (3.3) was found to be on par with that of half strength MS medium (3.7) and MSa + IAA 3.0 mg l^{-1} (4.4).

Length of shoots

Shoot length also varied significantly in different treatments. It ranged from 5.80 cm (MSa + IAA 3.0 mg l^{-1}), which was found to be on par with MSa + IAA 0.5 mg l^{-1} (6.40 cm), to 10.62 cm (MS full strength medium). The treatments MSa + IAA 2.0 mg l^{-1}, MSa + IAA 1.0 mg l^{-1} and half strength MS medium produced 9.09 cm, 9.26 cm and 9.52 cm shoot length, respectively.

Number of roots

Number of roots produced in different treatments varied significantly, ranging from 2.3 (MSa + IAA 0.5 mg l^{-1}) to 16.6 (full strength MS medium). The

treatment MSa + IAA 0.5 mg l^{-1} was found to be homogeneous with MSa + IAA 3.0 mg l^{-1} (2.6) and MSa + IAA 1.0 mg l^{-1} (3.6). Half strength MS medium produced 13.7 roots and MSa + IAA 2.0 mg l^{-1} showed 8.0 roots per culture.

Nature of roots

Long, creamy white roots with root hairs were observed in full strength MS medium and half strength MS medium. Slender and white roots, lacking in root hairs, were observed in the medium supplemented with IAA at different concentrations.

4.2.1.3.2 Effect of medium supplements

Effect of medium supplements [activated charcoal (0.1, 0.5 and 1.0 per cent) adenine sulphate (60, 80 and 100 mg l^{-1}), coconut water (5, 10 and 15 per cent) and casein hydrolysate (80, 100 and 120 mg l^{-1})] on shoot elongation was studied on MS basal medium with multiple axillary buds taken from the Stage 2, the results of which are presented in Table 15, Fig.4, Plate 6.

Days taken for shoot elongation

Significant variation among the treatments was observed with respect to the number of days taken for shoot elongation and ranged from 8.6 days in control to 13.8 days in the medium containing coconut water 15 per cent which was found to be on par with coconut water 5 per cent (13.6 days). In the treatments containing adenine sulphate 60 mg l^{-1}, adenine sulphate 100 mg l^{-1} and casein hydrolysate 80 mg l^{-1} shoot elongation was observed after 13.2, 12.8 and 11.6 days, respectively.

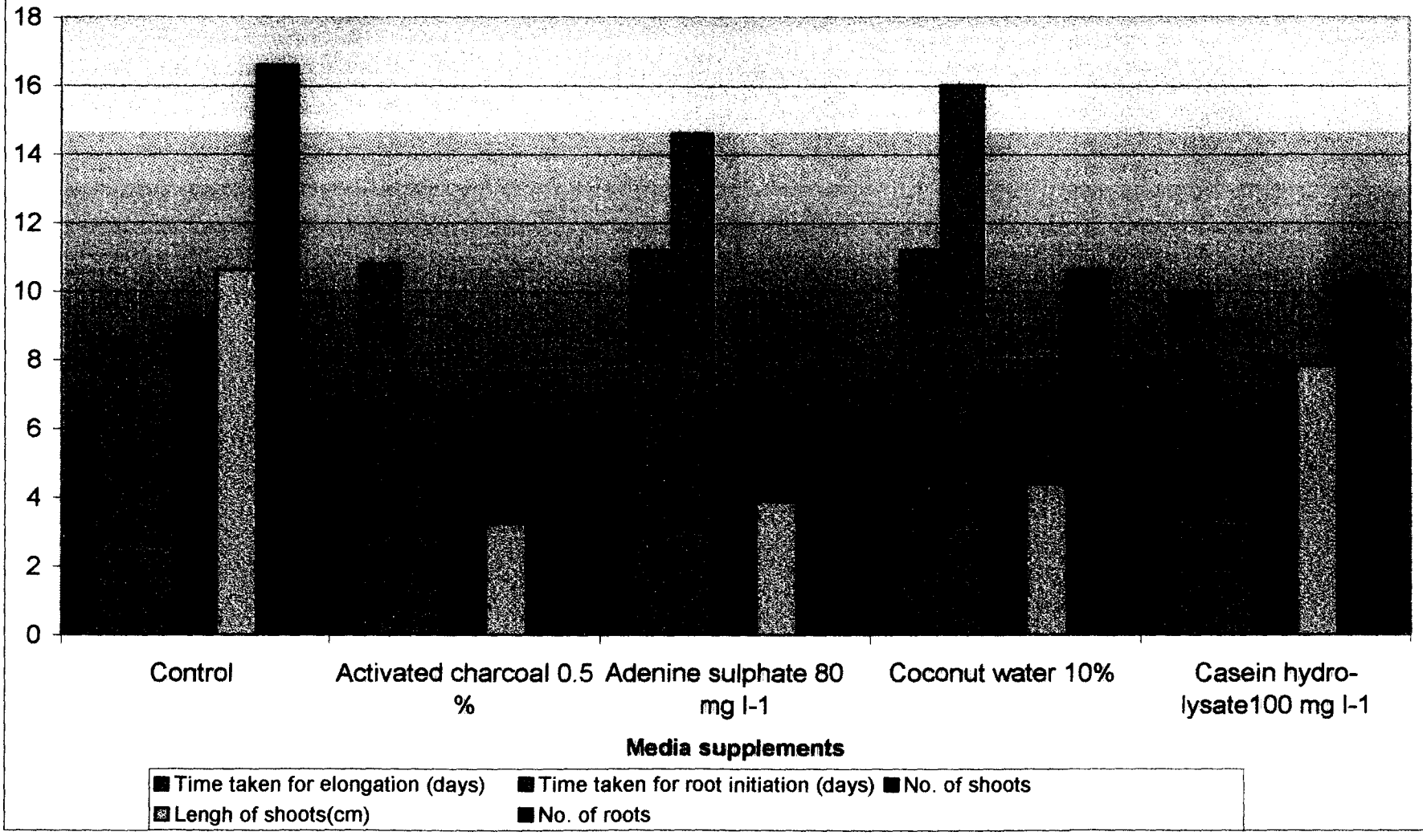
The number of days taken for shoot elongation in the medium containing activated charcoal 0.1 per cent was 11.4 which was found to be homogeneous

Table 15: Effect of media supplements on elongation of bud aggregates in cordyline

Basal medium : MS
Culture period : 3 weeks
Average of 10 cultures

Treatments		Time taken for elongation (days)	Time taken for root initiation (days)	No. of shoots	Length of shoots (cm)	No. of roots	Nature of roots
Control		8.60	8.40	9.2	10.62	16.6	Long, creamy white roots with root hairs
Activated charcoal	0.1 %	11.40	10.60	4.8	3.12	1.6	White slender and short
"	0.5 %	10.80	6.60	5.2	3.22	2.6	"
"	1.0 %	11.00	8.00	3.2	2.82	1.8	"
Adenine sulphate	60 mg l^{-1}	13.20	16.40	4.0	3.44	5.6	Slender creamy white
"	80 mg l^{-1}	11.20	14.60	5.8	3.88	6.4	"
"	100 mg l^{-1}	12.80	17.20	4.2	3.48	5.0	"
Coconut water	5%	13.60	16.40	4.4	3.67	9.4	Slender white and long
"	10%	11.20	16.00	7.2	4.38	10.6	"
"	15%	13.80	17.20	3.8	3.36	9.0	"
Casein hydrolysate	80 mg l^{-1}	11.60	10.00	4.2	6.56	8.6	Slender white and short
"	100 mg l^{-1}	10.00	9.20	6.0	7.82	10.2	"
"	120 mg l^{-1}	10.60	11.00	4.8	7.08	6.4	"
CD (0.05)		1.52	1.49	2.30	0.92	2.46	
SEm ±		0.54	0.52	0.81	0.32	0.87	

Fig. 4. Effect of media supplements on elongation of bud aggregates in cordyline



with that of the medium supplemented with coconut water 10 per cent and adenine sulphate 80 mg l^{-1} (11.2 days, each).

The treatments containing activated charcoal 1.0 per cent, activated charcoal 0.5 per cent and casein hydrolysate 120 mg l^{-1} showed shoot elongation after 11.0, 10.8 and 10.6 days, respectively. These three treatments were found to be statistically on par.

Days taken for root initiation

Significant variation was observed among the treatments. Time taken for root initiation varied from 6.6 days (activated charcoal 1.0 per cent) to 17.2 days (adenine sulphate 100 mg l^{-1}). The MS medium supplemented with adenine sulphate 100 mg l^{-1} took longest time (17.2 days) for root initiation and was found to be on par with the MS medium supplemented with coconut water 15 per cent (17.2 days), coconut water 5 per cent (16.4 days), adenine sulphate 60 mg l^{-1} (16.4 days), which differ significantly from to coconut water 10 per cent (16.0 days), adenine sulphate 80 mg l^{-1} (14.6 days), casein hydrolysate 120 mg l^{-1} (11.0) days), activated charcoal 0.1 per cent (10.6 days), casein hydrolysate 80 mg l^{-1} (10.0 days), casein hydrolysate 100 mg l^{-1} (9.2 days), control (8.4 days) and activated charcoal 1.0 per cent (8.0 days).

Number of shoots

The different treatments varied significantly with respect to the production of shoots, which ranged from 3.2 in 1.0 per cent activated charcoal to 9.2 in control (Plate 7). Maximum number of shoots (9.2) was produced by the treatment devoid of medium supplements, followed by the medium containing coconut water 10 per cent (7.2) and the medium containing casein hydrolysate 100 mg l^{-1} (6.0).

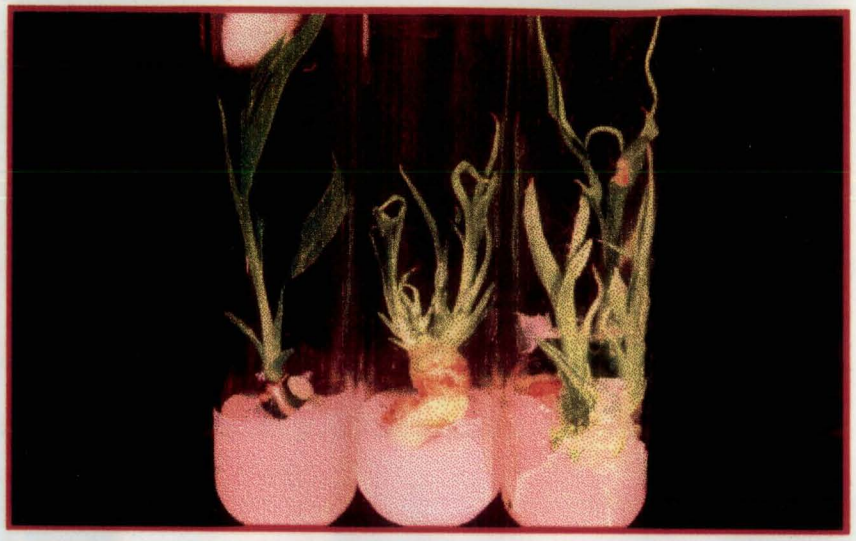


Plate. 5. Effect of media on multiple axillary bud production

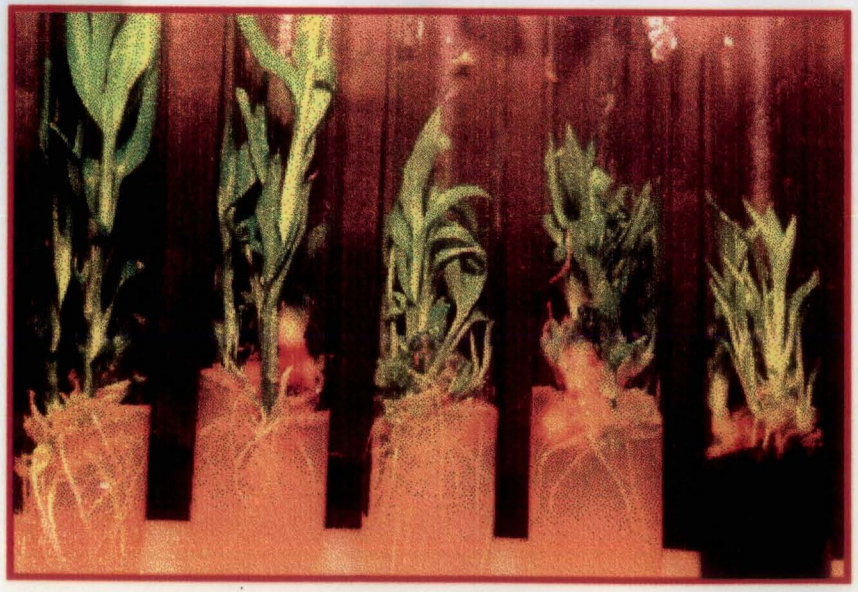


Plate. 6. Effect of media supplements on elongation

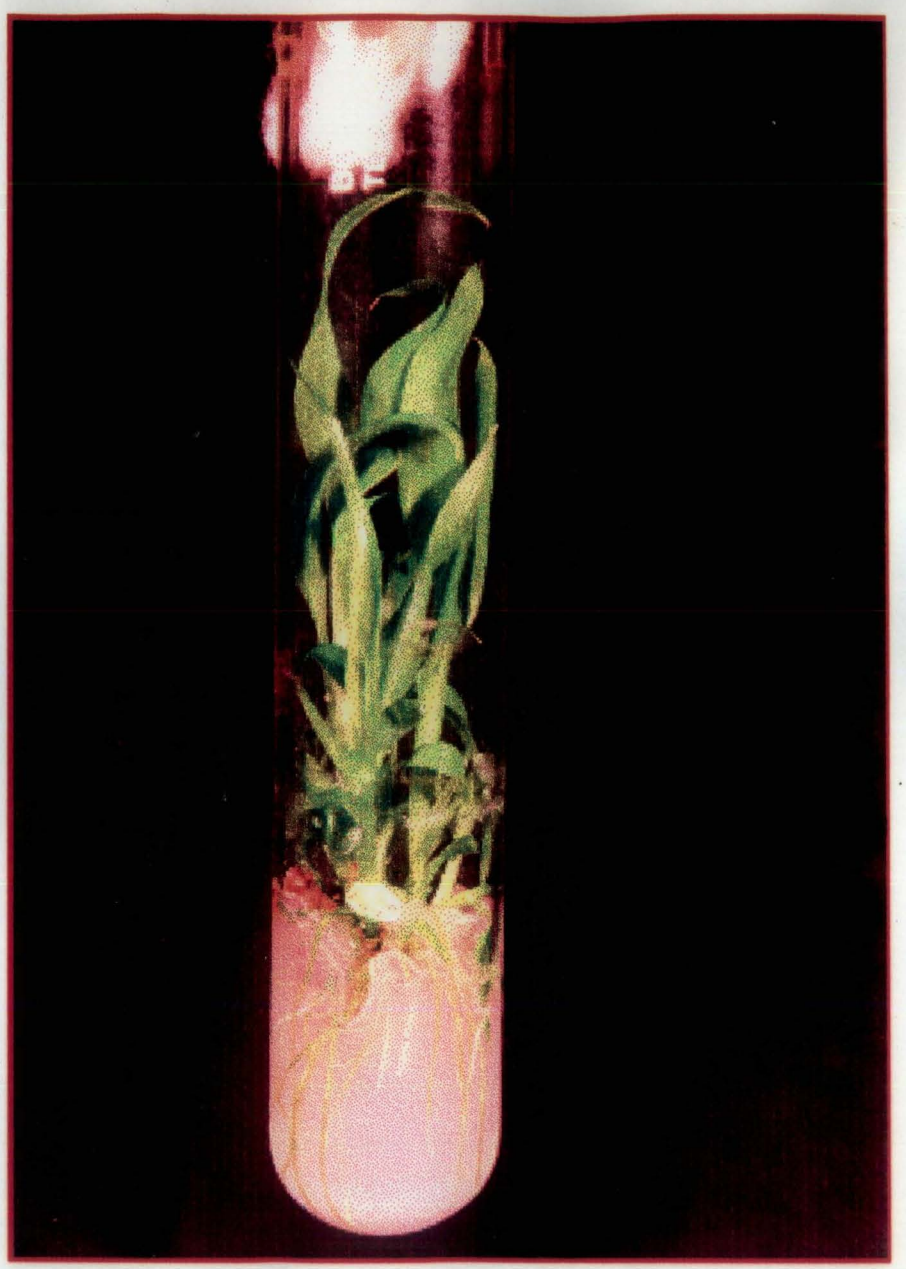


Plate. 7. Multiple axillary bud elongation in MS basal medium

The number of shoots produced by the medium containing adenine sulphate 80 mg l^{-1}, activated charcoal 0.5 per cent and 0.1 per cent and casein hydrolysate 120 mg l^{-1} were 5.8, 5.2, 4.8 and 4.8, respectively, and were found to be homogeneous. The treatments containing coconut water 5 per cent, adenine sulphate 100 mg l^{-1} and coconut water 15 per cent were found to be statistically on par in the production of shoots.

Length of shoots

Maximum shoot length (10.62 cm) was produced in control which was significantly superior to all other treatments.

Number of roots

The treatments differed significantly with respect to number of roots produced. It varied from 1.6 (activated charcoal 0.1 per cent) to16.6 (control). The number of roots produced in the medium containing coconut water 10 per cent (10.6) was on par with treatments with casein hydrolysate 100 mg l^{-1} (10.2) and coconut water 5 per cent (9.4).

Nature of roots

In all treatments medium supplements produced slender roots with out root hairs. Normal roots were produced in the MS medium devoid of medium supplements (control).

4.2.1.4 *In vitro* rooting

Elongated shoots from Stage 2 were used for *in vitro* rooting studies and the results are presented in Tables 16 to 18.

4.2.1.4.1 Effect of media

Data pertaining to the effect of MS, SH and WPM media on rooting are given in Table 16, Fig. 5, Plate 8.

Number of days taken for root initiation

The time taken for root initiation ranged from 8.4 days to 12.6 days. The shortest time (8.4 days) was taken by MS medium. Maximum days for root initiation was taken by SH medium (12.6) which was found to be homogeneous with that of WPM medium (12.5 days).

Number of roots

Number of roots produced differed significantly and varied from 5.0 to 16.6. Maximum number of roots (16.6) was observed in MS medium. The minimum number of roots (5.0) observed in SH medium was found to be on par with WPM medium (7.8).

Length of roots

Significant differences were observed among the treatments and the length ranged from 5.26 cm (SH medium) to 7.47 cm (MS medium). WPM medium with a root length of (5.74 cm) was found to be homogeneous with that of SH medium.

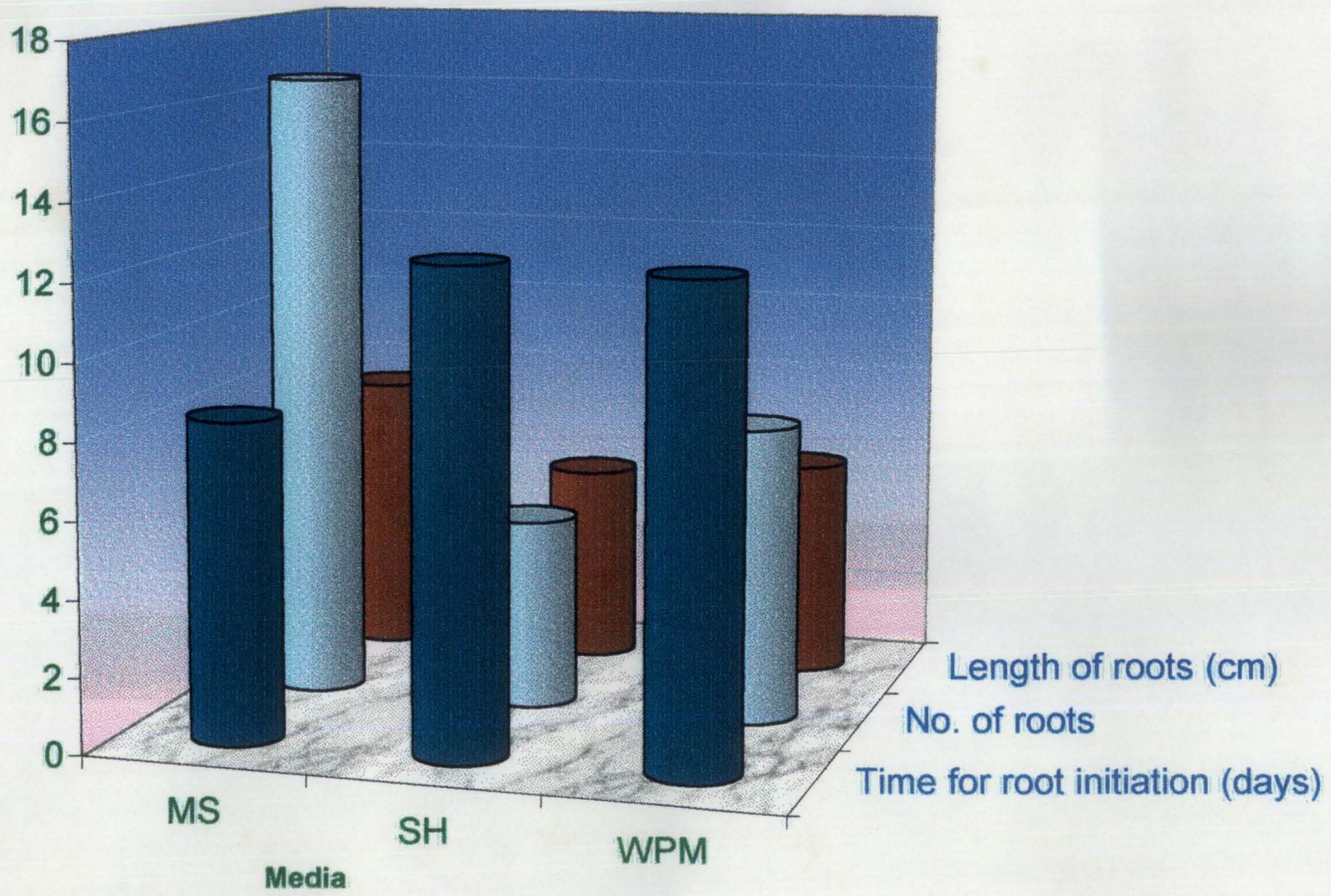
Nature of roots

Long creamy white roots with root hairs were produced in MS medium. Slender white roots were found in SH medium. WPM produced medium sized thick and white roots.

Table 16: Effect of media on rooting of elongated shoots of cordyline

Treatments	Time taken for root initiation (days)	No. of roots (Mean)	Length of roots (cm)	Nature of roots
MS	8.40	16.60	7.47	Long, creamy white roots with root hairs
SH	12.60	5.00	5.26	Slender and white
WPM	12.50	7.80	5.74	Medium thick and white
CD (0.05)	0.92	3.57	1.42	
SEm ±	0.32	1.23	0.49	

Fig. 5. Effect of media on rooting of elongated shoots of cordyline



4.2.1.4.2 Effect of auxins on rooting

Data generated from the studies on the effect of auxins, such as IAA, NAA and IBA (each at 1.0, 2.0 and 3.0 mg l^{-1}) are presented in Table 17, Fig. 6.

Number of days taken for root initiation

Number of days taken for rooting of *in vitro* shoots varied from 8.6 to 17.0 in different treatments. The treatment containing IBA 1.0 mg l^{-1} produced roots earlier (8.6 days) and was on par with IBA 3.0 mg l^{-1} (8.8 days) and differed significantly from other treatments, followed by IBA 2.0 (11.4 days), which was on par with NAA 2.0 (11.6 days).

Maximum days (17.0) for root initiation was taken by the treatment having IAA 3.0 mg l^{-1}. The treatments having NAA 1.0 mg l^{-1}, IAA 2.0 mg l^{-1}, IAA 1.0 mg l^{-1} and NAA 3.0 mg l^{-1} produced roots after 14.8, 14.2, 13.4 and 12.2 days, respectively.

Number of roots

The number of roots produced ranged from 2.2 to 10.4 and showed significant variation among the treatments. Minimum number of roots (2.2, each) was produced in the media supplemented with NAA 3.0 mg l^{-1} and IBA 1.0 mg l^{-1} and was found to be homogeneous with the treatments IAA 3.0 mg l^{-1} (2.6), NAA 2.0 mg l^{-1} (3.2), NAA 1.0 mg l^{-1} (3.4), IAA 1.0 mg l^{-1} (3.6).

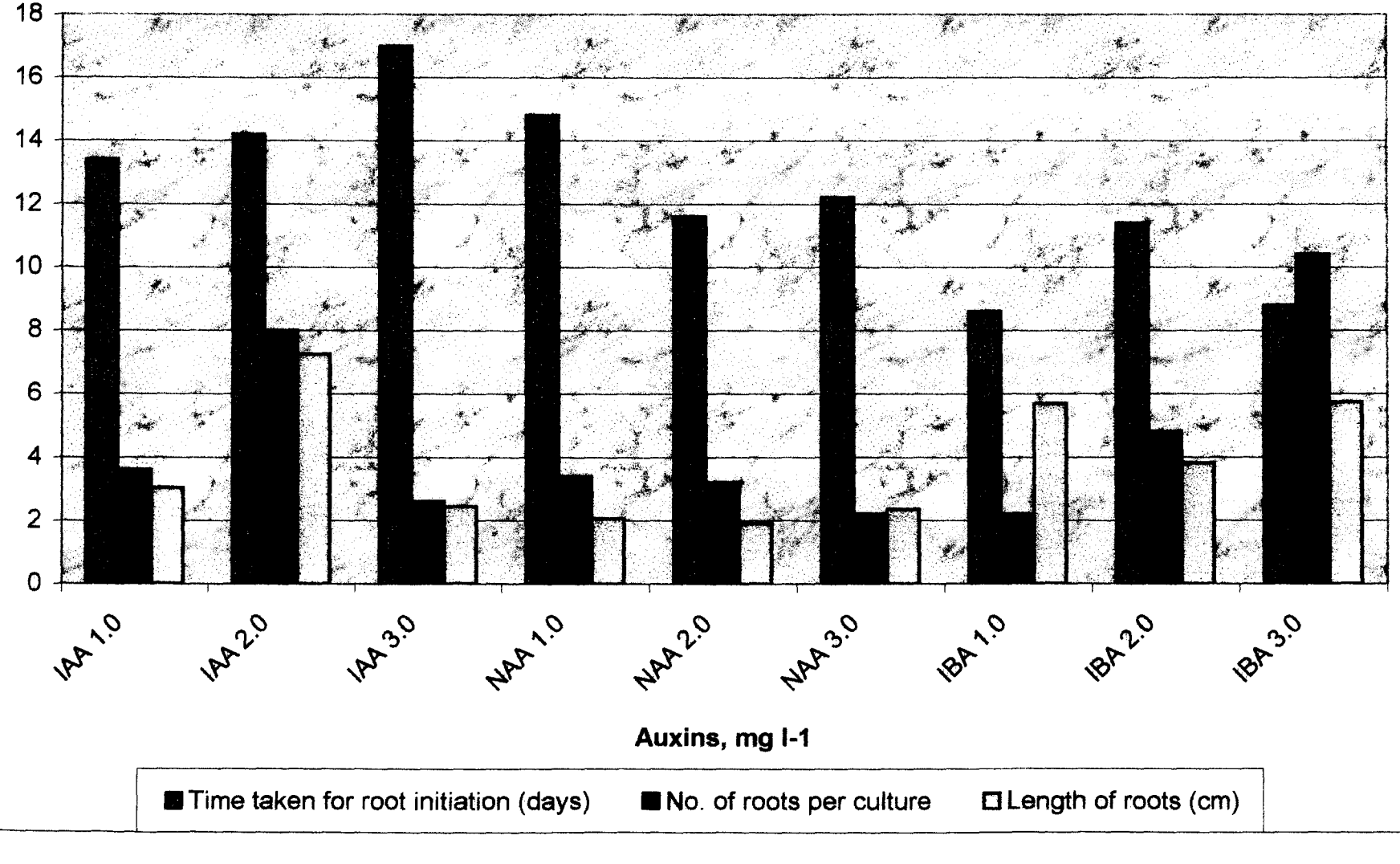
Maximum number of roots (10.4) was found in the medium with IBA 3.0 mg l^{-1}, which is superior to media supplemented with IAA 2.0 mg l^{-1} (8.0) and IBA 2.0 mg l^{-1} (4.8).

Table 17: Effect of auxins on rooting of elongated shoots of cordyline

Basal medium : MS
Culture period : 3 weeks

Treatments ($mg\ l^{-1}$)	Time taken for root initiation (days)	No. of roots per culture	Length of roots (cm)	Nature of roots
IAA 1.0	13.40	3.60	3.04	Slender and white
IAA 2.0	14.20	8.00	7.26	"
IAA 3.0	17.00	2.60	2.44	"
NAA 1.0	14.80	3.40	2.06	Callus formation at the base, roots thick, short and cream
NAA 2.0	11.60	3.20	1.90	"
NAA 3.0	12.20	2.20	2.36	"
IBA 1.0	8.60	2.20	5.70	Thick, white roots with branching
IBA 2.0	11.40	4.80	3.82	"
IBA 3.0	8.80	10.40	5.74	"
CD (0.05)	2.34	3.38	0.80	
SEm ±	0.82	1.18	0.28	

Fig. 6. Effect of auxins on rooting of elongated shoots of cordyline



Length of roots

The length of roots ranged from 1.9 cm to 7.26 cm and differed significantly in various treatments. The maximum length of roots (7.26 cm) was observed in the treatment having IAA 2.0 mg l^{-1}, followed by the medium with IBA 3.0 mg l^{-1} (5.74 cm), which was on par with that of the medium supplemented with IBA 1.0 mg l^{-1} (5.70 cm).

The minimum root length (1.9 cm) was observed in the treatment with NAA 2.0 mg l^{-1}, which was found to be homogeneous with the medium containing NAA 1.0 mg l^{-1} (2.06 cm) and it was followed by the media supplemented with NAA 3.0 mg l^{-1} (2.36 cm), IAA 3.0 mg l^{-1} (2.44 cm), IAA 1.0 mg l^{-1} (3.04 cm) and IBA 2.0 mg l^{-1} (3.82 cm).

Nature of roots

Media supplemented with IAA at different concentrations produced slender and white roots. Presence of NAA in the medium produced callus at the base before root formation. The roots produced were normal with branching habit and were thick and white in the medium supplemented with IBA.

4.2.1.4.3 Effect of activated charcoal on rooting

The elongated shoots from Stage 2 were separated and kept for rooting in MS medium containing different levels of activated charcoal to study the effect of charcoal and the results are presented in Table 18, Plate 8.

Number of days taken for root initiation

The number of days taken for root initiation ranged from 6.6 days to 10.6 days. Minimum number of days for root initiation was observed in the medium supplemented with 0.5 per cent activated charcoal (6.6 days). Longest time for

Table 18: Effect of activated charcoal on rooting of elongated shoots of cordyline

Basal medium : MS
Culture period : 3 weeks

Treatments Activated charcoal (%)	Time taken for root initiation (days)	No. of roots	Length of roots (cm)	Nature of roots
0.0	8.4	16.6	7.47	Long, creamy white roots with root hairs
0.1	10.6	1.6	2.84	White, slender short
0.5	6.6	2.6	3.42	"
1.0	8.0	1.8	2.57	"
CD (0.05)	1.04	1.16	0.87	
SEm ±	0.36	0.40	0.30	

root initiation (10.6 days) was taken in the medium with activated charcoal 0.1 per cent, followed by basal medium (8.4 days), which was on par with the medium containing 1.0 per cent activated charcoal (8.0 days).

Number of roots

The number of roots ranged from 1.6 to 16.6. Maximum number of roots was observed in the medium devoid of activated charcoal (control). The number of roots under various levels of charcoal was not significant.

Length of roots

The length of roots ranged from 2.57 cm to 7.47 cm. Maximum length of roots was observed in the medium devoid of activated charcoal. The length of roots under various levels of charcoal was not significant.

4.2.1.4.4 Effect of triadimefon and IBA on rooting

Data relating to the effect of triadimefon (1.0, 2.0, 3.0 and 4.0 mg l^{-1}) in combination with IBA (2.0, 3.0, 4.0 and 5.0 mg l^{-1}) are presented in Table 19, Plate 9.

Number of days taken for root initiation

The time for rooting of *in vitro* shoots ranged from 10.6 to 23.0 days in different treatments. The medium supplemented with triadimefon 1.0 mg l^{-1} and IBA 5.0 mg l^{-1} took minimum days for rooting (10.6).

The treatments having triadimefon 2.0 mg l^{-1}, + IBA 5.0 mg l^{-1}, triadimefon 1.0 mg l^{-1} + IBA 3.0 mg l^{-1}, triadimefon 1.0 mg l^{-1} + IBA 4.0 mg l^{-1}, triadimefon 2.0 mg l^{-1} + IBA 4.0 mg l^{-1}showed root initiation after 16.8, 18.0, 18.2, 18.5 days, respectively.

Table 19: Effect of triadimefon and IBA on rooting of elongated shoots of cordyline

Basal medium : MS
Culture period : 3 weeks
Average of 10 cultures

Treatments		Time taken for root initiation (days)	No. of roots	Length of roots (cm)	Nature of roots
Triadimefon (mg l^{-1})	IBA (mg l^{-1})				
1.0	2.0	NC	NC	NC	Callus formation
2.0	2.0	NC	NC	NC	"
3.0	2.0	NC	NC	NC	"
4.0	2.0	NC	NC	NC	"
1.0	3.0	18.0	8.0	5.0	Thick brown roots, showed branching, root hairs and slight callusing
2.0	3.0	20.0	5.5	3.2	"
3.0	3.0	23.0	2.3	3.0	Callusing observed, no root growth
4.0	3.0	NC	NC	NC	"
1.0	4.0	18.2	8.5	6.5	Slight callusing
2.0	4.0	18.5	6.8	5.5	"
3.0	4.0	22.0	5.6	3.6	"
4.0	4.0	NC	NC	NC	Callusing, no root growth
1.0	5.0	10.6	11.5	6.6	Thick brown hard roots with root hairs
2.0	5.0	16.8	6.0	5.2	"
3.0	5.0	NC	NC	NC	-
4.0	5.0	NC	NC	NC	-

NC: No Change

Longest time (23.0 days) for root initiation was taken by the treatment having triadimefon 3.0 mg l^{-1} and IBA 3.0 mg l^{-1}, followed by triadimefon 2.0 mg l^{-1} + IBA 3.0 mg l^{-1} (20.0). Other treatments did not show any response.

Number of roots

The number of roots varied from 2.3 to 11.5 and showed significant variation among treatments. Treatments having triadimefon 1.0 mg l^{-1} + IBA 5.0 mg l^{-1} was found to be superior with maximum number of roots (11.5) and was significantly different from other treatments.

Minimum number of roots (2.3) was noticed in the medium supplemented with triadimefon 3.0 mg l^{-1} + IBA 3.0 mg l^{-1} followed by the media supplemented with triadimefon 2.0 mg l^{-1} + IBA 3.0 mg l^{-1} (5.5), tiradimefon 3.0 mg l^{-1} + IBA 4.00 mg l^{-1} (5.6), triadimefon 2.0 mg l^{-1} + IBA 5.0 mg l^{-1} (6.0), triadimefon 2.0 mg l^{-1} + IBA 4.0 mg l^{-1} (6.8), triadimefon 1.0 mg l^{-1} + IBA 3.0 mg l^{-1} (8.0) and triadimefon 1.0 mg l^{-1} + IBA 4.0 mg l^{-1} (8.5).

Length of roots

The length of the roots ranged from 3.0 cm to 6.6 cm. The maximum length of roots (6.6 cm) was observed in the treatment having triadimefon 1.0 mg l^{-1} + IBA 5.0 mg l^{-1}, which was on par with that of the medium supplemented with 1.0 mg l^{-1} triadimefon + 4.0 mg l^{-1} IBA (6.5 cm).

The minimum root length was observed (3.0 cm) in the treatment having 3.0 mg l^{-1} triadimefon + 3.0 mg l^{-1} IBA and was on par with that of the media having 2.0 mg l^{-1} triadimefon + 3.0 mg l^{-1} IBA (3.2 cm) and 3.0 mg l^{-1} triadimefon + 4.0 mg l^{-1} IBA (3.6 cm).

Nature of roots

Callus formation was observed in all the treatments except at higher levels of IBA (5.0 mg l^{-1}). The treatment combination of 1.0 mg l^{-1} triadimefon + 5.0 mg l^{-1} IBA produced thick brown hard roots with roots hairs.

Thick brown roots with root hairs and slight callus were observed in the treatment having triadimefon 1 mg l^{-1} + IBA 3.0 mg l^{-1}.

4.2.1.5 Planting out and acclimatization (Stage 4)

The rooted plantlets (Plate 10) were carefully removed from the tubes and morphological observations were made.

The height of the plantlets at the time of planting out ranged from 1.6 to 5.5 cm with a mean height of 3.55 cm. The number of leaves ranged from 5.0 to 16.0 with a mean value of 10.5. Number of roots ranged from 10 to 26 and the mean number of roots was18.0. The length of the roots ranged from 4.5 to 8.8 cm with a mean value of 6.65 cm.

The plantlets were treated with 0.1 per cent Bavistin solution for 30 minutes. These plantlets were then planted out and subjected to different acclimatization treatments and the results are presented in the Table 20.

Observations were recorded on the survival percentage after two weeks, four weeks, six weeks and eight weeks.

When the plantlets were kept in open condition, all the treatments except peat + sand recorded complete death of the plants within 2 weeks. Ten per cent survival was recorded in peat + sand when given a pretreatment with 0.2 per cent carbendazim (50% WP). These survived plantlets failed to grow further and collapsed within four weeks.

Table 20: Effect of hardening techniques on post transplanting survival of tissue cultured plantlets of cordyline

Treatments		Pre planting treatments	Post planting treatments	Survival (%) after			
				2 weeks	4 weeks	6 weeks	8 weeks
A. Open condition							
Containers	Media						
Mud pot	Fine sand	Carbendazim 0.2 %	Watering at 2 days interval	0.0	-	-	-
"	Coconut fibre	"	"	0.0	-	-	-
"	Peat moss	"	"	0.0	-	-	-
"	Vermiculite	"	"	0.0	-	-	-
"	Peat + sand	"	"	10.0	-	-	-
"	Vermiculite + sand	"	"	0.0	-	-	-
B. Plastic cover							
Mud pot	Fine sand	"	"	0.0	-	-	-
"	Coconut fibre	"	"	0.0	-	-	-
"	Peat moss	"	"	20.0	10.0	-	-
"	Vermiculite	"	"	15.0	10.0	-	-
"	Peat + sand	"	"	60.0	50.0	50.0	30.0
"	Vermiculite + sand	"	"	20.0	10.0	-	-
C. Net house							
Mud pot	Fine sand	"	"	40.0	20.0	20.0	0.0
"	Coconut fibre	"	"	10.0	0.0	-	-
"	Peat moss	"	"	30.0	30.0	20.0	10.0
"	Vermiculite	"	"	40.0	30.0	30.0	20.0
"	Peat + sand	"	"	50.0	40.0	40.0	30.0
"	Vermiculite + sand	"	"	40.0	20.0	20.0	10.0
"	Fine sand	Carbendazim 0.2 % + Triadimefon 1 mg l^{-1} in rooting media	Watering at 2 days interval + Triadimefon 20 mg l^{-1} foliar spray	60.0	30.0	30.0	10.0
"	Coconut fibre	"	"	20.0	10.0	0.0	-
"	Peat moss	"	"	50.0	30.0	30.0	20.0
"	Vermiculite	"	"	50.0	40.0	30.0	20.0
"	Peat + sand	"	"	70.0	60.0	40.0	40.0
"	Vermiculite + sand	"	"	50.0	40.0	30.0	20.0
"	Fine sand	"	Triadimefon 20 mg l^{-1} drenching	60.0	30.0	30.0	15.0
"	Coconut fibre	"	"	20.0	10.0	0.0	-
"	Peat moss	"	"	60.0	40.0	40.0	30.0
"	Vermiculite	"	"	60.0	40.0	30.0	20.0
"	Peat + sand	"	"	80.0	70.0	60.0	60.0
"	Vermiculite + sand	"	"	60.0	40.0	40.0	30.0

When the pots were covered with polythene cover for two weeks, survival percentage of 60.0 was recorded after 2 weeks in peat + sand treated with 0.2 per cent carbendazim (50% WP). Even after eight weeks 30.0 per cent survival was observed. Under same conditions, peat moss, vermiculite + sand showed 20.0 per cent, each, survival and vermiculite showed 15.0 per cent survival after 2 weeks.

When plantlets were kept under net house (50% shade), survival percentage of 50.0, 40.0, 40.0, 40.0, 30.0 and 10.0 were recorded up to 2 weeks in the treatments peat + sand, fine sand, vermiculite, vermiculite + sand, peat moss and coconut fibre, respectively, after giving a pre-planting treatment with 0.2 per cent carbendazim (50% WP).

The treatments with different media (fine sand, coconut fibre, peat moss, vermiculite, peat + sand, vermiculite + sand) were kept under net house. The plantlets were given a pre-planting treatment with 1 mg l^{-1} triadimefon in rooting medium and 0.2 per cent carbendazim (50% WP) dip for 30 minutes and a post planting treatment with 20.0 mg l^{-1} triadimefon at weekly intervals.

The maximum survival percentage (60.0 %) after eight weeks was recorded in peat + sand (20.0 mg l^{-1} triadimefon drenching) followed by 40.0 per cent survival in the same medium with triadimefon foliar spray. Both peat moss, vermiculite + sand showed a survival level of 30.0 percent.

Plantlet survival up to two weeks was maximum (80.0 per cent) in the case of peat + sand with triadimefon drenching. Same medium with foliar spray of triadimefon showed 70.0 per cent survival after two weeks.

4.2.1.5.1 Standardisation of nutritional requirement of micropropagated plantlets

The results of the trial conducted to study the effect of 17: 17: 17 NPK mixture on plant height, number of leaves, leaf length and leaf breadth are given in Table 21.

Plant height after 30 days

Increment in plant height after 30 days ranged from 1.10 cm to 2.36 cm. Maximum increment in plant height (2.36 cm) was observed when the plantlets were drenched with NPK 17: 17: 17 at the rate of 0.75 g/plant, which was significantly superior to 0.5 g/plant (1.86 cm) and 0.75 g/plant foliar spray (1.84 cm).

Minimum increment in plant height (1.10 cm) was observed in control. Treatments like 0.50 g/plant drenching (1.18 cm), 0.25 g/plant foliar spray (1.16 cm) and 0.50 g/plant foliar spray (1.16 cm) were found to be on par with the control.

Number of leaves, leaf length and leaf breadth

Increase in number of leaves after 30 days varied from 2.0 to 3.2. It was maximum (3.2) with 0.75 g/plant drenching treatment with NPK 17:17:17 and it was found to be homogeneous with the treatments like 1.0 g/plant drenching (3.0) and 0.75 g/plant foliar spray (3.0).

Minimum increase in number of leaves was observed (2.0, each) in the treatments like 0.25 g/plant foliar spray and control.

Leaf length and leaf breadth ranged from 0.32 cm to 0.36 cm and 0.16 cm to 0.20 cm, respectively, and did not differ significantly.

Table 21: Standardisation of nutritional requirement of micropropagated plantlets of cordyline

Medium : Mud pot with peat + sand

Treatments 17: 17:17 NPK mixture (g/plant)	Increment after 30 days				Increment after 60 days			
	Plant height (cm)	No. of leaves	Leaf length (cm)	Leaf breadth (cm)	Plant height (cm)	No. of leaves	Leaf length (cm)	Leaf breadth (cm)
0.0	1.10	2.0	0.34	0.16	2.84	5.2	0.52	0.30
Drenching								
0.50	1.18	2.4	0.36	0.18	2.96	5.6	0.63	0.30
0.75	2.36	3.2	0.36	0.20	4.84	6.2	0.68	0.36
1.00	1.86	3.0	0.32	0.18	4.00	6.0	0.64	0.32
Foliar spray								
0.25	1.16	2.0	0.36	0.16	2.88	5.2	0.64	0.30
0.50	1.16	2.8	0.36	0.20	2.84	6.0	0.60	0.38
0.75	1.84	3.0	0.34	0.20	3.88	6.0	0.74	0.34
CD (0.05)	0.47	0.88	0.08	0.08	0.62	1.24	0.10	0.09
SEm ±	0.16	0.30	0.03	0.03	0.21	0.43	0.03	0.03

Plant height after 60 days

Plants after 60 days were the tallest (4.84 cm) when drenched with 0.75 g of 17:17:17 NPK (Plate 11), which was superior to 1.0 g/plant drenching (4.00 cm) and 0.75 g/plant foliar spray (3.88 cm).

Minimum increase in plant height (2.84 cm) was observed in the treatments like 0.50 g/plant foliar spray and control, and were found to be homogeneous with the treatments like 0.25 g/plant foliar spray (2.88 cm) and 0.5 g/plant drenching (2.96 cm).

Number of leaves, leaf length and breadth

Increment in number of leaves, leaf length and leaf breadth after 60 days as influenced by the various treatments ranged from 5.2 to 6.2, 0.52 cm to 0.74 cm and 0.30 cm to 0.38 cm, respectively. The differences were not significant with respect to increment in number of leaves and leaf breadth. Maximum increase in leaf length (0.74 cm) was observed in 0.75 g/plant foliar spray.

4.2.2 Organogenesis

4.2.2.1 Explant choice

The results of the trial conducted to find out the response of various explants of cordyline to organogenesis are given in the Tables 22, 23a, 23b and 23c.

Cultures of nodal segments and shoot tips showed 100 per cent response when MS medium was supplemented with 2,4-D 0.5 mg l^{-1} + BAP 0.5 mg l^{-1}. The response of nodal segments and shoot tips was 80 per cent, each, in treatments containing 2,4-D 0.5 mg l^{-1}, 2,4-D 1.0 mg l^{-1}, 2,4-D 0.5 mg l^{-1} + BAP 1.0 mg l^{-1}, 2,4-D 0.5 mg l^{-1} + BAP 2.0 mg l^{-1}, 2,4-D 0.5 mg l^{-1} + KIN 0.5 mg l^{-1} and 2,4-D 0.5 mg l^{-1} + KIN 1.0 mg l^{-1}.

Plate. 8. Effect of activated charcoal, media on *in vitro* rooting

Plate. 9. Effect of triadimefon and IBA on *in vitro* rooting

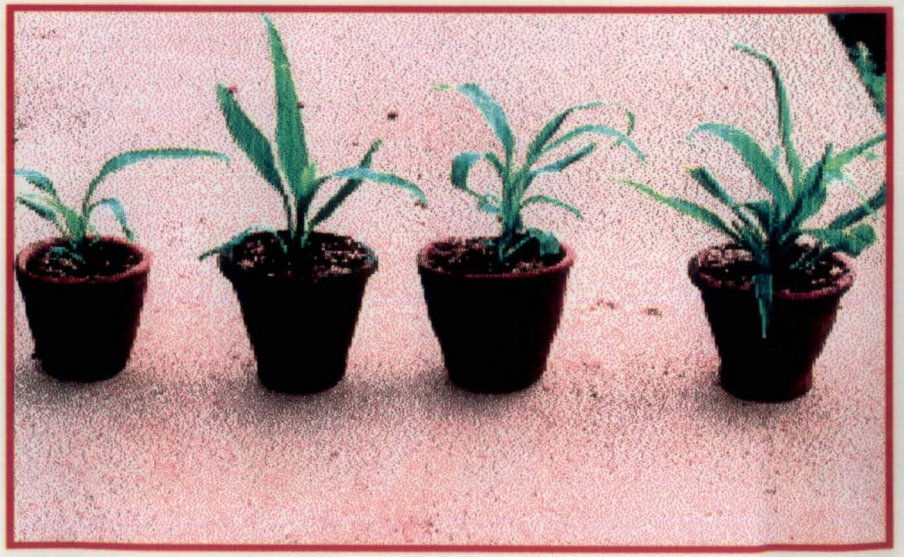


Plate. 10. Micro propagated plantlets

Plate. 11. Effect of nutrient application

Control

Table 22: Response of various explants of cordyline on callus initiation

Basal medium : MS medium
Average of 10 cultures

Treatments (mg l^{-1})	Percentage cultures showing callus				
	Nodal segments	Axillary buds	Shoot tips	*In vitro* roots	Leaf segments
2, 4 D 0.5	80.0	60.0	80.0	-	-
2, 4 D 1.0	80.0	60.0	80.0	-	-
2, 4 D 0.5 + BAP 0.5	100.0	80.0	100.0	-	-
2, 4 D 0.5 + BAP 1.0	80.0	60.0	80.0	-	-
2, 4 D 0.5 + BAP 2.0	80.0	60.0	80.0	-	-
2, 4 D 0.5 + BAP 3.0	90.0	60.0	90.0	-	-
2, 4 D 0.5 + KIN 0.5	80.0	60.0	80.0	-	-
2, 4 D 0.5 + KIN 1.0	80.0	60.0	80.0	-	-
2, 4 D 0.5 + KIN 2.0	50.0	40.0	50.0	-	-
2, 4 D 0.5 + KIN 3.0	0.0	0.0	0.0	-	-
2, 4 D 1.0 + NAA 0.5	-	-	-	-	40.0
2, 4 D 1.0 + NAA 1.0	-	-	-	-	40.0
2, 4 D 1.0 + NAA 5.0	-	-	-	-	100.0

Cultures of axillary bud explants also recorded high callus initiation in treatments 2,4-D 0.5 mg l^{-1} + BAP 0.5 mg l^{-1} (80%), 2,4-D 0.5 mg l^{-1} (60), 2,4-D 1.0 mg l^{-1} (60%), 2,4-D 0.5 mg l^{-1} + BAP 1.0 mg l^{-1} (60%), 2,4-D 0.5 mg l^{-1} + BAP 3.0 mg l^{-1} + (60%), 2,4-D 0.5 mg l^{-1} + KIN 0.5 mg l^{-1} (60%) and 2,4-D 0.5 mg l^{-1} + KIN 1.0 mg l^{-1} (60%).

The *in vitro* roots failed to initiate callus in any of the treatments. Leaf segments recorded (100%) callus initiation in treatment containing 2,4-D 1.0 mg l^{-1} + NAA 5.0 mg l^{-1}. In treatments involving 2,4-D 1.0 mg l^{-1} +NAA 0.5 mg l^{-1} and 2,4-D 1.0 mg l^{-1} + NAA 1.0 mg l^{-1} the callus initiation was 40 per cent, each.

Maturity of leaf, portion of the leaf and position of the leaf were found to influence callusing, data pertaining to which are given in the Tables 23a to 23c.

4.2.2.1.1 Effect of maturity of leaf

Days taken for callusing

Number of days taken for callusing ranged from 18.5 to 22.0 and treatments differed significantly. Immature leaves showed minimum days (18.5) for callus induction followed by young leaves (19.4 days). Mature leaves took the longest time (22.0 days) for callus induction (Table 23a, Plate 12).

Percentage callusing

All the cultures originating from immature and young leaves responded to callusing. Mature leaves initiated callus only in 60 per cent of the cultures.

Callus intensity and callus index

Callus intensity and callus index ranged from 2.0 to 4.0 and 120 to 400, respectively and differed significantly. Maximum callus intensity (4.0) and callus

Table 23a: Effect of maturity of leaf of cordyline on callusing

Medium: MS + 2, 4-D 1.0 mg l^{-1} + NAA 5.0 mg l^{-1}
Average of 10 cultures

Treatments	Time taken for callusing (days)	Percentage callusing	Callus intensity	Callus index	Callus texture	Callus colour
Immature leaf	18.50	100	4.0	400	Soft and friable	Creamy white
Young leaf	19.40	100	3.5	350	Soft	Slightly brown
Mature leaf	22.00	60	2.0	120	"	"

Table 23b: Effect of portion of the leaf on callusing in cordyline

Medium: MS + 2, 4-D 1.0 mg l^{-1} + NAA 5.0 mg l^{-1}
Average of 10 cultures

Treatments	Time taken for callus-ing (days)	Percentage callusing	Callus intensity	Callus index	Callus texture	Callus colour
P_1	18.5	100	4.0	400	Soft and friable	Creamy white
P_2	19.4	100	3.0	300	Soft	Slightly brown
P_3	21.9	60	1.5	90	"	"

P_1 : Basal portion of the leaf
P_2 : Middle portion of the leaf
P_3 : Top portion of the leaf

Table 23c: Effect of position of leaf on callusing in cordyline

Medium: MS + 2, 4-D 1.0 mg l^{-1} + NAA 5.0 mg l^{-1}
Average of 10 cultures

Positon of the leaf on the medium	Response
Adaxial surface	100 per cent callus formation
Abaxial surface	Sparse callusing (30.0 %), drying of leaves (50.0 %), no change (20.0 %)

index (400) were found in immature leaves, followed by young leaves (3.5, 350). Mature leaves showed minimum callus intensity (2.0) and callus index (120).

4.2.2.1.2 Effect of portion of leaf

Basal portion of the leaf showed minimum days for callusing (18.5), followed by middle portion of the leaf (19.4 days). Maximum number of days for callusing (21.9) was taken by top portion of the leaf (Table 23b).

Callusing was total in the cultures of bottom portion of the leaf and middle portion of the leaf. Top portion of the leaf showed 60 per cent callusing.

The treatments differed significantly with respect to regarding callus intensity and callus index and ranged from 1.5 to 4.0 and 90 to 400, respectively. Maximum callus intensity and callus index were observed in the medium containing bottom portion of the leaf. Top portion of the leaf showed minimum callus intensity (1.5) and callus index (90).

The texture of the callus was soft and the colour was creamy white in the case of bottom portion of the leaf. Middle and top portion of the leaf produced soft and slightly brown callus.

4.2.2.1.3 Effect of position of the leaf

Position of the leaf influenced callusing to a great extent (Table 23c). Adaxial surface of the leaf on the medium gave 100 per cent callus formation. Abaxial surface of the leaf on the medium showed sparse callusing in 30.0 per cent cultures. In 50.0 per cent cultures leaves dried up. Rest of the cultures retained the green colour of the leaf without induction of callus.

4.2.2.2 Direct organogenesis

4.2.2.2.1 Effect of IAA and 2,4-D

Data pertaining to the effect of IAA and 2,4-D on the direct organogenesis of the shoot tips, axillary buds and nodal segments are presented in Table 24.

Rhizogenesis was maximum (80.0% cultures) in the treatments having IAA 2.0 mg l^{-1}. In the medium supplemented with IAA 1.0 mg l^{-1} and IAA 3.0 mg l^{-1}, 40.0 per cent cultures produced direct rhizogenesis

Treatments like 2,4-D 1.0 mg l^{-1}, 2,4-D 2.0 mg l^{-1} and 2,4-D 3.0 mg l^{-1} showed rhizogenesis in 20.0 per cent cultures. All the treatments failed to show morphogeneiss.

4.2.2.2.2 Effect of BAP, KIN and 2ip

Data relating to the effect of BAP, KIN and 2ip on the direct organogenesis are given in Table 25.

All the treatments failed to show morphogenesis. Maximum rhizogenesis (40.0 per cent) was observed in the treatments having 2ip 2.0 mg l^{-1} and 2ip 3.0 mg l^{-1} followed by BAP 3.0 mg l^{-1} and 2ip 1.0 mg l^{-1} (30.0 per cent, each). Rhizogenesis was minimum (20.0 per cent) in the treatments having KIN 2.0 mg l^{-1} and KIN 3.0 mg l^{-1} each. Treatments having BAP 1.0 mg l^{-1}, KIN 1.0 mg l^{-1} and BAP 2.0 mg l^{-1} showed neither morphogenesis nor rhizogenesis.

4.2.2.3 Callus mediated organogenesis

4.2.2.3.1 Callus initiation

Nodal segments, shoot tips showed 100.0 per cent callusing when inoculated to the medium containing 2,4-D 0.5 mg l^{-1} + BAP 0.5 mg l^{-1}. Leaf

Table 24: Effect of IAA and 2, 4-D on direct organogenesis of shoot tips, axillarybuds and nodal segments of cordyline

Basal medium : MS
Culture period : 4 weeks
Average of 10 cultures

Treatments (mg l^{-1})	Time taken to respond (days)	Cultures showing morphogenesis (%)	Cultures showing rhizogenesis (%)
IAA 0.5	0.00	0.0	0.0
IAA 1.0	13.40	0.0	40.0
IAA 2.0	14.20	0.0	80.0
IAA 3.0	17.00	0.0	40.0
IAA 4.0	18.50	0.0	30.0
2, 4-D 0.5	0.00	0.0	0.0
2, 4-D 1.0	10.80	0.0	20.0
2, 4-D 2.0	13.60	0.0	20.0
2, 4-D 3.0	20.80	0.0	20.0

Table 25: Effect of BAP, KIN and 2ip on direct organogenesis of nodal segments of cordyline

Basal medium : MS
Culture period : 4 weeks
Average of 10 cultures

Treatments (mg l^{-1})	Time taken to respond (days)	Cultures showing morphogenesis (%)	Cultures showing rhizogenesis (%)
BAP 1.0	NC	NC	NC
BAP 2.0	NC	NC	NC
BAP 3.0	20.2	0.0	30.0
KIN 1.0	NC	NC	NC
KIN 2.0	26.5	0.0	20.0
KIN 3.0	28.2	0.0	20.0
2ip 1.0	18.0	0.0	30.0
2ip 2.0	16.7	0.0	40.0
2ip 3.0	15.6	0.0	40.0

NC : No change

segments, when inoculated on to medium with 2,4-D 1.0 mg l^{-1} + NAA 5.0 mg l^{-1} showed 100.0 per cent callusing. These three explants were used for callus mediated organogenesis.

4.2.2.3.1.1 Effect of 2,4-D in combination with BAP and KIN on nodal segments and shoot tips

Data relating to the influence of 2,4-D in combination with BAP and KIN on callus induction and growth of nodal segments and shoot tips are presented in Table 26.

Days taken for callus induction

The average time taken for callus production ranged from 6.5 days to 10.5 days. Minimum days for callus initiation was taken by the medium supplemented with 2,4-D 0.5 mg l^{-1} + BAP 0.5 mg l^{-1} (Plate 13) Where as the medium supplemented with 2,4-D 0.5 mg l^{-1} + BAP 1.0 mg l^{-1} took the longest time.

Callusing percentage

All the cultures callused in the treatments with 2,4-D 0.5 mg l^{-1} + BAP 0.5 mg l^{-1}, followed by the treatment having 2,4-D 0.5 mg l^{-1} + BAP 3.0 mg l^{-1} (90.0 per cent). A callusing level of 80.0 per cent, each, was recorded in 2,4-D 0.5 mg l^{-1}, 2,4-D 0.5 mg l^{-1} + BAP 1.0 mg l^{-1}, 2,4-D 0.5 mg l^{-1} + KIN 0.5 mg l^{-1} and 2,4-D 0.5 mg l^{-1} + KIN 1.0 mg l^{-1}. No callusing was observed in the medium supplemented with 2,4-D 0.5 mg l^{-1} + KIN 3.0 mg l^{-1}.

Callus index

Callus index ranged from 100 to 400 in different treatments. Callus index was maximum (400) when the medium was supplemented with 2,4-D 0.5 mg l^{-1} + BAP 0.5 mg l^{-1}, followed by the callus index of 240, each, in the treatments

Table 26: Effect of 2,4-D in combination with BAP and KIN on callus induction and growth of nodal segments and shoot tips of cordyline

Basal medium : MS
Culture period : 3 weeks
Average of 10 cultures

Treatments (mg l^{-1})	Time taken for callusing (days)	Callus-ing (%)	Callus intensity	Callus index	Callus texture	Callus colour
2, 4-D 0.5	9.0	80	2	160	Soft	Dull white
2, 4-D 0.5 + BAP 0.5	6.5	100	4	400	Soft and Semi firm	Creamy white
2, 4-D 0.5 + BAP 1.0	10.5	80	3	240	Soft	"
2, 4-D 0.5 + BAP 2.0	9.0	80	3	240	"	Slightly brown
2, 4-D 0.5 + BAP 3.0	9.0	90	2	180	"	"
2, 4-D 0.5 + KIN 0.5	8.0	80	3	240	Semi firm	Dull white
2, 4-D 0.5 + KIN 1.0	8.5	80	3	240	"	"
2, 4-D 0.5 + KIN 2.0	8.5	50	2	100	"	Slightly brown
2, 4-D 0.5 + KIN 3.0	-	-	-	-	-	-

2,4-D 0.5 mg l^{-1} + BAP 1.0 mg l^{-1}, 2,4-D 0.5 mg l^{-1} + BAP 2.0 mg l^{-1}, 2,4-D 0.5 mg l^{-1} + KIN 0.5 mg l^{-1} and 2,4-D 0.5 mg l^{-1} + KIN 1.0 mg l^{-1}.

Callus index of 180 was recorded in the treatment with 2,4-D 0.5 mg l^{-1} + BAP 3.0 mg l^{-1}. Minimum callus index (100) was noticed in the medium containing 2,4-D 0.5 mg l^{-1} + KIN 2.0 mg l^{-1}.

4.2.2.3.1.2 Effect of coconut water and 2,4-D

Data on the influence of coconut water and 2,4-D on the callus induction and growth of explants from shoot tips and nodal segments are given in the Table 27.

Days taken for callus induction

The days taken for callus induction ranged from 7.0 to 10.5. The treatment having 10 per cent coconut water + 2.0 mg l^{-1} 2,4-D took the shortest time. Maximum days for callus induction was taken by the media supplemented with coconut water 10 per cent alone and coconut water 5 per cent + 2,4-D 0.5 mg l^{-1}.

Callusing percentage

The extent of cultures callused in various treatments ranged from 60 to 90 per cent.

Maximum callusing was obtained in the MS medium supplemented with coconut water 10 per cent + 2,4-D 2.0 mg l^{-1}. Percentage of cultures callused were the lowest in the medium supplemented with coconut water 5 per cent alone.

Callus index

Callus index ranged from 70 to 315 in different treatments. The highest callus index was obtained in MS medium supplemented with coconut water 10.0

Table 27: Effect of coconut water and 2, 4-D on callus induction and growth of nodal segments and shoot tips of cordyline

Basal medium : MS
Culture period : 3 weeks
Average of 10 cultures

Treatments		Time taken for callusing (days)	Callus intensity	Callusing (%)	Callus index	Callus texture	Callus colour
Coconut water (%)	2, 4-D (mg l^{-1})						
5.0	0.0	-	-	-	-	-	-
10.0	0.0	10.5	2.5	60	150	Soft	Creamy white
15.0	0.0	-	-	-	-	-	-
5.0	0.5	10.5	2.0	80	160	Semi firm	Slightly brown
10.0	0.5	9.0	2.0	80	160	"	"
15.0	0.5	9.0	2.0	70	140	"	"
5.0	1.0	10.0	1.5	80	120	Soft	Creamy white
10.0	1.0	8.5	2.0	90	180	Semi firm	Slightly brown
15.0	1.0	9.0	1.0	70	70	"	"
5.0	2.0	7.5	2.5	70	175	Soft	Dull white
10.0	2.0	7.0	3.5	90	315	Soft and Semi firm	"
15.0	2.0	9.0	2.5	70	175	"	"

per cent + 2,4-D 2.0 mg l^{-1}, followed by the treatments having coconut water 10 per cent + 2,4-D 1.0 mg l^{-1} (180), coconut water 5 per cent + 2,4-D 2.0 mg l^{-1} (175), coconut water 15.0 per cent + 2,4-D 2.0 mg l^{-1} (175), coconut water 5.0 per cent + 2,4-D 0.5 mg l^{-1} (160), coconut water 10 per cent + 2,4-D 0.5 mg l^{-1} (160), coconut water 15 per cent + 2,4-D 0.5 mg l^{-1} (140). Callus index was minimum in the MS medium supplemented with coconut water 15 per cent + 2,4-D 1.0 mg l^{-1}.

4.2.2.3.2 Callus differentiation

Callus derived from the nodal segments and shoot tips were inoculated in MS medium supplemented with cytokinins to study the differentiation of callus.

4.2.2.3.2.1 Effect of BAP and KIN

Data pertaining to the effect of BAP and KIN on the differentiation of the callus are shown in the Table 28, Plates14,16.

Days taken for differentiation

The time taken for differentiation of the callus ranged from 42.5 to 48.5 days and were significantly different.

MS medium supplemented with 1.0 mg l^{-1} BAP + 2.0 mg l^{-1} KIN took the minimum days (42.5) for differentiation when sub cultured at monthly intervals.

Longest time for differentiation of callus was taken by the medium supplemented with 2.0 mg l^{-1} BAP + 1.0 mg l^{-1} KIN or 2.0 mg l^{-1} BAP + 2.0 mg l^{-1} KIN (48.5 days) followed by BAP 2.0 mg l^{-1} + KIN 0.5 mg l^{-1} (48.0 days). Other treatments did not show any response.

Plate. 12. Effect of maturity of leaf on callus initiation

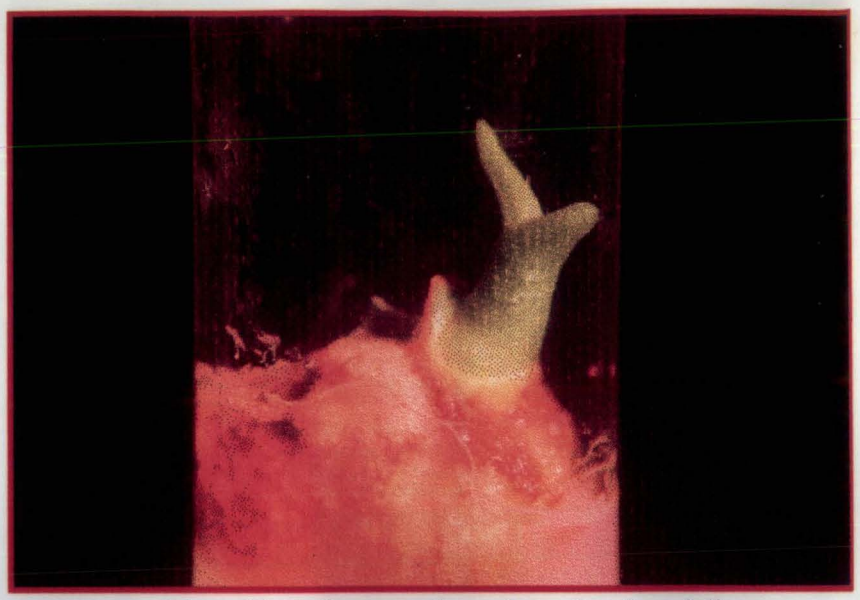


Plate. 13. Callus growth on shoot tip of cordyline

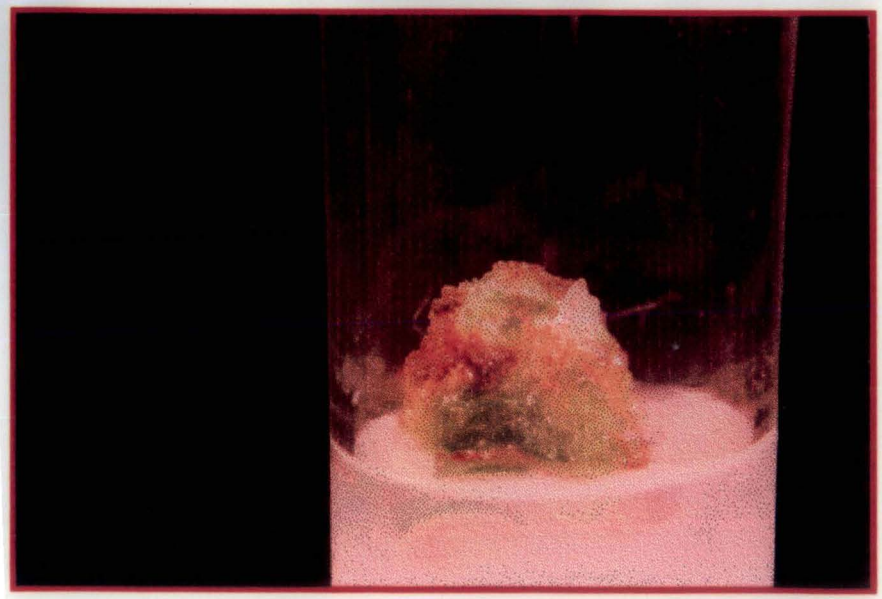


Plate. 14. Callus showing green colour with out differentiation

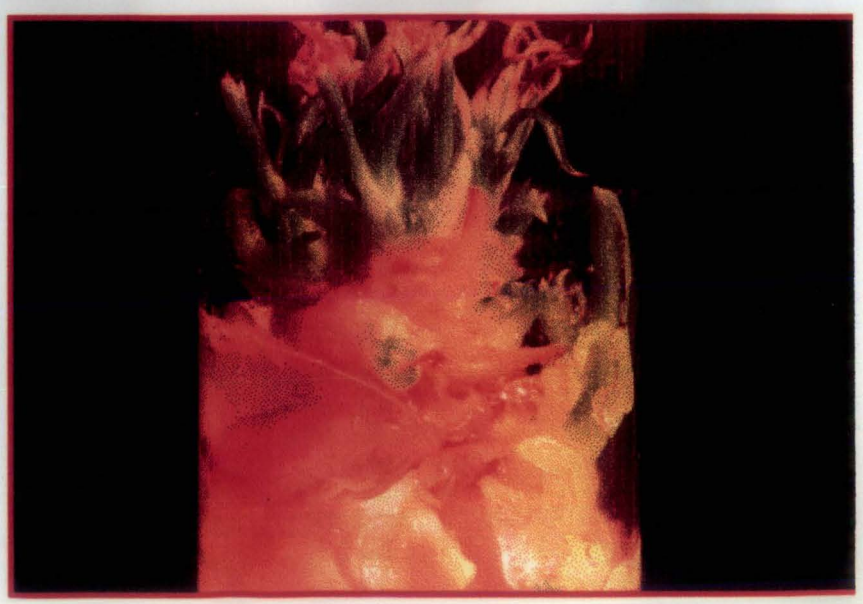


Plate. 15. Regeneration from callus in the presence of BAP+KIN

Table 28: Effect of BAP and KIN on shoot organogenesis from callus in cordyline

Basal medium : MS medium
Cultute period : 4 weeks
Average of 10 cultures

Treatments		Time taken for different-tiation (days)	No. of shoots	Response
BAP (mg l^{-1})	KIN (mg l^{-1})			
0.0	0.5	-	-	No shoot generation
0.0	1.0	-	-	Callus growth, no shoot regeneration
0.0	2.0	-	-	"
0.5	0.0	-	-	No shoot regeneration
0.5	0.5	-	-	Callus growth, no shoot regeneration
0.5	1.0	-	-	Callus proliferation, no shoot regeneration
0.5	2.0	-	-	Callus growth, no shoot regeneration
1.0	0.0	-	-	Callus growth, no shoot regeneration
1.0	0.5	-	-	Callus proliferation, no shoot regeneration
1.0	1.0	-	-	Callus turned green, no shoot regeneration
1.0	2.0	42.5	++++	Callus differentiation, multiple shoot formation
2.0	0.0	-	-	Callus proliferation, no shoot regeneration
2.0	0.5	48.0	++	Shoot regeneration, number of shoots medium
2.0	1.0	48.5	++	"
2.0	2.0	48.5	++	"

++++ Very high rate of shoot regeneration (≥ 31 shoots)
++ Medium rate of shoot regeneration (11-20 shoots)

Number of shoots

Very high rate of shoot buds were produced on frequent sub culturing in the MS medium supplemented with BAP 1.0 mg l^{-1} + KIN 2.0 mg l^{-1} (Plate 16) The treatments like BAP 2.0 mg l^{-1} + KIN 0.5 mg l^{-1}, BAP 2.0 mg l^{-1} + KIN 1.0 mg l^{-1}, BAP 2.0 mg l^{-1} + KIN 2.0 mg l^{-1} showed medium rate of shoot bud production. Other treatments did not show any response.

4.2.2.3.2.2 Effect of BAP and 2ip

Data pertaining to the effect of BAP and 2ip on the differentiation of the callus are given in the Table 29.

Minimum days (43.5) for differentiation was observed in BAP 2.0 mg l^{-1} + 2ip 1.0 mg l^{-1} when sub cultured at monthly interval. The medium with BAP 1.0 mg l^{-1} + 2ip 2.0 mg l^{-1} took maximum days for differentiation (48.0). Other treatments did not show any morphogenesis.

Treatments BAP 2.0 mg l^{-1} +2ip 1.0 mg l^{-1} (Plate 17) and BAP 1.0 mg l^{-1} + 2ip 2.0 mg l^{-1} showed medium rate of bud development. All the treatments showed rhizogenesis.

4.2.2.3.2.3 Effect of coconut water

Coconut water at different concentrations induced only rhizogenesis, but failed to induce morphogenesis.

4.2.2.3.2.4 Leaf segments

Data on the effect of 2,4-D and NAA on callus induction and growth of leaf segments of cordyline are given in the Table 30, Plate 18.

Table 29: Effect of BAP and 2ip on shoot organogenesis from callus in cordyline

Basal medium : MS
Culture period : 3 weeks
Average of 10 cultures

Treatments		Time taken for different-iation (days)	No. of shoots	Response
BAP (mg l^{-1})	2ip (mg l^{-1})			
0.0	0.5	-	-	Rhizogenesis
0.0	1.0	-	-	"
0.0	2.0	-	-	"
0.5	0.5	-	-	"
0.5	1.0	-	-	"
0.5	2.0	-	-	"
1.0	0.5	-	-	"
1.0	1.0	-	-	Rhizogenesis, callus proliferation
1.0	2.0	48.0	++	Rhizogenesis
2.0	0.5	-	-	Rhizogenesis, callus proliferation
2.0	1.0	43.5	++	"
2.0	2.0	-	-	"

Days taken for callus induction

The time taken for callus induction ranged from 18.50 days to 28.60 days.

The shortest time was taken when the medium was supplemented with 2,4-D 1.0 mg l^{-1} + NAA 5.0 mg l^{-1}. Maximum days for callus induction was taken by the treatment supplemented with 2,4-D 0.5 mg l^{-1} + NAA 0.5 mg l^{-1}.

Callusing percentage

Callusing percentage ranged from 20.0 to 100.0. Maximum callusing was obtained when the medium was supplemented with NAA 10.0 mg l^{-1} and 2,4-D 1.0 mg l^{-1} + NAA 5.0 mg l^{-1}, followed by the treatments NAA 8.0 mg l^{-1} and NAA 12.0 mg l^{-1} (70.0%) callusing.

When the explants were inoculated into the media containing 2,4-D 0.5 mg l^{-1} + NAA 0.5 mg l^{-1} and 2,4-D 0.5 mg l^{-1} + NAA 1.0 mg l^{-1} 20.0 per cent of cultures recorded callusing.

Callus index

Maximum callus index (350) was recorded in the medium supplemented with 2,4-D 1.0 mg l^{-1} + NAA 5.0 mg l^{-1}, followed by NAA 10 mg l^{-1} (300), 2,4-D 3.0 mg l^{-1} (180), NAA 8.0 mg l^{-1} (140), NAA 12.0 mg l^{-1} (140) and NAA 6.0 mg l^{-1} (120).

Callus index was minimum (20.0, each) in 2,4-D 0.5 mg l^{-1} + NAA 0.5 mg l^{-1} and 2,4-D 0.5 mg l^{-1} + NAA 1.0 mg l^{-1}.

4.2.2.3.2.5 Callus differentiation

Data pertaining to the effect of growth regulators and activated charcoal on the differentiation of the callus are given in the Table 31, Plate 19.

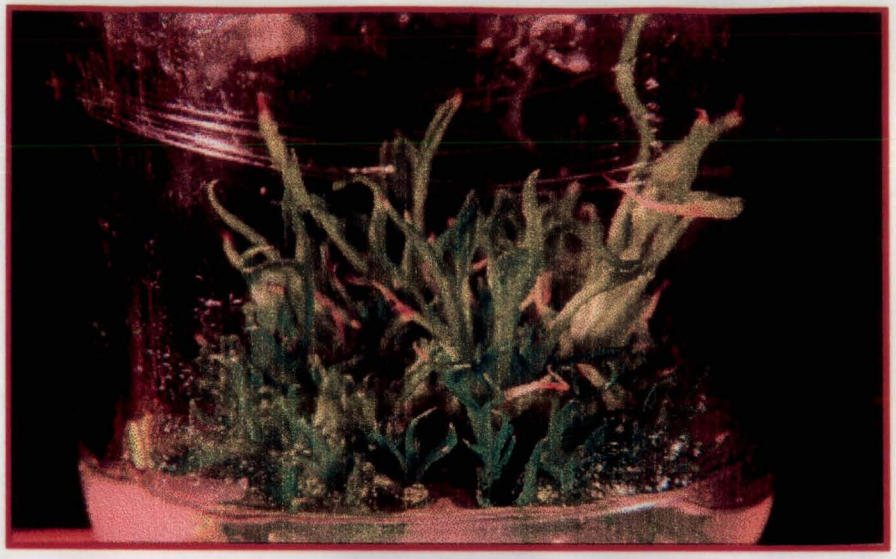


Plate. 16. Enhanced regeneration in presence of BAP+KIN

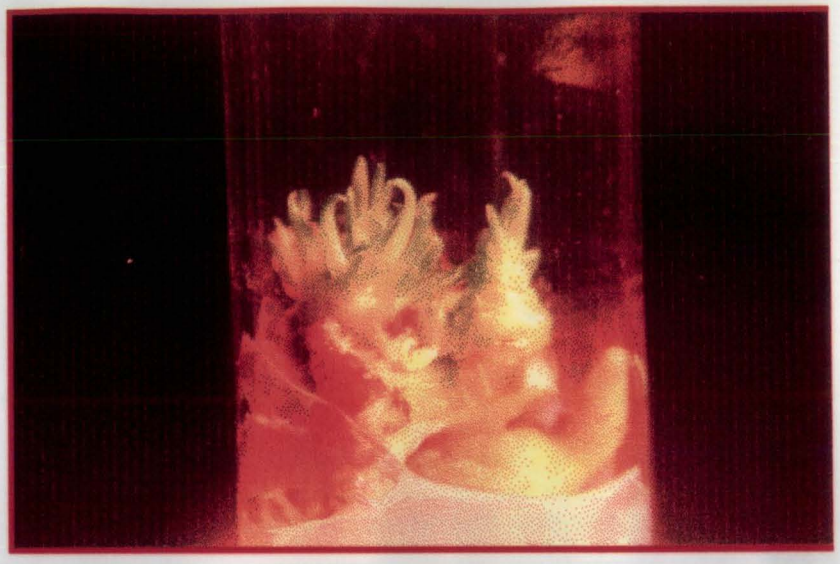


Plate. 17. Regeneration from callus in the presence of 2ip + BAP

Plate. 18. Different stages of callus growth

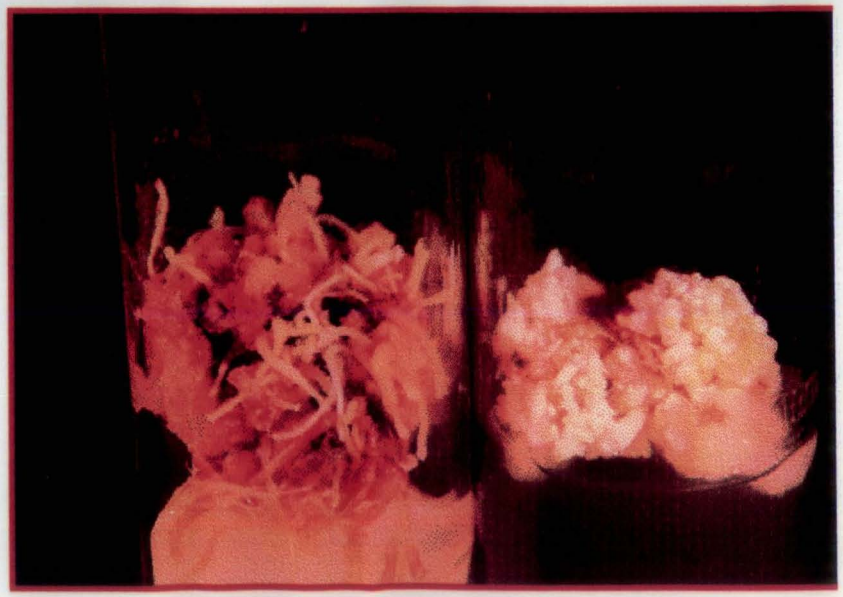


Plate. 19. Effect of activated charcoal on browning

Table 30: Effect of 2, 4-D and NAA on callusing of leaf segments of cordyline

Medium: MS basal
Culture period : 4 weeks
Average of 10 cultures

Treatments		Time taken for callusing (days)	Callusing (%)	Callus intensity	Callus index	Callus texture	Callus colour
2, 4-D ($mg\ l^{-1}$)	NAA ($mg\ l^{-1}$)						
0.0	6.0	23.00	60	2.0	120	Soft	Dull white
0.0	8.0	22.20	70	2.0	140	Soft and friable	"
0.0	10.0	19.90	100	3.0	300	"	Creamy white
0.0	12.0	20.50	70	2.0	140	"	Dull white
0.5	0.0	28.50	40	1.0	40	Firm	Pale brown
0.5	0.5	28.60	20	1.0	20	"	Slightly brown
0.5	1.0	28.50	20	1.0	20	"	Dull white
0.5	5.0	24.50	40	1.0	40	Semi firm	"
1.0	0.0	20.80	40	2.0	80	Firm	Slightly brown
1.0	0.5	23.30	40	1.5	60	Semi firm	"
1.0	1.0	22.20	40	1.5	60	"	Creamy white
1.0	5.0	18.50	100	3.5	350	Soft and semi firm	"
2.0	0.0	20.20	40	2.0	80	Semi firm	Slightly brown
3.0	0.0	19.30	60	3.0	180	"	"

One month old callus derived from the leaf segments was sub cultured on both half strength and full strength MS basal media. Both treatments resulted in browning of callus and rhizogenesis. Later, a series of MS medium supplemented with varying levels of BAP and KIN, alone and in combination with NAA, were used for callus differentiation. All the treatments showed browning of callus. Rhizogenesis was observed at lower levels of cytokinins.

Addition of activated charcoal prevented browning with out any morphogenesis of callus. Liquid medium supplemented with BAP or 2ip and activated charcoal also failed to show shoot organogeesis.

4.3 *In vitro* mutagenesis

Nodal and shoot tip explants were selected for irradiation *in vitro* using γ-rays at culture establishment stage, callus stage and shoot proliferation stage. The results obtained are given in Tables 32 to 34, Plates 20 to 22.

4.3.1 Effect of γ-irradiation on culture establishment stage

Time taken for shoot multiplication

Non irradiated stem segments took only 12 days for regeneration (Table 32). But, irradiated segments, depending on the dose, took 18-20 days for regeneration. Lower doses (1 Gy) took lesser days for multiple shoot production.

Number of shoots

Number of shoots produced by non-irradiated stem segments was the maximum (15.6 shoots/culture). The irradiated cultures produced lesser number shoots (1.0 to 2.5). Among the different doses of irradiation, 1 Gy recorded

Table 31: Effect of growth regulators on callus derived from leaf segment explants of cordyline

Media	Response
Full MS basal medium	Browning of callus and rhizogenesis
Half MS basal medium	"
Full MS + BAP 2.5 mg l^{-1}	"
Full MS + BAP 5.0 mg l^{-1}	"
Full MS + BAP 7.5 mg l^{-1}	"
Full MS + BAP 10.0 mg l^{-1}	Browning of callus with out rhizogenesis
Full MS + BAP 12.5 mg l^{-1}	"
Full MS + BAP 15.0 mg l^{-1}	"
Full MS + KIN 2.5 mg l^{-1}	Browning of callus and rhizogenesis
Full MS + KIN 5.0 mg l^{-1}	"
Full MS + KIN 7.5 mg l^{-1}	"
Full MS + KIN 10.0 mg l^{-1}	Browning of callus with out rhizogenesis
Full MS + 2ip 5.0 mg l^{-1}	Browning of callus and rhizogenesis
Full MS + 2ip 10.0 mg l^{-1}	Browning of callus with out rhizogenesis
Full MS + 2ip 15.0 mg l^{-1}	"
Full MS + BAP 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}	Browning of callus and rhizogenesis
Full MS + BAP 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}	"
Full MS + BAP 3.0 mg l^{-1} + NAA 0.5 mg l^{-1}	"
Full MS + BAP 4.0 mg l^{-1} + NAA 0.5 mg l^{-1}	"
Full MS + BAP 15.0 mg l^{-1} + activated charcoal 0.1%	No browning, no rhizogenesis, no morphogenesis
Full MS + BAP 15.0 mg l-1 + activated charcoal 0.5%	"
Full MS + BAP 15.0 mg l-1 + activated charcoal 1.0%	"
Liquid MS + activated charcoal 0.5 + BAP 1.0 mg l^{-1}	No browning and rhizogenesis
Liquid MS + activated charcoal 0.5 + BAP 2.0 mg l^{-1}	"
Liquid MS + activated charcoal 0.5 + BAP 3.0 mg l^{-1}	"
Liquid MS + activated charcoal 0.5 + BAP 4.0 mg l^{-1}	"
Liquid MS + activated charcoal 0.5 + 2ip 1.0 mg l^{-1}	"
Liquid MS + activated charcoal 0.5 + 2ip 2.0 mg l^{-1}	"
Liquid MS + activated charcoal 0.5 + 2ip 3.0 mg l^{-1}	"
Liquid MS + activated charcoal 0.5 + 2ip 4.0 mg l^{-1}	"

maximum number of shoots and the lowest number of shoots was minimum (1.0, each) with 15, 20 and 25 Gy (Table 32).

Length of the shoot and number of leaves

Length of the shoot (2.5 cm) and number of leaves (3-4) recorded by plantlets regenerated from cultures irradiated with 1 Gy and 5 Gy were comparable to those in plantlets from non-irradiated cultures (Table 32). Both irradiated and non-irradiated stem segments showed similar effect with reference to number of leaves.

4.3.2 Effect of γ-irradiation on Callus stage

Time taken for callus regeneration

Non irradiated callus took 42.5 days for regeneration. But irradiated callus, depending on the dose, regenerated from 52.5 days to 72.6 days. Lower doses took lesser days for callus regeneration (Table 33), Plates 20-22.

Percentage callus showing regeneration

Percentage callus showing regeneration was the maximum (90.0) in non-irradiated callus, followed by callus irradiated with 1 Gy (60.0 per cent) and 5 Gy (50.0 per cent). Callus irradiated with 10 Gy and 15 Gy, each, showed a regeneration percentage of 30.0.

4.3.3 Effect of γ-irradiation on shoot proliferation stage

As the irradiation dose increased, elongation of the shoots and number of roots decreased drastically. At higher doses (20 and 25 Gy) root formation was totally absent (Table 34).

Table 32: Response of nodal segments to different doses of γ- irradiation in cordyline

Medium MS + BAP 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}

Treatments (Gy)	Time taken for shoot multiplication (days)	No. of shoots/ culture	Length of the longest shoot	No. of leaves	Nature of response*
0.0	12	15.6	2.8	4-5	Green broad leaves
1.0	18	2.5	2.5	3-4	Pale green leaves
5.0	20	2.0	2.5	3-4	leaf tip curling
10.0	20	2.0	1.5	4-5	Narrow yellow leaves with green stripes at centre
15.0	NC	1.0	1.5	3-4	Pale yellow leaves
20.0	NC	1.0	1.0	3-4	"
25.0	NC	1.0	1.0	3-4	"

NC: No change

Table 33: Effect of γ-irradiation on regeneration from callus in cordyline

Treatments (Gy)	Time taken for callus regeneration (days)	Percentage callus showing regeneration	Nature of response*
0.0	42.5	90.0	Friable callus showed good regeneration
1.0	52.5	60.0	Callus proliferation, no regeneration
5.0	68.6	50.0	"
10.0	70.5	30.0	Less callus growth, no regeneration
15.0	72.6	30.0	"
20.0	-	0.0	Remained creamy white no change
25.0	-	0.0	"

Table 34: Effect of γ-irradiation on multiple shoot elongation in cordyline

Treatments (Gy)	Nature of response*
0.0	Normal long shoots and roots were observed
1.0	Medium shoots and roots were observed
5.0	"
10.0	Medium shoots and less number of roots
15.0	"
20.0	Length of shoot decreased, no root formation
25.0	"

The response mentioned was exhibited only in 10 – 30 per cent of the cultures

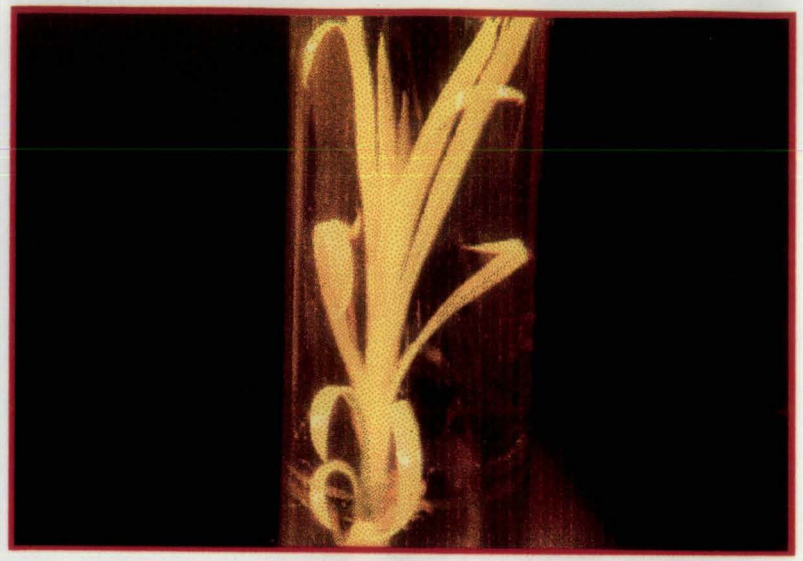


Plate. 20. Plant showing narrow leaves after irradiation

Plate. 21. Plant showing colour variation after irradiation

Plate. 22. Effect of γ irradiation on multiple shoots

Discussion

DISCUSSION

The present study on micropropagation and crop improvement of cordyline (*Cordyline terminalis* (L.) Kunth) was carried out at the plant tissue culture laboratory of the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara from 1998 to 2000.

Cordyline (*Cordyline terminalis* (L.) Kunth) is an attractive foliage plant belonging to the family Agavaceae which is widely used as an indoor plant. Propagation of cordyline is usually carried out through seeds, stem sections and suckers. Through this only restricted number of plants can be produced each year as large amount of planting material is required. Being vegetatively propagated the spectrum of variability is less and we cannot avoid the transmission of systemic microorganisms present in the plant. These problems necessitated an alternative method of propagation which helps in bye-passing season barrier, bulking up of new cultivars of disease free stocks and production of sufficient materials in a short period for field planting.

Micropropagation has become one of the important methods for propagation of ornamental plants for domestic as well as export market and to overcome the above problems in recent years (Bharathi, 2000). As the *in vitro* propagation methods were standardized for many of the foliage plants, investigations on these line also were started in cordyline by various workers (Kunisaki, 1975; Mee, 1978; Welander, 1988; Hvoslef-Eide, 1990 and 1993). But the reported works are scanty and relates to other members of the family Agavaceae. The present investigations have thus been carried out to find out the most ideal explant, medium, medium supplements, hardening techniques, dose of nutrient and dose of irradiation for cordyline. The results generated from the studies are discussed in this chapter.

5.1 Explants

Selection of proper explant is an important aspect of commercial tissue culture production as it amounts to the cost of production. Selection of a suitable explant, which will yield true to type plant and at the same time will not increase the cost considerably is considered ideal.

Of the various explants tried, shoot tips and nodal segments were found to be ideal. This was in conformity with the results of the studies by Kunisaki, 1975; Paek *et al.*, 1985; Mee, 1978; Podwyszynska and Olszewski, 1995 and Alla *et al.*, 1996 a. Considering the number of explants available and the cost involved preference can be given to nodal segments.

5.1.1. Standardisation of surface sterilization methods

The explants collected from the field harbour a variety of microorganisms which have to be removed before inoculation onto the culture medium. Though general sterilization procedures have been outlined by various workers (Dodds and Roberts, 1982; George and Sherrington, 1984), specific sterilization procedures have been evolved based on the tissues being handled. Hence, in the present study, the sterilization procedure with respect to the sterilant, its concentration and duration of exposure was standardized.

Among the different treatments tried, mercuric chloride (0.1 per cent) was found to be ideal. Though in literature use of sodium hypchlorite is more common in the surface sterilization of foliage plants like *Cordyline* (Kunisaki, 1975), *Saintpaulia* (Start and Cumming, 1976; Cooke, 1977), *Diffenbachia* (Voyiatzi and Voyiatzis, 1989) and *Agave* (Ruvalcaba *et al.*, 1999), in the present investigations it was found to be less efficient than mercuric chloride at the levels tried. The best treatment for nodal segments, axillary buds and shoot tips was dipping in 0.2 per cent carbendazim (50% WP) for 10 min followed by 70 per cent ethyl alcohol wiping and thereafter a dip in 0.1 per cent mercuric chloride for 18 min. Leaf

segments gave 100 per cent survival when surface sterilized with 0.1 per cent mercuric chloride for 10 min.

Mercuric chloride at 0.1 per cent has been effectively used as a surface sterilant in foliage plants by various workers (Binh *et al.* (1990) in agave, Das (1992) in *Agave sisalana* and Jasrai *et al.* (1999) in *Passiflora*). The results obtained in the present study agree with these reports in respect of the effectiveness of mercuric chloride as surface sterilant. It is advantageous cost wise also.

5.1.2 Seasonal influence on the *in vitro* establishment of explants

The percentage of contamination observed for the explants collected in the different months showed variation. The explants collected from November-March showed lower contamination as compared to those in other months. In Kerala the relative humidity is usually high, which provides a congenial condition for the growth and development of microorganisms. The situation is aggravated during the rainy months, which is evident from the meteorological data presented (Appendix – II).

Kukulczanka *et al.* (1977) also reported highest regeneration potential of leaves of *Peperomia scandens* taken from mother plants in summer, with less contamination.

5.2 The routes

5.2.1 Enhanced release of axillary buds

5.2.1.1. Culture establishment

Enhanced release of axillary buds is a common route followed for mass multiplication of several ornamental plants. Establishment of the cultures is the first and the most important *in vitro* step, which is influenced by several factors, the most important being the type of the basal media and growth substances like

cytokinins and auxins. The influence of cytokinins on culture establishment of nodal segments and shoot tips has been reported in various foliage plants like philodendron, Brassaia and Asplenium (Benezur and Riffer, 1990; Badzian *et al.*, 1989 and Higuchi and Amaki, 1989). However, the type of cytokinin and its combination with auxins, is often seen to influence culture establishment. In the present study, the effect of BAP and KIN alone and in combination with NAA on culture establishment of shoot tips and nodal segments was explored. Maximum percentage of establishment of nodal segments and shoot tips was observed in MS medium supplemented with BAP. Precocity in bud emergence was observed when BAP at 2.0 mg l^{-1} was incorporated in the medium. This resulted in the production of more number of shoots. This was in conformity with the results obtained by Evaldsson and Welander (1985).

BAP was superior with respect to the early bud break and number of shoots in case of nodal segments, compared to KIN. BAP was found to be the best for culture establishment in the case of *Syngonium* (Kozak and Dabski, 1995), *Philodendron* (Benezur and Riffer, 1990) and *Cordyline* (Beruto *et al.*, 1985) also.

In case of shoot tip explants, early bud break and more number of shoots were obtained in MS medium supplemented with KIN. A similar response has been reported by Paek *et al.* (1985). In the present study, KIN in combination with NAA was superior with respect to early shoot elongation as compared to BAP in combination with NAA. In *Yucca elephantipes* also best shoot growth of green and variegated cultivars was obtained on medium supplemented with KIN + NAA (Sakr *et al.*, 1999).

BAP in combination with NAA also gave good results in culture establishment of nodal segments and shoot tips. The favourable effect of BA + NAA on culture establishment has been reported in *Philodendron* (Zeping *et al.*, 1998), *Cyclamen* (Hawks and Wainwright, 1987) and *Aglaonema* (Maity *et al.*, 1994).

Increase in the concentration of NAA, however, could not further increase the number of shoots, but stimulated callus formation. Primarily this may be due to the supra optimal level of auxin, which usually discourages axillary bud formation resulting in formation of undifferentiated mass of tissues. The ability of NAA at higher levels to stimulate callus formation is also reported by Kunisaki (1975), Razdan (1993) and Alla *et al.*, (1996a).

In most of the reports on foliage plants, the medium used was Murashige and Skoog (1962) medium. In the present study also, for culture of nodal segments and shoot tip explants, MS medium was found to be better than SH and WPM with respect to early release of buds. MS medium has been reported to be the most suitable medium for *Dracaena, Philodendron, Begonia, Peperomia, Diffenbachia, Yucca* and *Agave* etc. (Maity *et al.*, 1994)

Nikam (1997) reported that optimum number of shoots was obtained from stem and rhizome explants of *Agave* in MS, SH, Gamborg and White's medium. The present study is also in conformity with this observation, where the number of shoots produced in Stage I did not differ significantly among the media. The advantage of MS medium is reported to be due to the desired level of nutrients, which helped in hastening the release of buds. Probably the number of shoots produced is controlled by the growth substances rather than the basal media.

5.2.1.2 Shoot proliferation

In the present investigation when the elongated buds from Stage I were inoculated to MS medium containing different levels of cytokinins (BAP, KIN, 2ip) and their combination with different levels of NAA, production of multiple axillary buds and callus was observed. The treatments having lower levels of BAP and other combinations with lower levels of NAA produced high rate of multiple axillary buds, with out any callus production. As the concentration of BAP and NAA increased, callus production was also increased with gradual

reduction in the rate of multiple axillary bud production. The results are in line with the findings of Welander (1988). The probable reasons for this are already stated.

It was also found that the rate of axillary bud production and callusing was lesser when KIN was used in combination with NAA. Medium rate of multiple axillary bud production was observed with KIN 3.0 mg l^{-1}. Murali *et al.* (1999) stimulated multiple shoot formation in cordyline when MS medium was fortified with KIN. Addition of NAA at higher levels with lower levels of KIN resulted in callusing and rhizogenesis. Sakr *et al.* (1999) also observed rhizogenesis when combinations of lower levels of KIN and higher levels of NAA were used in the media.

High rate of multiple axillary bud production was recorded in the medium with 2ip 2.0 mg l^{-1} in combination with NAA 0.5 mg l^{-1}. Low rate of callus production was observed at higher concentrations of 2ip. Callus production and rhizogenesis were also observed when auxins were incorporated along with 2ip. In *Cyclamen persicum,* higher concentration of 2ip either produced low amounts of callus or generated adventitious roots as well as shoots as reported by Bach *et al.* (1998).

Alla *et al.* (1996 a) reported that the number of multiple shoots per stem cutting explant was significantly greater with 0.5-4.0 mg l^{-1} BAP than with the same concentration of KIN or 2ip, in *Cordyline terminalis.* The present study confirms this observations. The difference in the effectiveness of three cytokinins, namely, BAP, KIN and 2ip is clearly evident from the result of the present study and from the reports of the other scientists. The response is more pronounced based on the relative concentration of NAA.

Of the three media tried, very high rate of axillary bud production within short time was observed in full strength MS medium. Similar results have been obtained in pineapple (Prabha, 1993). The complementary interaction of the

ingredients of MS medium with that of the growth substances might be responsible for the superiority of MS medium.

Investigations were carried out on the elongation of the bud aggregates using MS basal medium with different levels of IAA. Both full strength and half strength MS medium, without the growth regulators, induced elongation of shoots and production of normal roots. Full or half strength concentration of MS macronutrients resulted in increased shoot length in cordyline (Welander, 1988). In case of Begonia MS medium with a lower concentration of BAP resulted in enhanced shoot growth (Simmonds, 1984).

The bud aggregates used in the present study were derived from culture, where BAP and NAA were used in the medium. It is possible that the level of growth substances contained in the bud aggregates are lowered in the basal medium (MS full strength or half strength devoid of IAA). On the other hand the cytokinins contained in the bud aggregates might have nullified the effect of IAA contained in the other treatments.

Media supplements like coconut water, activated charcoal, adenine sulphate and casein hydrolysate were used for the elongation of bud aggregates. MS medium with out any additives induced elongation of shoots with in a short time and also resulted in induction of increased number of roots and shoots.

Addition of activated charcoal (0.5 and 1.0 per cent) to the medium resulted in early induction of roots, but number of shoots and length of shoots reduced drastically. Beneficial effect of activated charcoal are also reported in *Drosera spathulata,* where MS medium supplemented with activated charcoal + 5 per cent coconut milk resulted in bud formation and plantlet regeneration (Blehova *et al.*, 1990).

Adenine sulphate, coconut water and casein hydrolysate, at all the concentrations, delayed shoot elongation and root initiation. The increase in

number of shoots and length of shoots was less in the presence of media supplements, compared to basal medium devoid of additives. The response to rooting, when coconut water was used as the media supplement could be due to the effect of cytokinin, which it contains. This is in agreement with the statement of Yeoma (1986) who opined that all cytokinins inhibit root induction and that rooting of plants originating from cytokinin rich medium is delayed even if they are transferred to cytokinin free media. Among the different supplements tried, length of shoots was highest with casein hydrolysate and number of shoots was highest with 10 per cent coconut water. The beneficial effect of coconut water has been attributed to the presence of cytokinin like substances (Straus and Rodney, 1960). Mee (1978) also successfully used coconut water for organogenesis of callus in cordyline.

5.2.1.3 *In viro* rooting

Rooting of the micro shoots is an important stage in micropropagation. Earliness of induction of roots and morphological characters of the roots are of primary concern in *in vitro* rooting. Various studies conducted earlier on this aspect indicate that relative concentration of auxins and cytokinins, source of auxin, salt level, environmental factors, especially light etc contribute to *in vitro* rooting. Sriskandarajahi and Skirvin (1991) stated that root development in *Philodendron* was significantly depressed when the NH_4NO_3 content of the medium was increased from 1650 mg l^{-1} to 3300 mg l^{-1}. Macronutrient salts of MS inhibited lateral root formation in 95 per cent of preliminary adventitious roots of *Dracaena fragrans*. This root growth inhibition occurred as a result of inadequate balance of SO_4^{2-} with NO_3^- could be overcome by decreasing the concentration of all 5 macronutrient salts (Vinterhalter and Vinterhalter, 1992).

In the present study, the experiments were conducted on the elongated shoots of Stage 2 using different media (MS, SH and WPM). Earlier induction of more number of lengthy roots was observed in full strength MS basal medium.

This is in line with the findings of Binh *et al.* (1990), Welander (1988) and Sakr *et al.* (1999).

Morphological characters of the roots produced differed among the treatments tried. Roots induced in MS medium supplemented with IAA were slender and white in colour while those in medium supplemented with NAA were thick, short and creamish in colour. Besides, NAA in the medium induced callus formation at the base before root induction. The presence of callus on plantlets rooted on media with NAA was also reported by Kunisaki (1975). Roots produced in MS medium with IBA were thick and white with rootlets.

The studies indicate the relative advantage of IBA with respect to the quality of roots produced as compared to IAA and NAA. Various other workers have also obtained *in vitro* rooting in foliage plants using IBA. Beruto *et al.* (1985) reported the effectiveness of IBA on root induction of *in vitro* produced plants of *Cordyline*. In *Yucca* efficient rooting could be obtained in media supplemented with 1.0 mg l^{-1} IBA. Paek *et al.* (1985) reported that 3.0 mg l^{-1} IBA, if incorporated in MS medium promoted root growth in *Cordyline terminalis*. In the present study also, IBA at 3.0 mg l^{-1} induced rooting successfully.

Addition of activated charcoal to the basal MS medium resulted in early induction of roots, but number and length of roots were reduced drastically. Roots were slender, short and white in nature in presence of activated charcoal. Maene and Debergh (1985) reported that addition of activated charcoal to liquid Stage IIIa (bud elongation and preparation of rooting) medium eliminated the residual effect of BA applied during Stage II (bud induction and multiplication). This may be the reason for early induction of roots in the presence of activated charcoal in the present study.

Triazoles are generally growth inhibitors, the different forms of which are used in the commercial production of horticultural crops. Triadimefon, a

commonly used form of triazole in the hardening of tissue cultured plants, is also tried in the rooting of *in vitro* plants. In banana the positive effect of triadimefon at 1.0 mg l^{-1} on rooting has been reported by Murali and Duncan (1995). However, such works in ornamental foliage plants are not reported. In the present study different levels of triadimefon in combination with various levels of IBA were used in rooting media. Lower levels of IBA in presence of triadimefon resulted in callus formation without root formation. Higher levels of triadimefon (3.0 mg l^{-1} and 4.0 mg l^{-1}) resulted in the production of very short roots. Triadimefon concentration at 1.0 mg l^{-1} along with IBA resulted in more number of lengthy roots.

5.2.1.4 Hardening and acclimatization

The successes of tissue culture depends on the establishment of *in vitro* produced plants in natural condition. Under *in vitro* conditions the plants will be heterotrophs. During the first days after transplanting micropropagated plants to green house conditions, *in vitro* leaves are the only source to cover metabolic demands and to sustain the plants adaptation and regrowth (Huylenbroeck *et al.*, 1998). For this a period of humidity acclimatization is considered necessary.

Bhaskar (1991) and Balachandran (1993) have modified the techniques for hardening of *in vitro* banana plants. Better results could be obtained when the roots of the plantlets were dipped in 0.2 per cent carbendazim (50% WP) for 5 min and the plantlets covered with microscope cover after planting.

In the present investigation, maximum survival percentage of plantlets was obtained in the case of plantlets treated with triadimefon at 1.0 mg l^{-1} in rooting medium followed by 0.2 per cent carbendazim (50% WP) soon after removal from the culture vessels at the time of planting and subsequently giving a post planting treatment with 20 mg l^{-1} triadimefon drenching at weekly interval and then keeping the plantlets in net house. Under these conditions, up to 60.0 per cent survival was observed in the case of plantlets planted in peat + sand in mud pot.

Oliphant (1990) reported that application of paclobutrazol to the rooting medium can eliminate the need for a period of acclimatization before transfer. Hagiladi and Watad (1992) opined that the drenching of paclobutrazol was more effective than foliar spray. In the present study also soil drenching with triadimefon increased the survival percentage.

Survival of *in vitro* plants after planting out is largely dependent on the components of the media. Hence, standardisation of proper combination of media components assumes great importance. General considerations given for selecting a medium is the physical character, which determines the drainage and water holding capacity. Sterile nature and light weight are other important considerations. Accordingly coarse sand, vermiculite, peat based media etc are popular as planting out materials. According Kunisaki (1975) rooted plantlets of cordyline when transplanted into vermiculite and kept under 50 per cent light intensity in the green house resulted in more than 99 per cent survival.

Welander (1988) transferred *in vitro* rooted plantlets of *Cordyline* successfully to a peat based compost. Sakr *et al.* (1999) obtained increased plant height and number of leaves per plant in the green cultivar of *Yucca* in peat moss + sand (1:1 v/v) under green house conditions.

Another important consideration of the media is the nutrient composition. Usually it becomes necessary to apply nutrients in a balanced form, either as soil drench or as foliar spray. Apples Diaz (1984) reported that application of slow release fertilizers considerably increased shoot production in *Cordyline terminalis*. In the present study 0.75g/plant/week NPK (17: 17: 17) produced tallest plants, compared to other treatments. Number of leaves, leaf length and leaf breadth did not differ significantly among various treatments. Alla *et al.* (1996a) and Ding *et al.* (1997) have also reported tallest plant production with a 15: 15: 15 and 16: 16: 16 NPK formulation, respectively, in the case of *Cordyline*.

5.2.2 Organogenesis

5.2.2.1 Explant choice

Among the various explants tried for organogenesis nodal segments and shoot tips were found to be ideal with respect to callus initiation, growth and callus differentiation. Maity *et al.* (1994) also stated that apical meristems and stem segments were ideal explants for callus induction and differentiation. The suitability of leaf segments was limited to callus initiation only.

Mee (1978) used shoot apices as suitable explants for callus induction and regeneration in *Cordyline*. Kobza and Vachunova (1989 and 1991) also obtained best callus induction and regeneration in *Dracaena* when stem explants were used. Callus formation from stem internodal pieces of *in vitro* derived shoots of *Azalea* was better in light (Economou *et al.*, 1988) as in the present study. Narayan and Jaiswal (1986) could induce callus cultures from leaf discs of *Ficus religiosa.* Callus from leaf stalks produced plantlets on MS medium containing KIN and NAA in *Saintpaulia ionantha* (Chen *et al.*, 1987).

Effect of maturity of leaf

Though mature leaf discs can be successfully used to induce callus in most of the foliage plants like *Ficus* (Narayan and Jaiswal 1986) and *Saintpulia ionantha* (Redway, 1991), in the present study immature leaves took less number of days for callus production and showed highest percentage of callusing and callus index.

Effect of portion of leaf

Of the different portions of the leaf tried, basal portion showed highest callusing percentage and intensity. Kukulczanka *et al.* (1977) also reported that

the basal part of the leaf had the highest regeneration capability, which is probably due to the basipetal displacement of endogenous auxins.

Callus tissues were formed from adaxial and abaxial epidermis, as well as from the palisade and spongy mesophyll cells in *Saintpaulia ionantha* (Redway, 1991). Lo (1997) cultured leaf discs of *Saintpaulia* successfully by placing upside down (abaxial surface to air). This is in conformity with the results of present study, where adaxial surface facing medium induced 100 per cent callusing.

5.2.2.2 Direct organogenesis

Shoot tips, axillary buds and nodal segments were used as explants with different levels of IAA, BAP, KIN and 2ip. Direct morphogenesis was not observed in any of the treatments.

Direct rhizogenesis was observed in many treatments, the highest being in the MS medium having 2.0 mg l^{-1} IAA. In the case of cytokinins, 2ip showed maximum rhizogenesis at higher concentrations, followed by BAP. Minimum rhizogenesis was observed in the presence of KIN.

5.2.2.3 Callus mediated organogenesis

5.2.2.3.1 Nodal segments and shoot tips

Callus initiation

Shoot tips and nodal segments produced highest percentage of callus in the MS medium supplemented with lower levels of 2,4-D and BAP, both in 12h photoperiod, within a short period. Similar results were obtained by Meshkova *et al.* (1999), where lower concentrations of 2,4-D and BAP induced callus in *Cordyline*. Kobza and Vachunova (1991) could proliferate callus from stem explants of *Dracaena* on MS medium supplemented with 2,4-D 0.5 mg l^{-1}. In the present study coconut water at 10 per cent concentration gave highest callus

intensity and callus index, compared to other concentrations. Mee (1978) also obtained callus on cut surface of shoot apices of *Cordyline terminalis* in MS medium supplemented with 10 per cent by volume coconut water and 2,4-D 3.0 mg l^{-1}.

Callus differentiation

In the present study, MS medium supplemented with BAP (1.0 mg l^{-1}) and KIN (2.0 mg l^{-1}) showed early differentiation of callus into shoot buds, compared to other treatments. Higher levels of BAP (1.0 and 2.0 mg l^{-1}) in combination with KIN in the media could also induce callus differentiation. Similar results have been obtained in *Agave arizonica,* where higher levels of BAP resulted in shoot proliferation (Powers and Backhaus, 1989).

Maity *et al.* (1994) could also differentiate callus derived from stem segments by transferring the callus to MS basal nutrient liquid medium supplemented with 2,4-D or NAA (0.01 mg l^{-1} – 1.0 mg l^{-1}) and BAP (10 mg l^{-1} – 20 mg l^{-1}).

Different levels of 2ip in combination with BAP also showed differentiation of callus, but the rate of shoot production was medium. The number of shoots was greater in the medium having BAP + KIN compared to that having BAP + 2ip. Rhizogenesis was observed on medium fortified with 2ip.

In the present study, the callus was maintained by subsequent subcultures at monthly intervals on medium without any auxins. Similar results were reported by Mee (1978). He stated that callus growth was maintained by transferring callus at monthly intervals two to three times on basal medium and then allowing to differentiate on the medium without any auxins. He used 10 per cent coconut water for differentiation of callus, but in the present study coconut water failed to induce differentiation.

5.2.2.3.2 Leaf segments

Callus initiation

Leaf segments taken from immature leaves callused and gave maximum callus index of 350 when cultured on the medium with 2,4-D 1.0 mg l^{-1} + NAA 5.0 mg l^{-1} followed by NAA 10 mg l^{-1} (300). Presence of 2,4-D in the medium induced callusing of leaf segments in ficus (Narayan and Jaiswal, 1986) and Boston fern (Byrne and Caponetti, 1992).

It was observed that callus derived from the leaf segments failed to differentiate into shoots. The treatments tried showed rhizogenesis and browning of callus. Addition of activated charcoal prevented browning, but could not show any morphogenesis. This may be due to removal of toxic compounds (e.g., phenols) produced during the culture and adsorption of phytohormones (Razdan, 1993) to activated charcoal. Higher concentration of cytokinins reduced rhizogenesis.

Leaf explants showed no response in culture in the case of *Dracaena deremensis* (Deberg, 1975) and *Agave sialana* (Das, 1992).

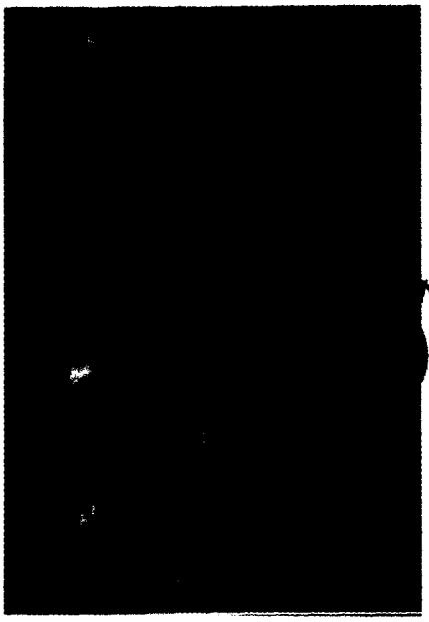
5.3 *In vitro* mutagenesis

To know the optimum dose and correct stage of irradiation nodal segments and shoot tip explants were subjected to different doses of γ-irradiation at culture establishment stage, callus stage and shoot proliferation stage.

Irradiated shoots took more number of days for shoot multiplication. The number of shoots and length of the shoots were also less. A γ-irradiation dose of 10 Gy produced plantlets having narrow yellow leaves with green line at the centre. After planting out the plantlets reverted to normal behaviour and did not show any variation. This could be because the effect of irradiation did not reach

all the cells and that after the changes initially exhibited by the affected cells, the unaffected cells took over.

Irradiated callus showed delayed regeneration. Percentage callus showing regeneration was also reduced. As the irradiation dose increased, elongation of the shoots and number of roots decreased drastically. Culture establishment stage and callus stage could resist slightly higher doses of irradiation compared to shoot multiplication stage, probably due to the tenderness of shoots at this stage.



SUMMARY

SUMMARY

Investigations on micropropagation and crop improvement of cordyline were carried out in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during 1998-2000. The main objectives were to study the response of various explants of cordyline and to identify the most suitable explant and media combination for *in vitro* propagation. Attempts were also made to find out the optimum dose of γ - irradiation, for inducing variation. The results and salient findings are summarized hereunder.

The response of various explants of cordyline was attempted through enhanced release of axillary buds and organogenesis. The shoot tips and nodal segments were found to be ideal explants for the enhanced release of axillary buds and for indirect organogenesis.

The effect of the surface sterilization treatments varied with respect to the source of explant. The best sterilization treatment was found to be 70 per cent ethyl alcohol wiping, followed by a dip in 0.1 per cent mercuric chloride for 18 min. for shoot tips, nodal segments and axillary buds. For leaf segments, treatment with 0.1 per cent mercuric chloride for ten minutes alone was found to be enough.

The percentage of contamination observed for the explants collected in different months showed variation. The nodal segment explants collected in January, March, November and December showed no contamination, while those collected during the months of June and July showed the highest contamination rate of 100 per cent. Survival percentage was maximum in the months of November and December. In the case of shoot tip explants least contamination was noticed during the period from August to April.

Studies on the culture establishment of nodal segments and shoot tips using various levels of growth regulators indicated that early release of buds (4.4

days after inoculation) and further growth of buds was good in MS medium supplemented with BAP 3.0 mg l^{-1}. Maximum shoot proliferation was observed in MS medium with 2.0 mg l^{-1} BAP.

In the case of shoot tips MS medium having KIN 3.0 mg l^{-1} showed the least time (5.5 days) for the bud emergence. Maximum number of shoots (2.5) was also obtained in the same medium.

MS medium supplemented with BAP in combination with NAA also proved better for culture establishment of nodal segments and shoot tips. Maximum number of shoots was observed in MS medium with NAA 0.5 mg l^{-1} + BAP 1.0 mg l^{-1}. Presence of KIN in combination with NAA in the medium was found to be superior with respect to early shoot elongation.

Of the three different media tried, MS medium was found to be effective for early release of buds in nodal segments and shoot tips. Number of shoots produced did not differ significantly among media.

The elongated buds from Stage 1 were inoculated to MS medium containing different levels of cytokinins (BAP, KIN, 2ip) alone, and in combination with different levels of NAA to know the best medium for shoot proliferation. Very high rate of multiple axillary bud production was observed in MS medium with BAP 1.0 mg l^{-1} + NAA 0.5 mg l^{-1} and BAP 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}.

Some of the combinations also produced callus. High rate of callus production was in MS medium having 1.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA and 2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA. The rate of axillary bud production and callusing was less with KIN in combination with NAA.

Irrespective of the media, rhizogenesis was observed when higher levels of 2ip were incorporated in the media. Of the three cytokinins used (BAP, KIN

and 2ip) BAP was found to be superior with respect to multiple axillary bud production.

Among MS, SH and WPM media, full strength MS medium gave very high rate of multiple axillary bud production within a short time (12.2 days).

Investigations were carried out to study the elongation of bud aggregates using MS basal medium, either with different levels of IAA or with media supplements. Elongation of the multiple axillary buds with normal shoot growth and root growth was recorded in full strength MS medium devoid of growth regulators. None of the media supplements (activated charcoal, adenine sulphate, coconut water and casein hydrolysate) was effective for multiple axillary bud elongation.

Elongated shoots were transferred to Stage 3 media for induction of rooting. Among the three different basal media tried, more number of lengthy roots was observed in full strength MS medium. The number of days taken for root initiation was also less in full strength MS basal medium.

It was observed that among the different auxins tried, IBA was superior for root induction. Callus formation was observed at the base when NAA was incorporated in the media. Morphological characters of the roots produced also differed with different auxins. Slender and white roots were produced in IAA while NAA gave thick, short and creamish roots. Use of IBA resulted in thick white roots with rootlets.

More percentage of rooting, early rooting and more number of lengthy roots could be obtained in full strength MS basal medium than in any other media tried for *in vitro* rooting.

Addition of activated charcoal in the medium did not show any positive response of root number and root length, but it reduced the number of days taken for root initiation.

Inclusion of triadimefon into the rooting medium slightly increased the number of days taken for root initiation and decreased the number of roots/culture and length of roots. It also enhanced the survival percentage during hardening. Higher concentration of triadimefon inhibited the root formation and growth.

Maximum survival percentage of the plantlets was obtained, when the plantlets rooted in the medium containing triadimefon 1.0 mg l^{-1} + IBA 5.0 mg l^{-1} were treated with 0.1 per cent Bavistin for 30 minutes soon after removal from the culture vessels and subjected to a post planting treatment with triadimefon (20 mg l^{-1}) drenching at weekly intervals and kept in a shade house. Sixty per cent survival of plantlets was obtained under this condition when planted in mud pots containing peat + sand and watered at two days interval.

Nutrient application significantly influenced the plant height of tissue cultured plantlets. Tallest plants were produced when the plantlets were supplied with 0.75 g 17:17:17 NPK mixture per week as soil drench after planting out. But application of NPK did not show any significant influence on leaf number, leaf length and leaf width.

Nodal segments, shoot tips, axillary buds and leaf segments were tried as explants for organogenesis. Nodal segments and shoot tips were found to be the most ideal explants for callus initiation, growth and differentiation. Leaf segments also showed callusing.

Age of the explant was found to influence the intensity of callus in case of leaf segments. Callus intensity was maximum when bottom portion of the immature leaves with midrib were cultured. The orientation of leaf segment was

also found to influence the percentage callusing and callus intensity, the best response being with their adaxial surface touching the medium.

Direct organogenesis could not be obtained in different treatment combinations tried with different explants.

Shoot tips and nodal segments produced higher percentage of callus and showed maximum callus index when MS medium was supplemented with lower levels of 2,4-D (0.5 mg l^{-1}) and BAP (0.5 mg l^{-1}), both in 12 h photoperiod.

No positive response was observed with the addition of coconut water on callus induction and differentiation, but 10 per cent concentration of coconut water gave maximum callus intensity, compared to other concentrations tried.

Differentiation of callus derived from nodal segment and shoot tip explants was made possible in MS medium supplemented with BAP and KIN or BAP and 2ip. Earliest differentiation of callus was possible in MS medium supplemented with BAP 1.0 mg l^{-1} + KIN 2.0 mg l^{-1} (42.5 days).

Very high rate of shoot production was observed in the medium supplemented with BAP 1.0 mg l^{-1} + KIN 2.0 mg l^{-1}. A combination of BAP + KIN gave high rate of shoot production, compared to BAP + 2ip combination.

Various treatment combinations failed to induce morphogenesis in leaf derived callus. Additon of activated charcoal prevented browning of callus upon subculturing. Higher concentration of cytokinins prevented rhizogenesis.

In vitro mutagenesis was attempted to know the optimum dose and correct stage for induction of variation. Of all the doses of γ-irradiation, a dose of 10 Gy at culture establishment stage produced plants having narrow yellow leaves with green line at the centre, but after planting out the plantlets reverted to normal characters. The safest doses of irradiation at culture establishment stage,

callus stage and multiple shoot proliferation stage were 25 Gy, 25 Gy and 15 Gy, respectively.

The protocol for mass multiplication of cordyline as derived from the present study is given below

Nodal segments (1-5 nodes from apex of shoots)

↓

Sterilise with 0.1 per cent mercuric chloride for 18 minutes

↓

Inoculate onto MS medium + BAP 2.0 mg l^{-1}

↓

Subculture onto MS medium + BAP 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}

↓

Transfer to MS basal medium for elongation and rooting

↓

Planting out (in sand + peat medium, under 50 per cent shade)


References

REFERENCES

Alla, A.H. and Staden, J.V. 1997. Micropropagation and establishment of *Yucca aloifolia*. *Pl. Cell Tissue Organ Cult.* **48**: 209-212

Alla, A.H. and Van, S.J. 1996. Somatic embryogenesis from leaves of *Yucca aloifolia* L. *J. Southern Afr. Soc. hort. Sci.* **6** (1): 4-7

Alla, A.H., Zaghloul, M.A., Waly, A.K. and Khattab, S.H. 1996a. Micro-propagation of some ornamental plants. 1. *In vitro* culture, establishment and effect of NPK fertilization on *ex vitro* of *Cordyline terminalis* cv. Atom. *Ann. agric. Sci.* **34** (2): 691-707

Alla, A.H., Zaghloul, M.A., Waly, A.K. and Khattab, S.H. 1996b. Micro-propagation of some ornamental plants. 3. *In vitro* culture and establishment of *Dracaena marginata* var. Tricolor. *Ann. agric. Sci.* **34** (3): 1153-1162

Anand, M. and Bir, S.S. 1984. Organogenetic differentiation in tissue culture of *Dalbergia lanceolaria*. *Current Sci.* **53**: 1305-1307

Ancora, G., Belli-Donini, M.L. and Cuozzo, L. 1981. Globe artichoke plants obtained from shoot apices through rapid *in vitro* micropropagation. *Scientia Hort.* **14**: 207-213

Apples Diaz, M. 1984. Utilization of slow-release nitrogen fertilizers for pot plants. *Comunicaciones de la III reunion de ornamentales.* Jornadas tecnicas, Almeria, Spain, pp. 96-101

Bach, A., Malik, M. and Zolneczko, B. 1998. organogenesis and somatic embryogenesis in cultures of *Cyclamen persicum* Mill. F_1 'medium'. *Acta Biologica Cracoviensia Series Botanica* **40**: 47-51

Badzian, T., Kern, F.J., Hennen, G.R. and Oglesby, R.P. 1989. The influence of 6-benzyladenine and thidiazuron on organogenesis of *Brassaia actinophylla* Endl. cv. Amate shoot explants. *Acta Hort.*(251): 191-197

Balachandran, M. 1993. Induction of genetic variability in *Musa* sp. var. Nendran by *in vitro* methods. M.Sc. (Hort.) thesis, Kerala Agricultural University, Vellanikkara, Kerala.

Beckett, K.A. 1987. *The RHS Encyclopedia of House Plants Including Green House Plants*. Swallow Editions Ltd., London, p.175

Benezur, J.E. and Riffer, M.A. 1990. *In vitro* propagation of *Philodendron tuxlanum* Bunting with benzylaminopurine. *Acta Agronomica Hungarica* **39** (3-4): 341-348

Beruto, M. Curir, P., Lercari, G.B. and Giusta, R. 1985. The *in vitro* propagation of *Cordyline terminalis* L. "Calypso". *Annali dell Instito sperimentale per la floricoltura* **14** (1): 37-46

Bharathi, N. 2000. Problems and prospects of micropropagation in horticulture crops. National seminar on Hi-tech Horticulture. National Academy of Agricultural Sciences and Indian Institute of Horticultural Research, Bangalore. *Abstract of papers.* p. 20

Bhaskar, J. 1991. Standardisation of *in vitro* propagation techniques in banana. M.Sc. (Hort.) thesis, Kerala Agricultural University, Vellanikkara, Kerala.

Bhojwani, S.S. and Razdan, M.K. 1983. *Plant Tissue Culture : Theory and Practice*. Elsevier, New York, p. 502

Binh, L.T., Muoi, L.T., Oanh, H.T.K., Thang, T.D. and Phong, D.T. 1990. Rapid propagation of agave by *in vitro* tissue culture. *Pl. Cell Tissue Organ Cult.* **23** (1): 67-70

Blehova, A., Somsakova, V. and Bobak, M. 1990. Anatomical studies of the development of new plants from the leaves of the sundew (*Drosera spathulata* L.) in *in vitro* conditions. *Physiol. Plant.* (26): 33-42

Byrne, T.E. and Caponetti, J.D. 1992. Morphogenesis in three cultivars of Boston fern II. Callus production from stolon tips and plantlet differentiation from callus. *Am. Fern J.* **82** (1): 1-11

Chen, Y.C., Lu, X.H. and Lin, H.Z. 1987. The effect of some growth regulators on pathways of morphogenesis in a somatic culture of *Saintpaulia ionantha*. *Acta Horticulturae Sinica* **14** (1): 57-61

Cooke, R.C. 1977. Tissue culture propagation of african violets. *Hort Sci.* **12** (6): 549

Das,T. 1992. Micropropagation of *Agave sisalana. Pl. Cell Tissue Organ Cult.* **31** (3): 253-255

Deberg, P. 1975. Intensified vegetative multiplication of *Dracaena deremensis. Acta Hort.* **54**: 83-92

Debergh, P.C. and Maene, L.J. 1981. A scheme for commercial propagation of ornamental plants by tissue culture. *Scientia Hort.* **14**: 335-345

Ding, M., Han, Y.X. and Yan, Z. 1997. The proportional fertilization in pot *Cordyline fruticosa* and *Rosa chinensis. Acta Horticulturae Sinica* **24** (1): 71-74

Dodds, J.H. and Roberts, L.W. 1982. *Experiments in Plant Tissue Culture.* Cambridge University Press, London, p. 28

Economou, A.S., Read, P.E. and Spanoudaki, M.J. 1988. Azalea regeneration from callus culture. *Acta Hort.* **I** (226): 209-216

Evaldsson, I.E. and Welander, N.T. 1985. The effects of medium compositon on *in vitro* propagation and *in vivo* growth of *Cordyline terminalis* cv. Atom. *J. hort. Sci.* **60** (4): 525-530

Evans, D.A., Sharp, W.R., Ammirato, P.V. and Yamada, Y. 1983. *Handbook of Plant Cell Culture Vol. I. Techniques for Propagation and Breeding.* Macmillan Publishing Co., New York, p. 620

Evans, D.A., Sharp,W.R. and Finck, C.E. 1981. Growth and behaviour of cell cultures. Embryogenesis and organogenesis. *Plant Tissue Culture : Methods and Applications in Agriculture.* (ed. Thorpe, T.A.) Academic Press, New York, pp. 45-114

Fereol, L., Louis, S. and Luce, L. 1996. Effects of gamma radiation on *in vitro* plantlets of *Alpinia purpurata. J. hort. Sci.* **71**: 243-247

George, E.F. and Sherrington, P.D. 1984. *Plant Propagation by Tissue Culture.* Exegetics Limited, Basing stoke

Groenewald, E.G., Wessels, D.C.J. and Koeleman, A. 1977. Callus formation and subsequent plant regeneration from seed tissue of an *Agave* species (Agavaceae). *Zeitschrift fur Pflanzenphysiologie* **81** (4): 369-373

Hagiladi, A. and Watad, A.A. 1992. *Cordyline terminalis* plants respond to foliar sprays and medium drenches of paclobutrazol. *Hort Sci.* **27** (2): 128-130

Hawks, H.Y. and Wainwright, H. 1987. *In vitro* organogenesis of *Cyclamen persicum* Mill. seedling tissue. *Acta Hort.* **II** (212): 711-714

Henny, R.J., Knauss, J.F. and Donnan, A. 1981. Foliage plant tissue culture. *Foliage Plant Production.* (ed. Joiner, J.N.) Prentice-Hall, England, pp. 137-178

Higuchi, H. and Amaki, W. 1989. Effects of 6-benzylaminopurine on the organogenesis of Asplenium nidus L. through *in vitro* propagation. *Scientia Hort.* **37** (4): 351-359

Hu, C.Y. and Wang, P.J. 1983. Meristem, shoot tip and bud cultures. *Handbook of Plant Cell Culture Vol. I. Techniques for Propagation and Breeding.* (ed. Evans, D.A., Sharp, W.R., Ammirato, P.V. and Yamada, Y.) Macmillan Publishing Co., New York, pp. 177-227

Huylenbroeck, J.M.V., Piqueras, A. and Debergh, P.C. 1998. Photosynthesis and carbon metabolism in leaves formed prior and during *ex vitro* acclimatization of micropropagated plants. *Pl. Sci.* **134** (1): 21-30

Hvoslef-Eide, A.K. 1990. The effect of irradiance and temperature on *in vivo* cultures of *Nephrolepis exaltata* (L.) Schott and *Cordyline fruticosa* (L.) A. chev. *Gartenbauwissenschaft* **55** (6): 259-264

Hvoslef-Eide, A.K. 1993. Influence of environmental conditions on *Cordyline fruticosa* (L.) A. chev. mother plants and on the subsequent growth of *in vitro* explants. *Gartenbauwissenschaft* **58** (2): 89-94

Jasrai, Y.T., Mudgil, Y., Remakanthan, A. and Kannan, V.R. 1999. Direct shoot regeneration from cultured leaves of *Passiflora caerulea* L. and field performance of regenerated plants. *Phytomorphology* **49** (3): 289-293

Jasrai, Y.T., Rao, T.V.L.N., Bhat, P.N. and Mehta, A.R. 1994. Radiation-induced variation in ornamental *Kalanchoe mortagei*. *Floriculture Technology, Trades* and *Trends* (ed. Prakash, J. and Bhandary, K.R.) Oxford and IBH Publishing Co., Bombay, pp. 345-347

Joiner, J.N. 1981. *Foliage Plant Production.* Prentice Hall, New Jersey, p. 614

Kobza, F. and Vachunova, J. 1989. Propagation of *Dracaena in vitro I.* Propagation of *Dracaena deremensis* Engl. *Acta Universitatis Agriculturae, Facultas Horticulturae* **4** (2): 33-37

Kobza, F. and Vachunova, J. 1991. Propagation of *Dracaena in vitro II.* Propagation of *Dracaena concina* Kunth. *Acta Universitatis Agriculturae, Facultas Horticulturae* **6** (1): 51-55

Kozak, D. and Dabski, M. 1995. Influence of some cytokinins on shoot development of *Syngonium podophyllum* cv. White Butterfly *in vitro. Annales Universitatis Mariae curie-sklodowska* **3**: 75-77

Kukulczanka, K., Klimaszewska, K. and Pluta, H. 1977. Regeneration of entire plants of *Peperomia scandens* Ruiz. from different parts of leaves *in vitro. Acta Hort.* **78**: 365-371

Kunisaki, J.T. 1975. *In vitro* propagation of *Cordyline terminalis* (L.) Kunth. *Hort Sci.* **10**: 601-602

Lakso, A.N., Reisch, R.I., Mortensen, J. and Roberts, M.N. 1986. Carbondioxide enrichment for stimulation of growth of *in vitro* propagated grape vines after transfer from culture. *J. Am. Soc. hort. Sci.* **111**: 636-638

Leffring, L., Vonk, C.R. and Ribot, S.A. 1985. Hormonal effects of shoot formation of *Cordyline* cultivars. *Acta Botanica Neerlandica* **34** (2): 238

Letouze, R. and Beauchesne, G. 1969. Action d'eclairements monochromatiques surla rhizogenese de tissues de Topinambour. *C. R. Acad. Sci.* **269**: 1528-1531

Lloyd, B. and Mc Cown, B. 1980. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia* by use of shoot tip culture. *Comb. Proc. Int. Pl. Prop. Soc.* **30**: 421-427

Lo, K.H. 1997. Factors affecting shoot organogenesis in leaf disc culture of African violet. *Scientia Hort.* **72**: 49-57

Maene, L. and Debergh, P. 1985. Liquid medium additions to established tissue cultures to improve elongation and rooting *in vivo. Pl.Cell Tissue Organ Cult.* **5** (1): 23-33

Maene, L.J. and Debergh, P.C. 1987. Optimisation of the transfer of tissue cultured shoots to *in vivo* conditions. *Acta Hort.* **I** (212): 335-348

Maity, I., Ghosh, P.D., Jana, B.K. and Bose, T. 1994. Micropropagation of some house plants. *Floriculture Technology, Trades and Trends* (ed. Prakash, J. and Bhandary, K.R.) Oxford and IBH Publishing Co., Bombay, pp. 357-366

Mee, G.W.P. 1978. Propagation of *Cordyline terminalis* from callus culture. *Hort Sci.* **13**: 660

Meshkova, N.V., Makarova, R.V., Borisova, T.A. and Lapotyshkina, L.I. 1999. The role of exogenous auxins and cytokinins at different stages of the *in vitro* propagation of *Cordyline terminalis. Biotekhnologiya* **15** (1): 71-79

Murali, T.P. and Duncan, E.J. 1995. The effects of *in vitro* hardening using triazoles on growth and acclimatization of banana. *Scientia Hort.* **64**: 243-251

Murali, T.P., Rangan, V.V. and Rajeevan, P.K. 1999. *In vitro* propagation of *Cordyline terminalis.* National Symposium on Emerging Scenario in Ornamental Horticulture in 2000 A.D. and Beyond. Indian Society of Ornamental Horticulture, Division of Floriculture and Landscaping, IARI, New Delhi. *Abstract of papers*

Murashige, T. 1974. Plant propagation through tissue culture. *Annual Rev. Pl. Physiol.* **25**: 135-166

Murashige, T. 1977. Clonal propagation of horticultural crops through tissue culture. *Plant Tissue Culture and its Biotechnological Applications.* (ed. Barz, W., Reinhard, E. and Zenk, M.H.) Springer-Verlag, New York, pp. 392-403

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

Narayan, P. and Jaiswal, V.S. 1986. Differentiation of plantlets from leaf callus of *Ficus religiosa* L. *Indian J. exp. biol.* **24** (3): 193-194

Nikam, T.D. 1997. High frequency shoot regeneration in *Agave sisalana. Pl. Cell Tissue Organ Cult.* **51** (3): 225-228

Oliphant, J. 1990. The use of paclobutrazol in the rooting medium of micropropagated plants. *Combined Proceedings.* International Plant Propagator's Society. Auckland, New Zealand, pp. 358-360

Paek, K.Y., Oh, M.O. and Choi, J.K. 1985. Mass propagation of *Cordyline* and *Scindapsus in vitro. J. Korean Soc. hort. Sci.* **26** (1): 83-92

Panse, V.G. and Sukhatme, P.V. 1985. *Statistical Methods for Agricultural Workers.* (4th ed), ICAR, New Delhi, p. 347

Paola, M.K.D.I., Siri, A., Fossi, D. and Tognoni, J. 1986. *In vitro* propagation of *Dieffenbachia amoena Hort. Rivista della ortoflorofrutticoltura Italiana* **70** (4): 245-252

Pierik, R.L.M. and Steegmans, H.H.M. 1983. Vegetative propagation of variegated Yucca in growth flasks. *Vakblad voor de Bloemisterij* **38** (1): 32-33

Podwyszynska, M. and Olszewski, T. 1995. Influence of gelling agents on shoot multiplication and the uptake of macro elements by *in vitro* cultures of rose, cordyline and homolomena. *Scientia Hort.* **64** (1-2): 77-84

Powers, D.E. and Backhaus, R.A.1989. *In vitro* propagation of *Agave arizonica* Gentry and Weber. *Pl.Cell Tissue Organ Cult.* **16** (1): 57-60

Prabha, J. 1993. *In vitro* multiplication and standardisation of hardening techniques in pineapple. M.Sc. (Hort.) thesis, Kerala Agricultural University, Vellanikkara, Kerala

Razdan, M.K. 1993. *An Introduction to Plant Tissue Culture.* Oxford & IBH Publishing Co. Pvt. Ltd., Calcutta, p.397

Redway, F.A. 1991. Histology and stereological analysis of shoot formation in leaf callus of *Saintpaulia ionantha* Wendl. *Pl. Sci.* **73** (2): 243-251

Reinert, J. 1959. Uber die kontroller der Morphogenese and die Induktion Von Advientive embryonen and gewebekulturen anskarotten. *Planta* **15** (3): 318-333

Robert, M.L., Herrera, J.L., Contreras, F. and Scorer, K.N. 1987. *In vitro* propagation of *Agave fourcroydes* Lem. (henequen), *Pl. Cell Tissue Organ Cult.* **8** (1): 37-48

Rodriguez-Garay, B., Gutierrez-Mora, A. and Acosta-Duenas, B. 1996. Somatic embryogenesis of *Agave victoria-reginae* Moore. *Pl. Cell Tissue Organ Cult.* **46**: 85-87

Ruvalcaba, F.S., Pulido, H.G. and Garay, B.R. 1999. Efficient *in vitro* propagation of *Agave parrasana* Berger. *Pl. Cell Tissue Organ Cult.* **56** 163-167

Sakr, S.S., Khateeb, E.M.A. and Kareim, E.A.H. 1999. *In vitro* production of *Yucca elephantipes. Bulletin of Faculty of Agric.* **50** (2): 265-282

Samyn, G. 1995. Influence of the sucrose concentration in the growth medium on adventitious and axillary shoot production and on the stability of the chimeral *Cordyline fruticosa* (L.) A. chev. cv. 'Rosa'. *Bulletin des Rescherches Agronomiques de Gembloux* **30** (1-2): 67-75

Shenk, R.U. and Hilderbrandt, A.C. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous cell cultures. *Can. J. Bot.* **50**: 199-204

Simmonds, J. 1984. Induction, growth and direct rooting of adventitious shoots of Begonia × hiemalis. *Pl. Cell Tissue Organ Cult.* **3** (4): 283-289

Sriskandarajahi, S. and Skirvin, R.M. 1991. Effect of NH_4NO_3 level on *Philodendron* root development *in vitro. Hort. Technol.* **1** (1): 37-38

Start, N.D. and Cumming, B.G. 1976. *In vitro* propagation of *Saintpaulia ionantha* Wendl. *Hort Sci.* **11** (3): 204-206

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

Swarup, V. 1993. Floriculture industry in India. *J. Orn. Hort.* **1** (1): 18-26

Telgen, H.J.V., Van, M.A. and Kunneman, B. 1992. Effect of propagation and rooting conditions on acclimatization of micropropagated plants. *Acta Botanica Neerlandica* **41** (4): 453-459

Vinterhalter, D. and Vinterhalter, B. 1992. Effect of inorganic nutrition on the formation of lateral roots in *Dracaena fragrans* Ker-Gawl cultured *in vitro. Pl. Cell Tissue Organ Cult.* **28** (3): 267-274

Voyiatzi, C. and Voyiatizis, D.G. 1989. *In vitro* shoot proliferation rate of *Dieffenbachia exotica* cultvar 'Marianna' as affected by cytokinins, the number of recultures and the temperature. *Scientia Hort.* **40**: 163-169

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

Weathers, P.J., Cheetham, R.D. and Giles, K.L. 1988. Dramatic increases in shoot number and lengths for Musa, Cordyline and Nephrolepis using nutrient mists. *Acta Hort.* **230**: 39-44

Weis, J.S. and Jaffe, M.J. 1969. Photoenhancement by blue light of organogenesis in tobacco pith cultures. *Physiol. Plant.* **22**: 171-176

Welander, T. 1988. Micropropagation of *Cordyline terminalis* cv. Atom-a European cooperation project. *Rapport Institutionen for Tradgardsvetenskap* **54**: 13

Yeoma, M.M. 1986. *Plant Cell Culture Technology.* Blackwell Scientific Publications, Malabourne, p. 33

Zeping, J. Jiefeng, H. Genlin, L. and Jing, L.D. 1998. Factors affecting the growth and differentiation of *Philodendron erubescens* Red Emerald tube-shoots. *J. Jiangsu For. Sci. Technol.* **25** (4): 32-34

Ziaullah., Ali, N., Asghar, S., Amin, N. and Arshad, M. 1999. Genotypic variation in two *cordyline* species and their performance in different growth media. *Sarhad J. Agric.* **15** (3): 177-179

Ziv, M. 1991. The use of growth retardants for the regulation and acclimatization of *in vitro* plants. *Progress in Plant Growth Regulation.* (Proc. of 14th International Conference on Plant Growth Substances, Amsterdam) Kluwer Academic Publishers, Netherlands, pp. 809-817

Appendix I

Chemical composition of the media

Chemical	mg l^{-1}		
	MS	SH	WPM
Macronutrients			
KNO_3	1900.000	2500.00	--
NH_4NO_3	1650.000	--	400.00
KH_2PO_4	170.000	--	170.00
$NH_4H_2PO_4$	--	300.00	--
$MgSO_4.7H_2O$	370.000	400.00	370.00
K_2SO_4	--	--	990.00
$Ca(NO_3)_2.4H_2O$	--	--	556.00
$CaCl_2.2H_2O$	440.000	200.00	96.00
Micronutrients			
H_3BO_3	6.200	5.00	6.20
$MnSO_4.4H_2O$	22.300	10.00	22.30
$Zn\ SO_4.7H_2O$	8.600	1.00	8.60
$Na_2MoO_4.2H_2O$	0.250	0.10	0.25
$CoCl_2.6H_2O$	0.025	0.10	--
$CuSO_4.5H_2O$	0.025	0.20	0.25
KI	0.830	1.00	--
$FeSO_4.7H_2O$	27.800	15.00	27.80
Na_2EDTA	37.300	20.00	37.30
Vitamins			
Thiamine Hcl	0.100	5.00	0.10
Pyridoxine Hcl	0.500	0.50	0.50
Nicotinic acid	0.500	5.00	0.50
Others			
Glycine	2.000	--	2.00
Myo-inositol	100.000	1000.00	100.00
Sucrose	30.000	30.00	30.00
pH	5.8	5.0	5.8

MS – (Murashige and Skoog, 1962)
SH – (Shenk and Hilderbrandt, 1972)
WPM – (Lloyd and Mc Cown, 1980)

Appendix – II

Meteorological parameters of the experimental site at the College of Horticulture, Vellanikkara, for the period from July 1999 to June 2000

Year	Month	Mean rainfall (mm)	Mean relative humidity (%)	Mean air temperature (°C)
1999	July	823.3	89	25.70
"	August	260.1	84	26.40
"	September	28.4	76	27.50
"	October	506.2	85	26.90
"	November	9.1	69	27.10
"	December	0.0	60	26.70
2000	January	0.0	60	28.10
"	February	4.6	67	28.10
"	March	0.0	67	29.80
"	April	67.9	74	29.30
"	May	117.2	72	29.10
"	June	602.0	86	26.20

MICROPROPAGATION AND CROP IMPROVEMENT OF CORDYLINE

(*Cordyline terminalis* (L.) KUNTH)

By

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ABSTRACT OF A THESIS

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ABSTRACT

Investigations on micropropagation and crop improvement of cordyline were carried out in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during 1998-2000. The main objectives were to study the response of various explants and to identify the most suitable explant and media combination for *in vitro* propagation. Attempts were also made to find out the optimum dose of γ - irradiation, for inducing variation.

The shoot tips and nodal segments of cordyline were found to be ideal explants for the enhanced release of axillary buds and for indirect organogenesis.

The best sterilization treatment was wiping with 70 per cent ethyl alcohol, followed by a dip in 0.1 per cent mercuric chloride for 18 min. for shoots, nodal segments and axillary buds. For leaf segments, treatment with 0.1 per cent mercuric chloride for ten minutes alone was enough.

The nodal segment explants collected in the drier months, namely, January, March, November and December showed no contamination. Survival percentage was the highest in the months of November and December. The shoot tip explants showed least contamination when collected during August to April.

Early release of buds (4.4 days after inoculation) and further growth of buds was better in MS medium supplemented with BAP 3.0 mg l^{-1}. Maximum shoot proliferation was observed in MS medium with 2.0 mg l^{-1} BAP. In the case of shoot tips MS medium having Kin 3.0 mg l^{-1} showed less time (5.5 days) for bud emergence. Maximum number of shoots (2.5) was also obtained when MS medium was supplemented with 3.0 mg l^{-1} KIN.

MS medium supplemented with BAP in combination with NAA also proved better for culture establishment of nodal segments and shoot tips. Maximum number of shoots was observed in MS medium with NAA 0.5 mg l^{-1} + BAP 1.0 mg l^{-1}. Of the different media tried, MS medium was found to be the

best for early release of buds in nodal segments and shoot tips. Number of shoots produced did not differ significantly in all the three media.

The elongated buds from Stage 1 showed very high rate of axillary bud production when inoculated in MS medium containing BAP 1.0 mg l^{-1} + NAA 0.5 mg l^{-1} and BAP 2.0 mg l^{-1} + NAA 0.5 mg l^{-1}. Among MS, SH and WPM media, full strength MS medium gave very high rate of axillary bud production within a short time (12.2 days). High rate of callus production was observed in MS medium having 1.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA and 2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA. The rate of axillary bud production and callusing was less with KIN in combination with NAA. Irrespective of the media, rhizogenesis was observed when higher levels of 2ip was incorporated in to the media.

Elongation of the multiple axillary buds with normal shoot and root growth was recorded in full strength MS medium devoid of growth regulators. None of the media supplements (activated charcoal, adenine sulphate, coconut water and casein hydrolysate) induced multiple axillary bud elongation.

Among the three basal media tried, MS medium was superior with respect to the number of lengthy roots and the time taken for root initiation. Among the auxins, IBA was superior for root induction. Callus formation was observed at the base when NAA was incorporated in the media.

Maximum percentage of rooting, early rooting and more number of lengthy roots were obtained in full strength MS basal medium. Addition of activated charcoal in the medium did not affect root number and root length, but reduced the number of days taken for root initiation.

Triadimefon in the rooting medium slightly increased the number of days taken for root initiation and decreased the number of roots/culture and length of roots. It also increased the survival percentage during hardening. Maximum survival percentage of the plantlets was obtained when the plantlets rooted in the medium containing Triadimefon 1.0 mg l^{-1} + IBA 5.0 mg l^{-1} were treated with 0.1 per cent Bavistin for 30 minutes soon after removal from the culture vessels and

subjected to a post planting treatment with triadimefon (20 mg l^{-1}) drenching at weekly intervals and kept in a net house having 50 per cent shade.

Plant height was the maximum when the plantlets after planting out were supplied with 0.75 g 17:17:17 NPK mixture per week as soil drench.

Among the various explants tried for somatic organogenesis, nodal segments and shoot tips were the most ideal for callus initiation, growth and differentiation. Callus intensity was maximum in the leaf segments when bottom portions of the immature leaves with midrib were cultured. Best response to callusing and callus intensity was obtained with the adaxial surface of the leaf touching the medium. Direct organogenesis could not be obtained in different treatment combinations or explants.

Shoot tips and nodal segments produced higher percentage of callus and showed maximum callus index when MS medium was supplemented with lower levels of 2, 4 D (0.5 mg l^{-1}) and BAP (0.5 mg l^{-1}), both in 12 h photoperiod. No positive response was observed with the addition of coconut water on callus induction and differentiation. Earliest differentiation of callus derived from nodal segment and shoot tip explants was possible in MS medium supplemented with BAP 1.0 mg l^{-1} + KIN 2.0 mg l^{-1}.

Very high rate of shoot production was observed in the medium supplemented with BAP 1.0 mg l^{-1} + KIN 2.0 mg l^{-1}. A combination of BAP + KIN gave high rate of shoot production, compared to BAP + 2ip combination.

Various treatment combinations failed to induce morphogenesis in leaf derived callus, but additon of activated charcoal prevented browning of callus upon subculturing. Higher concentration of cytokinins prevented rhizogenesis.

In vitro mutagenesis was attempted to know the optimum dose and correct stage for induction of variation. Of all the doses of γ-irradiation, a dose of 10 Gy at culture establishment stage produced plants having narrow yellow leaves with green line at the centre, but after planting out the plantlets reverted to normal behaviour.