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IN VITRO PRODUCTION OF *CORDYLINE TERMINALIS* FOR COMMERCIALIZATION

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Abstract

The current study envisages producing *Cordyline* plants on a large scale employing cost-effective *In vitro* protocols. The effect of various concentrations and types of plant growth regulators were observed to optimize the efficient media for propagation. Axillary buds excised from the greenhouse grown mother plants of *Cordyline terminalis* were cultured on MS medium supplemented with BAP (1.0-2.0 mg/L), Kinetin (2.0-4.0 mg/L) and NAA (0.1-0.5 mg/L). A rich solid mass of white-green callus was produced. Greatest number and length of shoots were produced in a medium containing kinetin (4.0 mg/L) and NAA (0.5mg/L). Rooting started on the same media when the plants had attained a height of 2.5 cm. Four weeks after a rich mass of roots were obtained the plants were transplanted individually in small clay pots and kept in close tunnels where the relative humidity was approximately 80%. Four weeks after acclimatization, the explants were shifted into green houses.

Introduction

Tissue culture technique is now used for propagation of numerous ornamental plants for commercial purposes. There has been a big market demand for exotic cultivars of *Cordyline* viz., Song of India and Song of Thai, and these are being imported in bulk quantities from countries like Thailand. Karachi, enjoying a subtropical coastal climate has full potential of growing this fabulous and exotic plant on a large scale which will play a significant role in the introduction of new varieties in the country. Micropropagation is a very economical means of multiplying a desirable plant species when time, space and personnel are often serious constraints. It is also possible to produce disease free, uniform propagules at needed quantity and at appropriate time of the year. *Cordyline* belonging to the family, Agavaceae originated from the tropical and sub tropical regions, East Himalayas, China, Malaysia and North Australia. In its normal habitat, it is a long slender bush up to 3m in height. This monocot species which has the cambium present in its stem, develops greenish-white roots that often form root suckers starting from their rhizomes. To maintain the genetic stability of the variegated leaves the conventional way of propagation is by rhizomes or by stem or top cuttings. The plants are grown in beds containing a mixture of leaves and peat so that there is sufficient space for the development of rhizomes. When the plants are approximately ready for sale, the rhizome is harvested which serve as new propagation material, and the saleable plants are potted and leave the nursery after a few weeks. The rhizome is cut into 1-2 cm pieces each and are planted in the surface of the substrate and kept warm. The axillary buds of the rhizome develop and produce new plantlets. This propagation procedure is rather labor intensive. Recently some new cultivars that do not develop new rhizomes have been introduced. It is thus necessary to use shoot cutting for them (Holttum, & Enoch, 1991).

The use of tissue cultured plant material can rationalize *Cordyline* culture because direct planting into pots without special manipulation for a large harvest of rhizome is possible. Moreover, the conventional mode of propagation enables us to produce only four daughter plants from one mother plant. Therefore micropropagation by means of *In vitro* techniques is of great interest in order to speed up the propagation rate and to reduce the need for mother plants (George & Sherrington, 1993).

The present study aims to optimize the micropropagation protocol for *Cordyline terminalis*, in order to make the propagation feasible and economical.

Materials and Method

The composition of medium used in this study consisted of MS (Murashige & Skoog, 1962) with full and half strength basal salts. Sucrose was added as the sole carbon source at 3% (w/v). The pH of the medium was adjusted using 0.1M NaOH or 0.1M HCl, to give a final value in the range 5.6-5.8. The medium was sterilized by autoclaving at 15 psi for 20 minutes. The medium was cooled and allowed to set before use.

The plant material of C*ordyline terminalis*, was obtained from the nursery of the HEJ Research Institute of Chemistry, University of Karachi. Terminal portions of young plants were taken as a source of explant. The terminal portions of the stem were cut into 5cm length, the leaves were removed by gentle peeling, leaving the base intact, and the stem was thoroughly washed under running tap water for 30-40 minutes. The explants were then soaked in a 250 ml sterile jar containing sterile distilled water with 2-3 drops of Tween 20. The jar was placed on a gyratory shaker for 15 minutes. The explants were given a quick dip in 70% ethanol for 5 seconds followed by placing in 20% NaOCl containing 3 drops of Tween 20 on continuous but very gentle stirring on a gyratory shaker for 30 minutes. This was followed by three consecutive rinses in sterile distilled water for five minutes each to remove traces of NaOCl. All the sterilization work was done under laminar flow cabinets in sterile environment. The stems were cut into transverse sections containing one nodal bud. They were cut into approximately 2cm long segments and used as explants for shoot initiation under *In vitro* conditions. For the multiplication experiments, the plants were subjected to different concentrations and combinations of BAP, Kinetin and NAA (Table 1) on full and ½ MS media with 6 replications. Cultures were maintained in a growth room at 24±2ºC with light provided by cool white florescent tube lights for a 16h photoperiod. Average number of shoots and increase in length (mm) were recorded after a period of 7 days on each jar for a successive period of 6 weeks. When newly initiated adventitious shoots reached a considerable height (>70mm), they were transferred to root initiating medium. Callus formed if any, were cut off from the shoot using a sterile scalpel before transfer. The callus was then subcultured on medium having the previous media composition, for further shoot production. The shoots were transferred onto fresh medium with the cut ends towards the medium. Data was recorded by counting the number and length of new shoots and roots per plant. Subsequently, the shoot proliferation rates were compared on solid and liquid medium (static condition). In order to induce rooting, the plants were subjected to IBA and NAA at concentrations of 0.25, 0.50, 1.00, 1.50 mg/L, in 1/2MS each with 6 replications. Cultures were incubated and maintained in a growth room. Average number of roots and increase in root length (mm) were measured after a period of 7 days on each jar for a successive period of 6 weeks. When a considerable network of adventitious roots had been established the plants were transferred to green house for acclimatization.

Table 1. Effect of BAP in combination with Kinetin and NAA on shoot proliferation on full and ½ MS medium.

Media	Kinetin (mg/L)	BAP (mg/L)	NAA (mg/L)	No. of shoots per bud explant		Length of shoots (mm)	
				MS	½ MS	MS	½ MS
M1	--	1.0	0.1	8.85±0.58	10.65±1.04	86±13.11	87±12.43
M2	2.0	--	0.1	9.85±0.60	11.85±1.50	89±23.23	70± 10.12
M3	--	2.0	0.1	7.95±0.22	8.50±1.14	94±43.21	81±41.24
M4	4.0	--	0.1	9.25±0.86	10.15±0.59	83±31.42	86±13.53
M5	--	1.00	0.5	10.95±0.22	12.75±0.86	97±12.12	80±32.11
M6	20	--	0.5	11.40±1.13	13.05±0.21	82±22.88	76±12.10
M7	--	2.0	0.5	10.89±0.59	13.65±1.04	97±20.23	77±10.13
M8	4.0	--	0.5	13.85±0.58	12.15±0.59	98±21.36	82±23.41

After 6 weeks, 20 explants were used for each treatment which is presented as mean ± SE.

Plantlets with roots between 20-40 mm in length were randomly selected for transferring into green house. Plantlets were removed from culture, and the roots were gently washed in luke warm distilled water to remove any residual gel or medium. These plants were placed into 250 mm plastic pots containing the following media: only sand, only charcoal, only fertilizer, sand + fertilizer (50:50), sand + charcoal (50:50), fertilizer + charcoal (50:50), fertilizer + charcoal + sand in equal parts. The sand employed was previously washed in 1 M HCl to adjust the pH to 5.5. The fertilizer used was cow dung. The pots were placed in green house where the humidity was approximately 80%. Data was recorded to determine which type of medium was optimum for acclimatisation. Over 4 weeks, relative humidity was slowly decreased by gradually shifting the plants into semi-shade areas. All experiments were of completely randomized design and repeated at least twice. Each treatment consisted of 80 explants (four explants per 250 ml jar). The numbers of shoots and roots were subjected to square root transformation prior to analysis. The number and lengths of both roots and shoots were also presented as mean values with a standard error. Data was analyzed employing SPSS version 4.0 (Scientific Enterprises, USA).

Results and Discussion

Shoot regeneration potential of various explants was investigated. The effect of a number of cytokinins and auxins in the growth media on explants was also investigated. The frequencies of sterile explants were increased when the latter were washed for 15-20 minutes in 20% NaOCl solution. This is considered better than $HgCl_2$ due to its lethal toxicity to plants. A rich mass of compact white callus was developed from the cut ends of the explants (Fig. 1). Profuse adventitious shooting was observed in media containing a combination of kinetin at 4.0 mg/L and NAA at 0.5 mg/L. Maximum increase in shoot number was 13.85 with a standard error of ± 0.58 (Table 1). Likewise, the maximum length of shoots was also obtained in the media having the same combination and concentration of plant growth regulators. Greatest increase in shoot length obtained was 98 mm with a standard error of 21.36. These tiny shoots, when transferred to fresh medium continued to produce adventitious shoots. Therefore, a cyclic production of shoots was possible, leading to continuous cultures and generation of large number of shoots. When a comparison was made against full and half MS media, a clear picture was drawn exhibiting that shoots in full MS showed better results (Fig. 2).

Fig. 1. Two weeks after cultures were subjected to subculture.

Fig. 2. Shoot elongation was observed 4 weeks after plants were subjected to medium containing KIN@ 4.00mg/L.

Fig. 3. Profuse natural rooting was observed on the shooting media.

For root induction, shoots were observed to have a natural tendency to produce roots in the same medium, at the onset of maturity (Fig. 3). The frequency of root induction and the rate of plantlet growth were slow when shoots were inoculated for rooting (liquid) under semi-submerged conditions. Trimming of older leaves of shoots prior to subculture promoted axillary shoot formation as this breaks apical dominance. Plantlets grown for 6-8 weeks *In vitro* were removed and thoroughly washed under running tap

water. Transfer of plantlets with sterile roots showed 100% survival in greenhouses. The established plants were exposed to the open environment where they showed rapid growth. The present study elucidates an *In vitro* multiplication of *Cordyline* clones and also indicates that *Cordyline* can be grown successfully in solid medium. No evidence of vitrification has been found during a 10-month period of continuous culture. Use of liquid medium was also successful but with a low and unstable multiplication rate of shoots.

The method of callus culture was preferred to shoot tip culture for micropropagation as it maximizes shoot multiplication, thus making this a very feasible protocol, which can be conveniently employed. Previous studies show that other varieties of *Cordyline* can be propagated *In vitro* on Murashige & Skoog (MS) medium supplemented with various combinations of cytokinin, as propagation was done in 3 steps viz., Callus induction, shooting and rooting. Along with different concentrations and combinations of hormones, each stage also requires different intensity of light. Instead of 3 different media, one media and one light intensity was sufficient for the micropropagation. This is in contrast to the findings of Debergh (1990) and Maene (1988), who employed a high light intensity of 100 μM (sec.m^2) for shoot elongation. Utilization of the procedure described here offers simple media and culture procedure for multiplication of Cordyline clones in a shorter duration.

Transfer of plantlets with sterile roots showed almost 100% survival in greenhouses. The established plants were exposed to open environment where they showed rapid growth. On conducting a series of experiments, it was found that an ideal soil type was one which contained soil and fertilizer (cow dung) in equal proportions. All the plants subjected to acclimatization are surviving in the green house and are ready to be transferred into the nursery for sale.

References

Debergh, P. 1990. Stimulation of axillary shoot development of *Cordyline terminalis* from Celestine Queen by foliar sprays . *HortScience*, 17: 344-345.

Ghose, S.P. 1993. Micropropagation of certain tropical horticultural crops. Adapted Propagation techniques for Commercial Crops of the Tropics. Proceedings of the Southeast Asian Regional Workshop of Propagation Techniques for commercial Crops of the Tropics. Ho Chi Minh City, Vietnam, 25-26.

George, E.F. and P.D. Sherrington. 1993. *Plant Propagation by Tissue Culture*. Exegetics Ltd., Press, pp. 709.

Holttum, R.E. and I. Enoch. 1991. Gardening in the tropics. Times Publications. Singapore. 6: 57-62

Maene, L. J. 1988. Rooting of tissue cultured plants under *In vivo* conditions. *Acta Hort.*, 131: 201-208

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant,* 9: 8-11.

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