

IN-VITRO PROPAGATION OF *CORDYLINE TERMINALIS* CV 'GOLD-COMPACTA' AND CV 'RED-COMPACTA'

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INTRODUCTION

Floriculture is the cultivation of ornamental plants for aesthetic purpose and for business. At present, there is a developing potential for floricultural items in both local and export markets.

Cordyline terminalis cv 'Gold-compacta' and *Cordyline terminalis* cv 'Red-compacta' are two popular ornamental plants (Duth, 1996) which have high export potential. Traditional method of propagating these plants is by sprouting of stem cuttings. Such method of propagation is often slow and requires a large number of mother plants (Sadhu, 1989). Hence, it is not an efficient method to satisfy the export demand. In this context *in-vitro* propagation is a viable alternative.

Plants produced through tissue culture are in high demand owing to their fascinating beauty, freedom of pests and diseases and uniformity (Dixon and Gonzales 1994). Their demand is increasing day by day. Therefore, this research was conducted to develop an *in-vitro* propagation protocol for the above *Cordyline* cultivars.

MATERIALS AND METHODS

Location

The experiment was conducted in the Tissue culture laboratory of Mike-Flora (Private) Limited, Rambukkana.

Explant Preparation

Top cuttings (soft wood and semi-hard wood) were taken from the healthy, vigorous mother plants grown in a net house. After removing the leaves the stems were washed with Teepol™ and kept under running tap water for more than 1 hour.

Surface sterilization of explants was done by using 10% sodium hypochlorite for 20 minutes. Normal MS medium was used for all the experiments and plant growth regulators were added where necessary. pH of the medium was kept 5.8 in all the experiments. Agar 3.5g/l was used to solidify the medium. Transferring was carried out in a laminar flow cabinet and cultures were kept in a culture room with light intensity of 700Wm^{-2} and temperature of 27°C .

Different BA concentrations on shoot multiplication

MS medium was supplemented with 0.5, 1, 2, 3, 5 mg/l BA as treatments. Each treatment was replicated for five times and 6 samples were included in each replicate. Treatments of 0.5, 1, 2 mg/l BA levels were repeated.

Different 2,4-D levels on callus induction of stem explants

The MS medium was supplemented with 1, 2 and 5 mg/l 2,4-D as treatments. Each treatment was replicated for five times and 6 samples were included in each replicate. Then, their callus initiation and growth was observed regularly.

Elimination of the Internal Bacteria in Stem Explants

Treatment I - After conventional sodium hypochlorite sterilization procedure, stem explants were shaken in 1g/l HgCl_2 for 10 minutes and washed 3 times with sterilized distilled water.

Treatment II - The stem explants were shaken in Teepol™ for 10 minutes, dip in fungicide (Carbendasim) solution for 30 minutes and shaken in few drops of Tween 20 in water for 10 minutes. Then, stem explants were washed with 70% ethanol for 1 minute, shaken in 2g/l HgCl_2 for 5 minutes and washed in sterilized water for 1, 2, 3, 5, 10 minutes.

Treatment III – After normal bleach sterilization procedure, stem explants were dipped in 100mg/l Streptomycin solution for 10 minutes.

Treatment IV - Streptomycin (100mg/l) was added to the autoclaved MS medium in the laminar flow cabinet.

RESULTS AND DISCUSSION

Standard sodium hypochlorite surface sterilization of explants

Standard sodium hypochlorite surface sterilization was not successful for *Cordyline terminalis* stem explants. It is because, internal bacteria were present in stem explants and they were not eliminated by standard surface sterilization procedure.

Different BA concentrations on shoot multiplication

According to statistical analysis, axillary bud development after 3 weeks was highest at 0.5 and 1 mg/l BA levels. (Table 1) There was no significant ($\alpha = 0.05$) difference between two varieties for the effect of BA levels. Same results were obtained in the repeated trial.

This result indicates that, most suitable BA level for axillary bud development lies between 0.5-1.0 mg/l. Further, experiments are necessary to find the exact value.

Table 1: Effect of BA level on axillary bud development

BA Level (mg/l)	0.5	01	02	03	05
Percentage of cultures with axillary buds (%)	90	91	59	41	22

Different 2,4-D levels on callus initiation of stem explants

Considering callus formation after 2 weeks of inoculation of stem explants the statistical analysis revealed ($\alpha = 0.05$.) 2,4-D levels of 1 and 2 mg/l are similarly effective on callus initiation, producing 89% of explants with callus. (Refer Table 2) Repeated experiments also showed the same results for both *Cordyline* cultivars.

According to the above finding, most appropriate 2,4-D concentration for callus initiation of stem explants lies between 1-2 mg/l.

Table 2: Effect of 2,4-D levels on callus initiation

2,4-D Level (mg/l)	01	02	05
Percentage of cultures with callus (%)	89	89	28

Elimination of the internal bacteria present in stem explants

According to statistical analysis, treatment II and III (Dip in 100mg/l streptomycin and shake with 2mg/l HgCl₂ after standard bleach sterilization) were more effective in controlling internal bacteria in *C. terminalis* cv 'Gold-compacta' and *C. terminalis* cv 'Red-compacta'. Treatment I and IV were also effective than conventional bleach sterilization procedure in controlling internal bacteria.

Responsiveness of stem explants of both the cultivars to low amount of BA gives an opportunity to carry out at least 12 to 15 sub cultures (Stafford and Warren 1991). Also *Cordyline* being an ornamental plant BA created bushy appearance and prolonged vegetative phase is very much acceptable (Deberg and Maene, 1990). Ability to regenerate through callus will also have path to crop improvement through somoclonal variations in *Cordylines*.

There were some internal bacteria present in the stem explants of *C. terminalis* cv 'Gold-compacta' and *C. terminalis* cv 'Red-compacta'. They can not be eliminated by conventional bleach sterilization procedure. They grew slowly and showed symptoms such as yellowing of medium, growing as a colony on medium and browning and death of the explant. But in some cultures, explants grew well while bacteria were there. Streptomycin and HgCl₂ are capable of controlling these bacteria effectively.

CONCLUSIONS

Most suitable BA level for axillary bud development lies between 0.5-1.0 mg/l. Most appropriate 2,4-D concentration for callus initiation lies between 1-2 mg/l. Dip in 100mg/l streptomycin and shake with 2mg/l HgCl₂ after standard bleach sterilization are effective in controlling internal bacteria in stem explants