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Direct Organogenesis of Seaside Heliotrope (*Heliotropium crassavicum*) Using Stem Explants

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Abstract: *Heliotropium crassavicum* L. is a sand binder salt marsh herb with enormous traditional value and widely found in South Asia America and Europe. In the direct method of regeneration from stem explants, we observed the maximum number of shoot regeneration after four weeks culture of MS elongation medium with 2.0 mg L^{-1} of 2, 4-D (17.27±0.51). It was clear that MS medium with 2.0 mg mL^{-1} 2, 4-D alone suitable for shoot multiplication as well as shoot elongation then compared to other combination of auxin and cytokinin. *In vitro* shoots were excised from shoot clumps and transferred to rooting medium containing 2, 4-dichlorophenoxy acetic acid (0.5-3.0 mg L^{-1}). The maximum number of root regeneration (6.4±0.416) and root length (6.08±0.07) were observed in MS rooting medium fortified with 2.5 mg L^{-1} of 2, 4-D after 2 weeks of culture. 85% of *in vitro* raised plantlets with well-developed shoots and roots were transferred to *ex vivo* conditions into polythene bag containing sterile compost with ratio (v/v/v) of organic fertilizer: sand: peat (1: 2: 2; 3: 1:0 or 2: 2: 1). Sixty five percent of acclimated plants were transferred to the pots under full sun where they grew well without any detectable phenotypic variations.

Key words: Direct method, 2,4-D, sterile compost, ste, salt marsh

INTRODUCTION

Heliotropium curassavicum is a species of heliotrope that is native to much of the South Asia, Europe and America, be found on other continents as an introduced species. It is known several common names, such as Seaside Heliotrope, Salt Heliotrope, Monkey Tail and Quail Plant (Wolff, 1977). Wild Heliotrope belongs to the Boraginaceae family also it is a low, spreading perennial herb which can take from a prostrate creeper with succulent leaves and small white flowers. It is often found in salty or alkaline places like dry streambeds (McAuley, 2007). Traditionally, it has been used for various external applications to ulcers, wounds, local inflammation, ringworm, rheumatism, urticaria (Kirtikar and Basu, 1998) and recently for cancer and diabetes (Sharma *et al*., 2009). Numerous techniques are available for rapid and extensive multiplication of elite and desirable plant species through *in vitro* conditions. Among these the Clonal multiplication become a major subject of investigation through plant tissue culture techniques; because investigators are developing interest on improvement of the biomass production of available resources of fast growing trees (Cavusoglu *et al*., 2011). Medicinally important Coastal plants *Citrullus colocynthis* (Satyavani *et al*., 2011) *Ruta graveolens* (Gurudeeban *et al*., 2012) and *Aegle marmelos* (Ramanathan *et al*., 2011) are also, micro propagated through direct and indirect organogenesis. Therefore, our present study focused to regenerate *Heliotropium curassavicum* through direct regeneration method from stem explants; it is the best and first report to our knowledge on large scale multiplication in a short period of time for conservation of medicinally important species *Heliotropium crassavicum*.

MATERIALS AND METHODS

Source of explants: *Heliotropium curassavicum* were freshly collected from Parangipettai (Southeast coast of Tamil Nadu, Lat 11°26'N; Log 79°46' E) India during November 2010 and kept under shade net (50%) house environment. The specimen was certified by Botanical Survey of India (BSI) Coimbatore and by the herbaria of C.A.S.in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India (Voucher No. Aucasmb 63/2010).

Surface sterilization: The stem was used as explant material for plantlet regeneration. The explants were

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washed in running tap water containing one drop of Tween 20 for 10 min and were rinsed three times in sterile distilled water. The stem explants were surface sterilized with ethyl alcohol for 1-5 min followed by three times rinsed with distilled water. Finally, the explants exposed to $HgCl_2$ for 3 min. The explants were then washed 4 times with sterile distilled water to remove traces of $HgCl_2$. The experimental chemicals were purchased from Hi-Media, Mumbai.

Media and incubation conditions: The stem explants used were planted for direct regeneration on MS medium Murashige and Skoog (1962). The media used for all purposes were supplemented with 3% (w/v) sucrose and solidified with 0.8% (w/v) agar. The pH of media was adjusted to 5.7 before autoclaving for 15 min at 121°C. The pH of the medium was adjusted with 0.1 N NaOH or 0.1 N HCl. Cultures were maintained in a growth chamber at 28°C±2°C with 16 h light/8 h darkness. The stem explants were excised with the help of sterile forceps and surgical blade. The stem explants were cut into 0.5 to 1.0 cm sized segments. The explants were cultured on different concentrations (1.0, 2.0 and 3.0 mg L^{-1}) of auxin (2,4-D and NAA) and 1.0 and 2.0 of cytokinin (6-BA) for respective shoot and root regeneration.

Micro propagation through direct regeneration techniques: The plantlet regenerate directly from the stem explants without undergoes for callus induction. 0.5 to 1.0 cm long stem explants cultured on MS elongation media containing various concentrations (0.5 and 1.0 mg L^{-1}) and combinations of auxin and cytokinin such as 2,4-dichlorophenoxy acetic acid (2,4-D), Napthyl Acetic Acid (NAA) and 6-Benzyl Adenine (6-BA) for shoot regeneration. Directly regenerative shoots 2 to 3 cm long with 5 to 6 leaves were induced to root *in vitro* by culture on agar-solidified MS rooting medium. Elongated and healthy regenerated multiple shoots from the elongation medium were rooted on of MS rooting medium fortified with 0.25-2.5 mg L^{-1} of 2,4-D for root development. The cultures were regularly subculture on fresh medium at two weeks interval and observation was recorded.

Rooting and acclimatization condition: After two weeks, shoots that had produced roots were recorded and transferred to sterile compost with ratio (v/v/v) of organic fertilizer: sand: peat (1:2:2; 3:1:0 or 2:2:1) in polythene bag a greenhouse. The plantlets after 10-15 days in green house; they were then placed in the normal environment for 1 h and assessed for sighs of wilting. The exposure was increased daily until the plants established fully under normal environment conditions, they then transferred into the field for normal growth.

Statistical analysis: Visual observation of culture was made every week. Data on shoot proliferation and root induction were recorded after three weeks of inoculation and used for calculation. For each treatment 15 explants were used for shoot proliferation and 15 shoots were used for rooting. All the treatments were repeated thrice. Significance of the treatment effects was determined using analysis of variance (ANOVA, $p \leq 0.05$) and comparison between mean values of treatments were made by Tukey's test.

RESULTS AND DISCUSSION

In the present study, revealed that cumulative work on plant tissue culture of *H. curassavicum* through direct method of regeneration from stem explant, it mainly depends on donor tissue and influenced by type of growth regulator and their concentration in the nutrient medium. Adventitious shoot formation is a reliable technique for clonal propagation as it prevents somaclonal variations in the cultures. In the present study, we observed the maximum number of shoot regeneration after four weeks culture of MS elongation medium with 2.0 mg L^{-1} of 2,4-D (17.27±0.51), then compared to other combination of hormones (Table 1). From the results, it was cleared that MS medium with 2.0 mg mL^{-1} 2, 4-D alone suitable for shoot multiplication as well as shoot elongation. Then compared to other combination of auxin and cytokinin (1.0 to 2.0 of 2, 4-D with 0.5 mg mL^{-1} of 6-BA and NAA). Then the above mentioned elongation medium was regularly sub cultured with 2.0 mg L^{-1} of 2, 4-D. Ramanathan *et al.* (2011) regenerated a medicinally important spiny tree (*Aegle marmelos*), they used MS media with Kn (1.5 mg L^{-1})+0.5 mg L^{-1} 2,4-D with NAA(1.0 mL L^{-1}) for maximum shoot formation. Among the auxins, 2, 4-dichlorophenoxy acetic acid is most efficient and widely used in tissue culture media. In our findings, the 2, 4-D alone plays an efficient role in the root regeneration with 2.5 mg L^{-1} in MS rooting medium. Jeyachandran *et al.* (2010) proved that the plant can be grown in single hormone up to *in vitro* flowering and rooting. *In vitro* shoots were excised from shoot clumps and transferred to rooting medium containing 2, 4-dichlorophenoxy acetic acid (0.5-3.0 mg L^{-1}) shown in Table 2. Among these, the maximum number of root regeneration (6.4±0.5) and root length (6.08±0.07) were observed in MS rooting medium fortified with 2.5 mg L^{-1} of 2, 4-D after 2 weeks of culture.

Figure 1 shows the *In vitro* direct plantlet regeneration of *H. curassavicum* from stem explants. However, this being the last stage of *in vitro* culture, it is important to transform the plant from heterotrophic to autotrophic mode of nutrition, the supply of exogenous

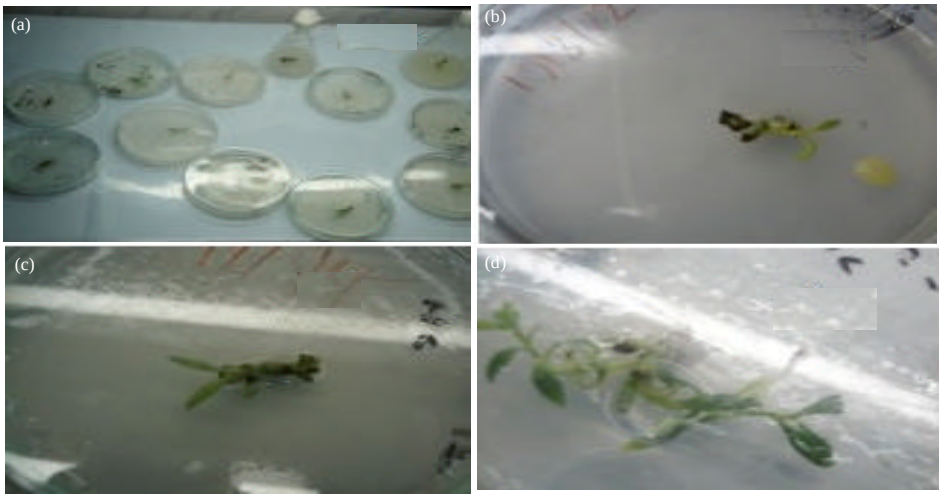

Fig. 1(a-d): *In vitro* direct plantlet regeneration of *H. curassavicum* from stem explants: (a) Stem explant inoculation, (a and b) Shoot induction and Shoot elongation in MS elongation medium with 2,4-D (2.0 mg L^{-1}) and (c) *In vitro* rooting of regenerated micro shoots in MS rooting medium with 2.5 mg L^{-1} of 2,4-D

Fig. 2: *Ex vivo* transplantation of *H. curassavicum* plantlets under environment shade net house in polythene bag containing sterile compost with ratio (v/v/v) of organic fertilizer: sand: peat (1: 2: 2; 3: 1:0 or 2: 2:1) in polythene bag a greenhouse

sugar should be reduced at this time. The rooting response differed according to different concentration and combinations of hormones (Satyavani *et al*., 2011). Eighty five percent of *in vitro* raised plantlets with well-developed shoots and roots were transferred to *ex vivo* conditions. During this period of acclimation the shoots elongated, leaves expanded and turned deep green looking good and healthier (Afrobz *et al*., 2009). *Ex vivo* transplantation of *H. curassavicum* plantlets under environment shade net house in polythene bag containing sterile compost with ratio (v/v/v) of organic fertilizer: sand: peat (1: 2: 2; 3: 1:0 or 2: 2: 1) in polythene bag a greenhouse shown in Fig. 2. Acclimatized after which the successfully sixty five percent of acclimated plants were transferred to the pots under full sun where they grew well without any detectable phenotypic variations.

In conclusion, the high production cost of nursery plants and the time required for restored plants to complete their life cycle are commonly considered the barriers to successful propagation, to overcome this problem our study provided the simple protocol with a

Table 1: Effect of plant growth regulators (2, 4-D and 6-BA) on shoot regeneration

Growth Regulators (mg L^{-1})			
2,4-D	6-BA	IAA	No. of shoots per explants (Mean±SD)
1.0	0.5	-	9.3±0.450
2.0	0.5	-	11.33±0.35
1.0	-	0.5	3.63±0.37
2.0	-	0.5	5.34±0.31
1.0	-	-	12.03±0.20
2.0	-	-	17.27±0.53
3.0	-	-	13.91±0.23

Effect of 2, 4-D and 6-BA on shoot regeneration from stem explants of *H. curassavicum* Mean±SD elongation medium after four weeks of culture

Table 2: Effect of 2, 4-D on root induction from *in vitro* raised shoots of *H. curassavicum*

Growth Regulators 2,4-D (mg L^{-1})	No. of roots/shoot (Mean±SD)	Root length (cm) (Mean±SD)
0.5	1.3±0.20	3.1±0.100
1.0	2.5±0.36	4.1±0.057
1.5	4.3±0.15	4.7±0.260
2.0	3.4±0.20	3.9±0.200
2.5	6.4±0.41	6.08±0.07
3.0	5.2±0.15	5.10±0.20

Effect of 2, 4-D on root induction from *in vitro* raised shoots of *H. curassavicum* after 4 weeks of culture. Values are represented as Mean±SD

low cost manner. Also it will useful for production of secondary metabolites, conservation of medicinally important endangered coastal flora, synthesis bio medically potential metallic nanoparticles and stress tolerant studies etc.

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