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MICROPROPAGATION OF THE HALOPHYTE *SUAEDA NUDIFLORA* MOQ. THROUGH AXILLARY BUD CULTURE

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SUMMARY

An *in vitro* micro propagation protocol has been developed for the halophyte *Suaeda nudiflora* Moq. Nodal explants were used for axillary shoot proliferation. The best results for shoot multiplication were recorded on MS medium suplemented with BAP (1.0 mg l^{-1}) and KN (0.2 mg l^{-1}). The MS medium supplemented with BAP in combination with auxins resulted very few shootlets and profuse callusing of the explants. Half strength MS liquid medium supplemented with IAA + IBA + NAA + IPA (0.5 mg l^{-1}) produced rooting in 70% of the *in vitro* regenerated shoots. Further root development was achieved in half strength MS agar medium without any auxins in two weeks. Plantlets were established on sterile vermiculite under controlled conditions and later transferred to pots or plastic bags containing 1: 1 mixture of soil and sand.

Key words : **Micropropagation, MS medium, salinity, *Suaeda nudiflora***

INTRODUCTION

For decades a major factor limiting crop production in arid environments has been the accumulation of salts due to irrigation. Hence the idea of sea water agriculture and domestication of wild salt tolerant plants native to saline habitat (halophytes) for use as food, fodder and oil seed crop appears to be more feasible and cost effective (Glenn *et al.* 1988). The halophytes and mangroves are also the last frontiers in a defence against the adverse consequences of rising sea levels. Due to various biotic and abiotic factors and human pressures like deforestation, waste disposal and coastal area development, the halophytic and mangrove populations are dwindling in an alarming scale. There is an urgent need to protect these valuable coastal bio-resources to conserve the coastal ecology. The present paper describes a micropropagation protocol for *Suaeda nudiflora*, a coastal halophyte of the family Chenopodiaceae.

Suaeda nudiflora Moq. is common to saline marshes and inland waters of the Gulf of Cambay (21° 45' N 70° 14'E) and can be a potential candidate for sea water agriculture. Though it is highly tolerant to salinity, it is very sensitive during the germination stage of development and needs alternate methods of propagation. Moreover, work on *in vitro* cultures of halophytes are limited and mostly restricted to callus or suspension cultures (Reddy *et al.* 1996, Blits *et al* 1993, Cherian 1998). Hence, employing tissue culture techniques for the clonal propagation and *in vitro* conservation of this highly salt tolerant species is highly desirable and useful for maintaining the population and coastal ecology.

MATERIALS AND METHODS

Juvenile shoots (15-20 cm) were collected from healthy plants of *S. nudiflora* growing in the natural stand. These shoots were first washed under running tap water

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and then treated with fungicide Cuman L (0.5% (v/v)) for 5 minutes and rinsed thoroughly with distilled water. The nodal segments were cut into small pieces of 2 to 3 cm and used as explants. The explants were surface sterilized with mercuric chloride solution (0.1v/v) for 5 minutes and rinsed serveral times with sterile distilled water under a laminar flow hood.

The Murashige and Skoog (1962) basal medium spplemented with 3%(w/v) sucrose and 0.6% (w/v) agar were used to initiate the culture. Depending on the experiment the basal medium was further supplemented with cytokinin (BAP or KN) or auxin (IAA, NAA or IBA) alone or in combinations. The cultures were maintained at 25± 2°C at a light intensity of 40-60 $\mu Em^{-2}S^{-1}$ provided by cool white fluorescent tubes (16 h/day photoperiod) and relative humidity of 55-65%.

The regenerated shoots were excised from primary cultures and sub-cultured on fresh basal medium supplemented with BAP (0.5 mg l^{-1}) and KN (0.1 mgl^{-1}) for shoot elongation. The elongated shoots measuring 5 cm or more were kept in half sterngth MS liquid medium supplemented with IAA, IBA, NAA and IPA at different concentrations (0.1-1.0 mgl^{-1}) in dark for root induction. The rooted shoots were transplanted to 10 cm diameter plastic bags containing autoclaved vermiculite. The plants were kept in controlled conditions (25 ± 2°C and 85% RH) and irrigated with sterile half strength MS nutrients for 3 weeks. Established plants were repotted in 20 cm diameter pots or plastic bags containing 1:1 mixture soil and sand.

RESULTS AND DISCUSSION

The potential for shoot proliferation in *S. nudiflora* appeared to be very strong in the presence of cytokinins in the culture medium. In most cultures where a combination of BAP and KN was used 1 to 2 shoots emerged per axillary bud soon after bud break. Subsequently, new axillary buds in varying numbers appeared around each node of the explant and developed into shoots depending upon the concentration of cytokinins used. Thus after a period of 6 weeks incubation the best result (16.13 ± 0.27 shoots/ explant) were recorded on medium supplemented with BAP (1.0 mg l^{-1}) and KN (0.2 mg l^{-1}) (Table 1). Depending on the concentrations and combinations of cytokinins tested, the proportion of explants showing bud break, mean shoot number and shoot length varied. The dependence of explants on various cytokinins for bud break response and shoot multiplication has already been establilshed (George and Sherrington 1984, Einset 1986). Furthermore, the relative benefit of BAP compared to other cytokinins in tissue cultures is well documented (Konan *et al.* 1997). In axillary shoot proliferation, cytokinin is utilized to overcome the apical dominance of shoots and to enhance the branching of lateral buds from leaf axils (Wang and Charles 1991). The overall pattern of shoot proliferation supperssed and the cultures produced basal callus when supplemented with cytokinin and auxins (Table 1). The presence of auxins in the culture medium has been shown to improve the culture growth (Yang *et al.* 1995, Patnaik and Debata 1996).

Half strength MS liquid as well as agar medium was employed for rooting of micro shoots. The micro shoots were kept on paper boats in half sterngth MS liquid medium containing a combination of IAA + IBA + NAA + IPA at a concentration of 0.5 mgl^{-1} each for 36 hours initiated rooting and found to be the best. At this combination the % rooting response (68.92 ± 0.73), the mean number of roots per shoots (6.5 ± 0.09) and mean root length (7.09 ± 0.21) were found to be optimum (Table 1). A combination of two or more auxins were reported to be more effective in root induction (Yadav *et al.* 1990, Sha Valli Khan *et al.* 1997). Further root development was achieved in half strength auxin free agar medium supplemented with or without charcoal (2.0 g l^{-1}) before transplantation (Fig. 1). Similarly, in order to provide the hard to root apple root stock with a strong root induction stimulus and to avoid callusing the shoots were first cultured in an auxin containing root initiation medium and then transferred to an auxin free root developing medium (James and Thurbon 1979). The procedure effectively prevented callus formation and resulted in 95% rooting, compared to those in continuous contact with auxin. Similar results were also reported in *Gypsophila* (Kusey *et al.* 1980).

The promotory effect of reducing the salt concentrations of MS media on *in vitro* rooting of shoots has been described in several reports (Constantine 1978, Upreti and Dhar 1996). Purohit and Dave (1996) reported

Table 1. Effect of different growth regulators on multiple shoot formation and rooting of *S. nudiflora* on MS medium. Values mean ± s.d. of three independent experiments of 20 explants each.

Growth regulator concentrations (mg l^{-1}) (BAP + Kinetin)	Number of shoots/ explant	Shoot length (cm)	% Response
BAP 0.25 +KN 0.2	4.61± 0.18	3.36±0.13	67
BAP 0.5 +KN 0.2	12.48±0.43	4.54±0.22	79
BAP 1.0 +KN 0.2	16.13±0.27	5.26±0.10	93
BAP 2.0 +KN 0.2	14.35±0.11	3.14±0.08	73
BAP 2.5 +KN 0.2	8.09±0.03	2.96±0.03	62
BAP 3.0 + KN 0.2	6.12±0.13	2.78±0.09	52

Growth regulator (mg l^{-1}) (BAP + IAA)	Mean number of shoots/explant	Growth regulator (mg l^{-1}) (BAP+NAA)	Mean number of shoots/explant
BAP 0.5 + IAA 0.025	3.3±0.52	BAP 0.5 +NAA 0.025	2.90±0.44
BAP 1.0 + IAA 0.025	3.78±0.56	BAP 1.0 +NAA 0.025	4.18±0.55
BAP 2.0 + IAA 0.025	5.00±0.76	BAP 2.0 +NAA 0.025	3.84±0.54
BAP 2.5 + IAA 0.025	4.86±0.44	BAP 2.5 +NAA 0.025	3.02±0.58
BAP 3.0 + IAA 0.025	3.14±0.33	BAP 3.0 +NAA 0.025	3.36±0.70
BAP 0.5 + IAA 0.05	2.76±0.33	BAP 0.5 +NAA 0.05	2.66±0.49
BAP1.0 + IAA 0.05	4.18±0.54	BAP 1.0 +NAA 0.05	3.0±0.41
BAP 2.0 + IAA 0.05	4.28±0.63	BAP2.0+NAA 0.05	3.22±0.07
BAP 2.5 + IAA 0.05	3.10±0.35	BAP 2.5 +NAA 0.05	2.94±0.65
BAP 3.0 + IAA 0.05	2.76±0.23	BAP 3.0 +NAA 0.05	3.82±0.23
BAP 0.5 + IAA 0.1	2.08±0.27	BAP 0.5 +NAA 0.1	2.14± 0.61
BAP 1.0 + IAA 0.1	3.20±0.38	BAP 1.0 +NAA 0.1	2.32±0.48
BAP 2.0 + IAA 0.1	3.52±0.42	BAP 2.0 +NAA 0.1	3.68±0.40
BAP 2.5 + IAA 0.1	3.04±0.20	BAP 2.5 +NAA 0.1	2.70±0.62
BAP 3.0 + IAA 0.1	2.76±0.20	BAP 3.0 +NAA 0.1	3.90±0.38

Rooting of *in vitro* offshoots

Growth regulator concentrations (mg l^{-1}) (BAP + IAA + IBA + NAA+ IPA)	Number of roots/ offshoot	Root length (cm)	% Response
0.1	0.00	0.00	0.00
0.25	27±0.07	3.2±0.32	29.00
0.5	6.5±0.09	7.09±0.21	68.92
1.0	4.7±0.05	5.2±0.57	57.71

best rooting in *Sterculia urens* when the salt concentration of MS was reduced to one quarter (1/4). Inclusion of activated charcoal in growth regulator free medium has been shown to promote shoot elongation in *Loblolly pine* (Jang and Tainter 1991) and in *Red spruce* (Lu *et al.* 1991) and increased rooting percentage in *Atriplex nummularia* (Reddy *et al.* 1996). In the present study we could establish a basic protocol for the micropropagation of *S. nudiflora* through axillary bud culture. This could be exploited as an alternate method for the large scale propagation of *Sueada* for coastal ecology conservation practices.

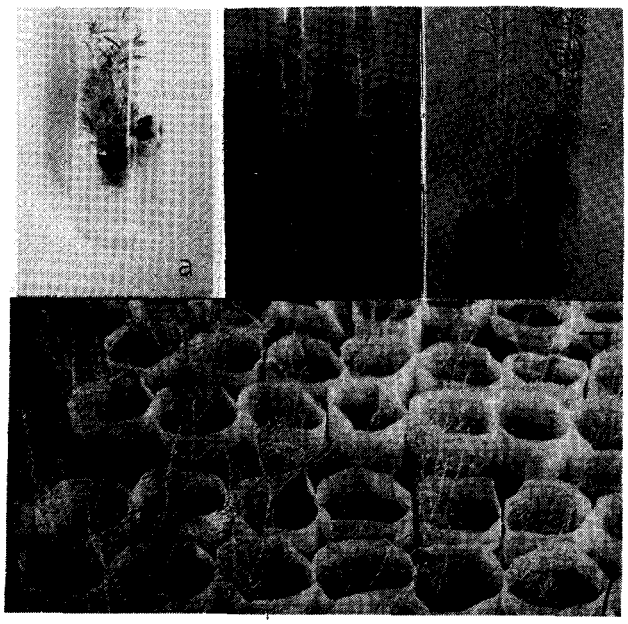

Fig.1. Different stages in the micropropagation of *Suaeda nudiflora* from nodal explants.

(a) Shoot proliferation from axillary buds after 6 weeks of culture on MS+BAP (1.0 mg l^{-1}) and Kinetin (0.2 mgl^{-1}).

(b) Elongation of microshoots on MS+BAP (0.5mg 1) and Kinetin (0.1 mgl^{-1})

(c) Rooting of offshoots in charcoal as well as chrcoal free 1/ 2 strength MS medium.

(d) *In vitro* plantlets after transplantation to potting mixture.

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