

Plant regeneration from callus cultures in *Suaeda nudiflora* (Wild.) Moq.

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Suaeda nudiflora plants, showing relatively high salt tolerance, were used for micropagation and generation of plantlets from callus cultures. Induction of somaclonal variability in the existing germplasm was another aim. Callus was initiated from young stem on MS medium supplemented with different concentrations of BAP and 2,4-D. Organogenesis was achieved by transferring callus on MS medium supplemented with BAP, KN and adenine. Well-grown shoots rooted easily on MS-half supplemented with IBA, IPA and NAA and 80-90% rooting was achieved. Plantlets got hardened by keeping them in hardening unit for few days. Hardened plants established very well in the nursery. Though originated from callus, these plants did not show any morphological variations and were similar to their parent donor plant.

Keywords: *Suaeda nudiflora*, callus culture, organogenesis, benzylaminopurine, root primordial, hardened plantlets

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Suaeda nudiflora, a halophytic species, grows wild in the highly saline, dry and extreme high tidal belt along the seacoast, mainly in Gujarat. As the seeds contain approx. 30-35% oil, plant has the potential as a future oil seed crop and is highly suitable for producing high protein biomass in saline soils due to C₄ photosynthesis. It is occasionally used as fodder for camels and goats¹. The tips of the branches in an oily medium are used to cure sores on camel backs. Its aqueous infusion is used as an emetic. Halophytes and mangroves have rarely been attempted for *in vitro* establishment of callus cultures and regeneration. The present paper describes *in vitro* initiation of callus culture from stem segments and subsequent regeneration of healthy plantlets.

Seeds of *S. nudiflora* were collected from the coastal areas. The young shoots (3-4 cm long) from mature plants raised in the institute's nursery, after

washing thoroughly in running tap water, were surface sterilized with 0.1% mercuric chloride for 5 min and rinsed with sterilized distilled water several times under aseptic conditions. Stem segments (5 mm long) from shoots were aseptically placed horizontally on MS media² (chemicals from Hi-Media, Mumbai).

The basal medium (BM1) was supplemented with BAP (1-benzylaminopurine, Sigma Chemicals, USA) and 2,4-D (2,4-dichloro phenoxyacetic acid, Sigma Chemicals, USA) for callus induction. Subsequently, callus was shifted to media supplemented with various concentrations and combinations of various plant growth hormones (Sigma Chemicals, USA) viz. BAP, NAA (1-naphthaleneacetic acid) and/or KN (kinetin) for regeneration and proliferation of shoots. Regeneration was obtained within 10-12 days and number of shoot primordia per culture was recorded. Grown up shoots were harvested and transferred to root inducing media enriched with IAA (indole 3-acetic acid), IPA (indole phenoxyacetic acid) or IBA (indole 3-butyric acid) alone or in combinations with 3% sucrose. To assess the effectiveness of the media, percentage rooting and number of roots per plantlet were recorded. Standard error was calculated to estimate the differences. Media pH was adjusted to 5.7 using 0.1 N HCl or 0.1 N NaOH before autoclaving and gelled with 0.6% (v/v) agar agar. Routinely 20 ml of molten medium was dispensed into culture tubes and steam sterilized at 1.06 kg cm⁻² for 15 min.

All the cultures were maintained at 25±2°C under white fluorescent light (3000 lux intensity) for 16 hrs photoperiod. However, for induction of callus, cultures were incubated in dark. For each treatment, 20 cultures were raised and experiment was conducted at least two times to confirm the results. The cultures were examined periodically to record morphological changes. Plantlets with well-developed root system were transferred to glass jars for hardening. After 30 days, hardened plants were successfully transferred in the nursery.

In *S. nudiflora* callus could be induced from most of the plant parts like leaf, stem, floral stem and flower buds as reported in bacopa³. During the present study, callus was initiated by placing the stem segments on MS media (BM1) supplemented with various concentrations of 2,4-D and BAP. These

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segments showed callus induction on the lower surface within 10 days in the medium supplemented with BAP (0.5 mg/l) and 2,4-D (1.0 mg/l).

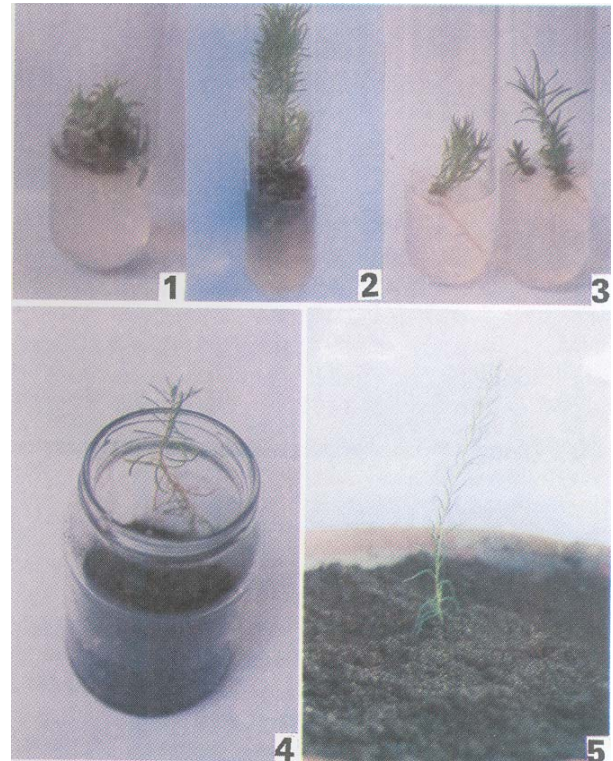
Callus was excised and subcultured on basal medium enriched with various combinations of BAP and NAA (Table 1). Though, organogenesis could be achieved on the medium supplemented with BAP (2.00 mg/l)+NAA (1.0 mg/l). Organogenesis in the form well differentiated shoot primordia with leaves were observed in 50-60% cultures after approx. 40 days in the medium supplemented with NAA (1 mg/l) and BAP (3 mg/l). Thus, relatively higher concentration of cytokinin improved regeneration potential of callus (Fig. 1), as also reported in *Niger*⁴.

Elongated shoots were separated and proliferated on media supplemented with BAP (0.5-1.5 mg/l), NAA (0.5-1.0 mg/l) and KN (0.5-1.0 mg/l) alone or in combinations. Any hormone did not promote multiple shoot formation individually. Although, BAP (0.5 mg/l) along with NAA (0.5 and 0.6 mg/l) and BAP (1.5 mg/l) with KN (0.5-0.6 mg/l) produced multiple shoots, no further growth was observed and culture dried after 15 days (Table 2). BAP (1.5 mg/l) with KN (0.8 mg/l) was found to be the most suitable combination for shoot proliferation and growth in the species with approx. 2-8 shoots per culture. This observation indicates specific requirement of growth hormones for enhancement of growth in the present material. Addition of adenine (0.5 mg/l) enhanced the growth of the shoots with dark green leaves (Fig. 2).

Elongated shoots were later separated individually and transferred to root induction media (MS ½) supplemented with IBA+IAA or IBA+IPA individually or in combination. IBA (0.5 mg/l) in combination with IPA (0.1 and 0.5 mg/l) or IAA (0.5 mg/l) failed to induce rooting in the species and induced only callusing at proximal end of the shoots

Table 1—Effect of BAP and NAA on regeneration of shoots from callus in *S. nudiflora*

BAP (mg/l)	NAA (mg/l)	Characteristics of callus	Callus showing regeneration (%)
1	0.5	Whitish callus	0.0
2	0.5	Greenish callus	0.0
3	0.5	Greenish callus	0.0
4	0.5	Brown callus	0.0
1	1.0	Green compact callus	0.0
2	1.0	Green and compact callus	9.0
3	1.0	Callus with shoots	50-60
4	1.0	Brown callus	0.0



Figs 1-5—*In vitro* propagation of *S. nudiflora*: 1) Regeneration of shoots from callus on MS media containing 2,4-D (1.0 mg/l) and BAP (0.5 mg/l); 2) Grown up multiple shoots on MS medium supplemented with BAP (3 mg/l) and NAA (1 mg/l); 3) Development of rooted plantlets after three weeks of culture on half strength MS+IBA (0.5 mg/l)+IPA(0.1 mg/l)+NAA(0.1 mg/l); 4) Rooted plant let transferred to glass jar for hardening in sterilized potting mixture of soilrite and FYM (1:1); and 5) Potted plant of *Suaeda* after 10 days of hardening.

Table 2—Effect of BAP, NAA and KN on proliferation and growth of shoots of *S. nudiflora*

BAP (mg/l)	NAA (mg/l)	KN (mg/l)	Mean number of multiple shoots per culture and SE
0.5	0.5	—	2.4*±0.21
0.5	0.6	—	2.3*±0.21
0.5	0.7	—	No growth
1.0	0.5	—	No growth
1.0	0.6	—	No growth
1.0	0.7	—	No growth
1.5	0.5	—	No growth
1.5	0.6	—	No growth
1.5	0.7	—	No growth
1.5	—	0.5	2.2*±0.22
1.5	—	0.6	2.3*±0.07
1.5	—	0.7	2.7**±0.07
1.5	—	0.8	5.5±0.35
1.5	—	0.9	1.8±0.05

*Shoots dried after 15 days

**Shoots survived but did not show any increase in length

Table 3—Influence of different auxins on rooting of *in vitro* developed shoots in *S. nudiflora*

IBA (mg/l)	IPA (mg/l)	NAA (mg/l)	IAA (mg/l)	Response of rooting (%) Mean±SE	No. of roots (Mean±SE)
0.5	—	—	0.5	Bc	00
0.5	—	—	1.0	Bc	00
0.5	0.1	—	—	Bc	36±1.2
0.5	0.5	—	—	Bc with brittle roots	30±1.5
0.5	0.5	—	0.5	Bc	00
1.0	1.0	—	1.0	Bc	00
0.5	0.1	0.1	—	60±0.3	25±0.2
0.5	0.1	0.2	—	90±0.05	15±.05
0.5	0.1	0.3	—	70±0.2	12±0.2
0.5	0.1	0.4	—	50±1.2	6±1.5

Bc= Callus at base

with callus at the base. Addition of NAA (0.1-0.4 mg/l) at the same concentrations developed true roots and simultaneously enhanced the root length. Among all the combinations, IBA (0.5 mg/l) along with low concentrations of IPA (0.1 mg/l) and NAA (0.2 mg/l) found to be most effective for root induction (Table 3) giving 90% rooting. Well-differentiated fibrous roots (approx.15) with hairs (Fig. 3) were observed in IBA (0.5 mg/l)+IPA (0.1 mg/l)+NAA (0.1 mg/l). The increase in the concentration of NAA reduced the rooting percentage drastically.

Plantlets with well developed root system were removed from culture tubes, washed thoroughly under tap water so as to remove the excessive media and transferred to glass jars filled with mixture of sterile soil and farm yard manure (FYM) in equal proportions (Fig. 4). Plantlets in the jar were irrigated with MS salts, covered with plastic bag and kept in hardening unit at 30°C with high humidity (60-70%)

for 10-15 days. Plantlets were transferred to earthen pots containing soil, sand and FYM (2:1:1), irrigated with MS salts (1/2) and kept in green house. After 30 days, hardened plants were transferred in the nursery under natural light (Fig. 5). Anatomical studies of leaf and stem also confirmed the gradual hardening of plantlets. All plants established successfully under natural conditions (up to 80%). Earlier, a few reports confirmed somaclonal variants derived from callus cultures in Peach⁶ and oil palm⁷. The regenerated plants did not show any detectable variations in the morphological characters as compared to donor plant.

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