Results

Plants are inevitably contaminated with a wide range of micro organism. The retention of these viable contaminants in association with a plant tissue after coming in contact with a nutrient medium (containing a source of carbon) lead to rapid proliferation of the contaminants & seriously affect the growth of callus. Occasionally antibiotics have been employed to prevent growth of bacteria (Monants, 1957). Sometime the antibiotics themselves may seriously impair the growth of the tissue. Different chemicals were use for surface sterilization.

A. *Suaeda nudiflora*

1. Sterilization of different plant parts

The process of surface sterilization was standardized by taking different concentrations of mercuric chloride for different timings. As can be seen from (Table 3), the 0.075 % $Hgcl_2$ was found to be the best for all the plant parts of Suaeda and Salicornia.. When the explants were treated with 50 % for 10 min only 40% culture survived. While 50% culture survived when explants were treated 0.050g for 15 minutes and hence concentration was discarded. Though, cultures survived after treatment for 20 min but growth rate was slow. When the explants

were treated with 0.075g/100ml $HgCl_2$for 10 min,15 min (Table 4) cultures survived to the extent of 50% and 80% respectively but 20 minutes period was lethal to the explants. The explants were also treated with 100mg/100ml for 10 min,15min and 20 minutes but only 10% cultures survived (Table 5), remaining treatments were found lethal to cultures. Hence 75 mg/100ml showed best survival and with minimal contamination

Table – 3 : Effect of $Hgcl_2$ on explant survival, treated at different timings.

Explant	HgCl2 mg/100ml	Time	Survival (%)
Stem/Floral stem/ leaf	50mg	10min	40%
Stem / Floral stem leaf	50mg	15min	50%
Stem/ Floral stem / leaf	50m g	10min	lethal

Table- 4: Effect of $Hgcl_2$ on explant survival treated at different timings.

Explant	HgCl2 mg/100ml	Time	Survival (%)
Stem/ Floral stem / leaf	75mg	10min	50%
Stem/ Floral stem / leaf	75mg	15min	80%
Stem/ Floral stem / leaf	75m g	10min	Lethal

Table- 5: Effect of $Hgcl_2$ on explant survival treated at different timings.

Explant	HgCl2 mg/100ml	Time	Survival (%)
Stem/ Floral stem / leaf	100mg	10min	10%
Stem /Floral stem / leaf	100mg	15min	Lethal
Stem /Floral stem / leaf	100m g	20min	Lethal

2. Effect of hormones on Callus induction and growth derived from different plant parts.

The effect of hormones on the growth of callus derived from different plant parts was studied. Leaf is the most common plant part used to generate sterile culture in a numbers of plants. Callus was generated on MS, media supplemented with different concentration of hormones 2,4-D, NAA and BAP. Leaf of *S. nudiflora* placed on. media supplemented with only 2,4-D (1- 4 mg/l). Later, addition of BAP was tried along with 2,4-D in the range of 0.5mg/l to 4.0 mg/l (Table 6). It was found that leaf placed on media supplemented by 2,4-D (2.0 mg/l) and BAP (0.5 mg/l) gave good callus growth with 4.2g fresh weight (Table 7). Callus showed increase in growth up to 72 days (exponential phase) after that stationary phase started leading to drying of callus. When leaf was placed on media supplemented with BAP and NAA. The young leaf generated callus while older leaf

T

able -6 :Response of Auxin/ cytokinin on callus induction from leaf

Auxin mg/l (2,4-D)	Cytokinin mg/l (BAP) 0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0	--	-	-	-	-	-	-		-
0.5	-	-	-	-	-	-	-		-
1.0	-	++	++	-	-	-	-		-
1.5	-	+++	++	-	-	-	-		-
2.0	-	+++	++	-	-	-	-		-
2.5	-	+	++	-	-	-	-		-
3.0	-		++	-	-	-	-		-
3.5	-	-	-	-	-	-	-		-
0.0	-	-	-	-	-	-	-		-

++++ Excellent , -- No response

+++ Best , ++ Better , + Good

Table -7. Effect of hormones on growth of callus of leaf.

2,4-D mg/l	BAP mg/l	Initial approx weight of leaf (g)	Wt after 24 days (g)	Wt after 48 days (g)	Wt after 72 days (g)	Wt after 96days (g)	Wt after 120days (g)
1.0	0.0	0.08	0.08	0.07±2.31	Dried	Dried	Dried
2.0	0.0	0.08	0.08	0.07±2.62	Dried	Dried	Dried
3.0	0.0	0.08	0.08	0.07±2.34	Dried	Dried	Dried
4.0	0.0	0.08	0.08	0.07±3.16	Dried	Dried	Dried
1.0	0.5	0.08	0.58	1.48±1.34	3.01±1.32	3.00±1.32	2.9±1.02
2.0	0.5	0.08	0.62	1.90±0.86	4.20±0.84	4.10±0.81	4.0±0.85
3.0	0.5	0.08	0.12	0.10±2.34	0.81	Dried	Dried
4.0	0.5	0.08	0.07	Dried	Dried	Dried	Dried
1.0	1.0	0.08	0.58	1.48±1.36	0.92±1.26	0.91±1.23	Dried
2.0	1.0	0.08	0.52	1.60±0.96	1.96±0.92	1.80±0.92	1.70±1.00
3.0	1.0	0.08	0.51	1.40±2.36	.82±2.41	1.20±2.34	1.10±2.51
4.0	1.0	0.08	0.07	Dried	Dried	Dried	Dried
1.0	1.5	0.08	0.09	0.1±2.31	.21±2.11	.20±2.01	.20±2.11
2.0	1.5	0.08	0.09	0.1±4.35	.08±3.24	Dried	Dried
3.0	1.5	0.08	0.09	0.1±4.35	.08±3.21	Dried	Dried
4.0	1.5	0.08	0.07	Dried	Dried	Dried	Dried
1.0	2.0	0.08	0.09	0.1±2.11	0.20±2.12	.23±2.11	.21±2.11
2.0	2.0	0.08	0.09	.1±4.32	.08±4.20	Dried	Dried

generated roots at different concentration of NAA & BAP. In case of floral stem, both 2,4-D and BAP individually failed to induce callus (Table 8). 2,4-D alone could just initiate callus but further growth was not observed. With the increases in concentration of BAP the callus growth decreased. BAP (0.5 mg/l) and 2,4-D (1.0mg/l) gave 3.2g callus in 96 days. The growth of callus from floral stem was observed up to 96 days than stationary phase started & callus started drying (Table 9) .

Table -8. Response of Auxin/ cytokinin on callus induction from Floral stem

Auxin mg/l 2,4-D	Cytokinines mg/l BAP 0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0	--	--	--	--	--	--	--	--	--
0.5	--	--	--	--	--	--	--	--	--
1.0	--	++++	++	--	--	--	--	--	--
1.5	--	+++	++	--	----	--	--	--	--
2.0	--	+++	+	--	--	--	--	--	--
2.5	--	++	+	--	--	--	--	--	--
3.0	--	++	+	--	--	--	--	--	--
3.5	--	--	+	--	--	--	--	--	--
4.0	--	--	+	--	--	--	--	--	--

Table -9 Effect of hormones on growth of callus of Floral stem .

2,4-D mg/l	BAP mg/l	Initial approx. weight of floral. (g)	Mean of wt after 24days & S.E (g)	Mean of wt of after 48days & S.E (g)	Mean of wt after 72 days & S.E (g)	Mean of wt after 96 days & SE (g)	Mean of wt after 120day & SE (g)
1.0	0.0	0.05	0.08	0.09±3.64	0.09±2.41	0.09±3.01	0.08±811
2.0	0.0	0.05	0.08	0.09±3.54	0.09±2.43	0.09±2.01	0.08±2.61
3.0	0.0	0.05	0.08	0.09±3.64	0.09±2.61	0.09±2.56	0.08±2.61
4.0	0.0	0.05	0.05	Dried	Dried	Dried 3.21	Dried
1.0	0.5	0.05	0.45	1.57±3.46	*2.7*±2.56	*3.2*±3.01	3.1±2.23
2.0	0.5	0.05	0.45	0.76±2.98	1.3±2.56	1.8±3.12	1.8±2.64
3.0	0.5	0.05	0.25	0.46±2.86	0.72±2.34	0.51±2.30	0.50±2.65
4.0	0.5	0.05	0.05	Dried	Dried	Dried 2.31	Dried
1.0	1.0	0.05	0.25	0.40±3.45	0.72±2.65	1.2±2.31	1.10±2.56
2.0	1.0	0.05	0.40	0.60±2.89	1.22±2.87	1.1±2.41	1.00±2.56
3.0	1.0	0.05	0.22	0.22±3.02	0.21±2.63	0.20±4.0	0.20±4..34
4.0	1.0	0.05	0.07	0.08±3.89	0.07±4.31	0.07±4.21	0.06±4.25

- Dried

Similar to floral stem, in case of flower buds also 2,4-D and BAP alone were unable to induce callus. Media supplemented with 2,4-D in range of (1 –2 mg/l) and BAP (0.5- 1mg/l) initiated callus. As the concentration of BAP increased the callus growth decreased (Table 10). 2,4-D (2.0mg/l) and BAP (0.5mg/l) increased fresh weight of callus up to about 2.91g (Table 11).

Table -10. Response of Auxins/ cytokinin on callus induction from flower.

Auxin mg/l 2,4-D	Cytokinines (mg/l) BAP 0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0	--	--	--	--	--	--	--	--	--
0.5	--	--	--	--	--	--	--	--	--
1.0	--	+++	+	--	--	--	--	--	--
1.5	--	+++	+	--	--	--	--	--	--
2.0	--	++++	+++	--	--	--	--	--	--
2.5	--	+++	+++	--	--	--	--	--	--
3.0	--	+	+	--	--	--	--	--	--
3.5	--	--	--	--	--	--	--	--	--
4.0	--	--	--	--	--	--	--	--	--

- Dried

Table-11. Effect of hormones on growth of callus of flower

2,4-D mg/l	BAP mg/l	Initial approx. weight of flo. (g)	Mean of wt after 24days & S.E (g)	Mean of wt after 48days and S.E (g)	Mean of wt after 72 days and S.E (g)	Mean of wt after 96days and S.E (g)	Mean of wt after 120day and S.E (g)
1.0	0.0	0.05	**	**	**	**	**
2.0	0.0	0.05	**	**	**	**	**
3.0	0.0	0.02	**	**	**	**	**
4.0	0.0	0.04	0.05±3.25	**	**	**	**
1.0	0.5	0.05	0.52±3.64	0.99+0.3	1.51+0.1	2.42+0.1	2.29±0.3
2.0	0.5	0.04	0.52±3.24	1.65+0.1	2.3+0.1	2.91+0. 2	2.71+0.1
3.0	0.5	0.05	0.09±2.89	0.15+1. 2	0.17+1.2	0.17+1. 3	0.16+
4.0	0.5	0.05	0.05±3.02	**	**	**	**
1.0	1.0	0.04	0.08±3.02	**	**	**	**
2.0	1.0	0.06	0.25±3.04	1.6+	2.6+	1.55+	**
3.0	1.0	0.05	**	**	**	**	**
4.0	1.0	0.05	**	**	**	**	**

* Dried

Attempts were also made to culture roots on media supplemented with 2,4-D (0.5- 4 mg/l0 and BAP (0.5- 4.0 mg/l) but all of them failed to respond.

Callus was initiated by placing the stem segments on MS media supplemented with various concentrations of 2,4-D and BAP (Table 12). Like floral stem and flower buds, 2,4-D & BAP alone failed to induce callus in stem. 2,4-D (1 – 3 mg/l) with BAP (0.5 –2.0 mg/l) found suitable for callus initiation. & growth. Within 72 days 2.6 g of callus was obtained (Table 13). These segments showed callus induction on the lower surface within 10 days in the medium supplemented with 2,4 D (1.0 mg./l) & BAP (0.5 mg/l) and organogenesis was observed after 40 days. When excised callus was sub cultured on basal medium supplemented with NAA (1mg/l) and BAP (3 mg/l) 60% of the cultures showed regenerated shoots (Table 14).These regenerated shoots were allowed to grow in the same media for 30 – 50 days.

The callus derived from leaf, inflorescence and flower on these combination and concentration of hormones did not show caulogenesis.

Table - 12 : Response of Auxin/ cytokinin on callus induction from stem

Auxin mg/l	Cytokinines mg/l BAP								
2,4-D	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0	--	--	--	--	--	--	--	--	--
0.5	--	--	--	--	--	--	--	--	--
1.0	--	+++ +	++	++	++	--	--	--	--
1.5	--	+++	++	++	++	--	--	--	--
2.0	--	+++	++	+	+	--	--	--	--
2.5	--	++	+	+	+	--	--	--	--
3.0	--	++	+	+	+	--	--	--	--
3.5	--	--	--	--	--	--	--	--	--
4.0	--	--	---	- -	--	--	--	--	--

Table- 13: Effect of hormones on callus induction and growth from stem

2,4-D mg/l	BAP mg/l	Initial approx. weight of stem.(g)	Mean of wt after 24days S.E (g)	Mean of wt after 48days S.E (g)	Mean of wt after 72 days S.E (g)	Mean of wt after 96days S.E (g)	Mean wt after 120day S.E (g)
1.0	0.0	0.06	**	**	**	**	**
2.0	0.0	0.06	**	**	**	**	**
3.0	0.0	0.06	**	**	**	**	**
4.0	0.0	0.06	**	**	**	**	**
1.0	0.5	0.06	0.63±2.13	1.37±2.34	2.61±3.21	2.61±2.45	**
2.0	0.5	0.06	0.63±3.14	0.82±3.45	1.21±3.41	1.01±3.45	**
3.0	0.5	0.06	0.23±3.45	0.53±	0.58±3.41	0.21±3.45	**
4.0	0.5	0.06	**	**	**	**	**
1.0	1.0	0.06	**	**	**	**	**
2.0	1.0	0.06	0.31±2.34	0.72±	1.01±2.31	0.92±2.34	**
3.0	1.0	0.06	0.1±2.56	0.2±	0.31±3.45	0.22±3.42	**
4.0	1.0	0.06	**	**	**	**	**
1.0	2.0	0.06	0.17±2.53	0.32±	0.21±3.45	0.56±3.12	**
2.0	2.0	0.06	0.08±2.56	0.60±	0.10±3.45	0.08±3.15	**
3.0	2.0	0.06	0.08±2.45	0.13±	0.123.45	0.09±2.56	**
4.0	2.0	0.06	**	**	**	**	**

**Dried

Table –14: Effect of BAP and NAA on regeneration of shoots from callus of stem 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 $\pm$2.68
3	1.0	Callus with shoots	60$\pm$2.35
4	1.0	Brown callus	0.0

3. Somatic embryogenesis.

Attempts were made to induce embryogenesis in the Callus thus generated from different plant parts, by sub culturing on MS, MS (1/2), N6 and B5 media but somatic embryos were observed only in leaf generated callus. No somatic embryos were detected on primary culture medium. Callus from leaf generated on MS media supplemented with hormones 2,4-D (2 .0mg/l) and NAA (0.5 mg/l) when transferred on B5 & N6 media supplemented with 2,4-D (2mg/l) & NAA (1.0mg/l), after 48 - 60 days somatic embryos were observed. Morphologically embryos were different, the embryos on B5 media (Fig.5) were greenish and small bulb like where as embryos on N6 media (Fig.6) were yellowish brown, long and heart shaped. These somatic embryos were sub cultured on B5 and MS full & MS 1/2 strength media for germination. But failed to grow on any of the media and callus formation was observed.

4. Organogenesis and regeneration

The callus when transferred from MS media enriched with BAP and NAA showed organogenesis after 30-40 days of incubation by developing dark green and dense points or eyelets in callus(Fig.10).From these points shoot primordia transformed after a period of 20-30 days. Elongated shoots were separated and

transferred on to media supplemented with various combination of BAP, NAA and KN (Fig.11). Shoots were cultured on media supplemented with BAP (0.5-2.0mg/l) in combinations with KN (0.5–1.0 mg/l) and NAA (0.5-0.7 mg/l). Among all combinations BAP (1.5 mg/l) with KN (0.8 mg/l) found to be the most suitable for proliferation and growth of shoots (Table 15). Approximately 4-5 shoots per culture could be obtained. Initially these shoots did not show any increase in height and hence to promote growth, adenine was added in the range of 0.1 - 2.0 mg/l. Addition of adenine improved the height and vigour of the shoots. Low concentration of adenine (0.5 mg/l) enhanced the growth of the shoots with dark green color leaves. However, higher concentrations of adenine adversely affected the shoot growth.

Table -15: 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*$\pm$ 0.21
0.5	0.6	--	2.3* $\pm$ 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 * $\pm$ 0.22
1.5	--	0.6	2.3* $\pm$ 0.07
1.5	--	0.7	2.4** $\pm$ 0.07
1.5	--	0.8	5.5 $\pm$ 0.35
1.5	--	0.9	1.8 $\pm$ 0.05

* shoots dried after 15 days.

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

Elongated shoots were later separated individually and transferred to root induction media. MS (full strength, ½ strength and 1/4) containing IBA, IPA, NAA and IAA in various combinations was used. MS full strength with IBA alone or in combination. IBA+ IAA or IBA + IPA showed callus development at the base of shoots and no rooting was observed (Table 16). MS (1/2) (Table 17) supplemented with IBA, IAA also showed basal callus but by addition of IPA, root primordia were observed. These roots did not showed any increase in length even if, they were allowed to remain in media for 30 days. Later, addition of NAA in the media supplemented with IBA, IPA showed increase in root length (Table 17). This shows that NAA is responsible for increase in root length. Addition or omission of IAA did not affect the root growth adversely. Even increase in the concentration of hormones in the media did not further improve the rooting. Media supplemented with IBA, IPA, and NAA showed fibrous type of 10 to 12 roots after 9 to 11 days of

Table- 16: Effect of MS full strength and Auxins on rooting percentage.

IBA mg/l	IPA mg/l	NAA mg/l	IAA mg/l	Percentage of rooting & S.E	Mean number of root & S.E
0.5	0.0	0.0	0.5	--	---
0.5	0.1	0.0	0.0	Nodular basal callus	---
0.5	0.5	0.0	0.0	Callus with root 2+1.32	3+1.27
0.5	0.1	0.1	0.0	20+4.31	4+1.11
0.5	0.5	0.5	0.5	Basal callus	----
1.0	1.0	1.0	1.0	Basal callus	----

Table-17: Effect of MS 1/2 strength and Auxins on rooting percentage

IBA mg/l	IPA mg/l	NAA mg/l	IAA mg/l	Percentage of rooting	Mean number of root &S.E	Root length (cm)
0.5	0.0	0.0	0.5	Basal callus	--	--
0.5	0.1	0.0	0.0	Basal callus with root primordia	--	--
0.5	0.5	0.0	0.0	Callus with root 20±1.32	30 ± 0.21	2 –3
0.5	0.1	0.1	0.0	60±0.31	25 ± 0.2	4 -5
0.5	0.1	0.2	0.0	90±0.05	15 ± 0.05	4 -5
0.5	0.1	0.3	0.0	70±0.20	15 ± 0.2	--
0.5	0.5	0.5	0.0	60±1.27	30 ± 0.21	4 -5
0.5	0.5	0.5	0.5	Basal callus	--	--
1.0	1.0	1.0	1.0	Basal callus	--	--

inoculation. Roots of light pink color, slender with differentiated root cap and root hair were observed. The growth rate of root observed was 1 cm/3 days. Among all the combinations tried, IBA (0.5mg/l), IPA (0.1mg/l) and NAA (0.1mg/l) found to be most effective for root induction and growth, giving 90% rooting (Table 17). MS (1/4) with the same hormonal concentration showed only 50 % rooting, slow shoot growth with thin roots (Fig.12).

Plantlets with well developed root system were removed from culture tubes and washed thoroughly under tap water so as to remove the remaining media attached to roots and transferred to glass jars filled with sterile soilrite (Fig.13). Initially for few days plant lets were irrigated with MS salts & 20% sucrose, covered with plastic bags and kept in hardening unit at 30^0C with high humidity (70-80%) for approx. 20 days to reduce desiccation. Later, these plantlets were gradually brought to low humidity conditions (30-40%). After keeping for few days, transferred to earthen pots containing soil:sand:farm yard manure (2:1:1), irrigated with MS salts (1/2) and kept in green house (Fig.154. After 30 days hardened plants were transferred in the

nursery under natural light. All plants established successfully under natural conditions (up to 90 %.)

Morphogenetic changes :

1. *During regeneration of shoot from callus*.

Thickenings of cell walls leading to xylem formation was observed (Fig.4) in media supplemented with NAA and BAP. Well developed xylem vessels were observed in media supplemented with BAP (2 – 3 mg/l) + NAA(0.5 – 1.0 mg/l). BAP below 2mg/l in combination with NAA below 0.5 mg/l failed to show xylogenesis (Fig.9) in the cells. The cell size was approx. 250 μm both in the callus generated on media supplemented with 2,4-D and lower concentration of BAP and NAA and in friable callus. While normal cell size reduced to 60 – 70 μm in the regenerated callus while xylem cells were of 120 – 130 μm size. The embryonic cells were observed in clumps (Fig.7).

2. Anatomical changes during hardening process.

Anatomical changes occurring during hardening process were also studied to note the gradual process of acclimatization. Transverse section of leaf from field plant showed epidermis with many unicellular hairs and double layer of hypodermis (Fig. 20). While transversal section of leaf of Tissue

cultured plant let showed epidermis with only 1-2 hair on it (Fig. 15). Well differentiated hypodermis was not seen. The cell size of epidermis was large as compared to normal plants.

The per unit leaf area of tissue cultured plant showed 35 stomata & stomatal pores were wide open (Fig. 21). Where as in naturally occurring plant 25 stomata were observed per unit leaf area . After hardening in tissue culture plant also 20-26 stomata were observed (Fig.22).

The T. S of stem (Fig.17) of naturally occurring plant showed well developed cuticle layer, epidermis with many hairs on it and well differentiated hypodermis were little thick walled. The cells of hypodermis were little thick walled as small as compared to tissue culture plant. But the T.S of stem (Fig.18) of tissue culture plant let showed thin epidermal layer with out cuticle. Only few (2 –3) hairs were seen on it. Hypodermis was under developed and undifferentiated from parenchymtous cells inside. Parenchymtous cells were larger in size culture plant was found larger as compare to normal occurring plant the similar results were reported by Selvaraj et al. (1995). The cell size was reduced in hardened plant (Fig.19).

5. Effect of macro nutrient on growth of callus from different plant parts .

MS basal media (2,4-D 2.0 mg/l and BAP 0.5 mg/l) was considered as control for studying the effect of macro nutrient on callus growth and dynamics. By changing the concentration of macro nutrient following results were observed. Leaf, stem, inflorescence responded to change in macro nutrient but roots did not responded to any variation.

A. The effect of macro nutrient on growth of callus from leaf.

The BM media was supplemented with different concentration of NH_4NO_3. By increasing the concentration of NH_4NO_3,(Table 19) it was found that initiation of the callus enhanced to 8^{th} day instead of 10 to 12 days. While lower concentration delayed callus initiation. Higher concentration (>1950 mg/l) could just initiate the callus of leaf but further growth was ceased as indicated by no increase in fresh weight (0.3 g within 48 days).

The BM media was supplemented with different concentration of KNO_3. It was found that lower concentration of KNO_3 showed poor callus growth. The increase in weight was only 0.1g in 48 days after which stationary phase was observed. KNO_3

at 1950 mg/l slowed down the callus growth. Hence best callus growth was observed at KNO_3 1900 mg/l (Table 20).

Table -18: Effect of MS 1/4 concentration and Auxins on rooting percentage.

IBA mg/l	IPA mg/l	NAA mg/l	IAA mg/l	Percentage of rooting	N0 of roots Mean number of root &S.E	Root length (cm)
0.5	0.0	0.0	0.5	Basal callus	---	---
0.5	0.1	0.0	0.0	Nodular basal callus	---	---
0.5	0.5	0.0	0.0	Callus with root 20+1.32	30+0.21	2 –3
0.5	0.1	0.1	0.0	60+1.31	12+0.11	2-3
0.5	0.5	0.5	0.0	40+1.53	----	1-2
0.5	0.5	0.5	0.5	Basal callus	----	---
1.0	1.0	1.0	1.0	Basal callus	----	---

Table-19 :Effect of Ammonium nitrate on growth of callus from leaf.

Sr. NO.	NH_4NO_3 Mg/l	Callus initiation (days)	Initial approx wt. of leaf (g)	Mean wt after 24days &S.E (g)	Mean wt after 48days & S.E (g)	Mean wt after72 days & S.E (g)	Mean wt after 98days & S.E (g)
1.	1550	15	0.08	0.50±2.21	0.58±2.51	0.61+ 3.42	0.51+3.51
2.	1650	12	0.08	0.61±1.53	2.12±1.73	4.24+1.60	4.33+1.52
3.	1700	12	0.08	0.61±3.81	2.11±3.72	4.23+ 3.53	3.62+3.51
4.	1750	10	0.08	0.61±2.89	2.37±2.78	3.54+2.85	3.21+3.54
5.	1800	10	0.08	0.61±2.79	2.36±2.98	3.51+2.95	3.22+2.83
6.	1850	8	0.08	0.65±2.83	2.45±2.83	3.51+2.97	3.21+2.85
7	1900	8	0.08	0.67±2.51	2.43±2.86	3.52+2.87	3.23+2.67
8	1950	9	0.08	0.32±3.87	0.34±3.85	0.29+3.54	0.20+3.68
9	2000	25	0.08	0.22±3.56	0.32±3.64	**	**

Table -20: Effect of potassium nitrate on growth of callus from leaf.

Sr. No.	KNO_3 mg/l	Callus initiation (days)	Initial approx wt. of leaf (g)	Mean wt after 24days and S.E (g)	Mean wt after 48 days and S.E (g)	Mean wt after 72 days and S.E (g)	Mean wt after 98 days and S.E (g)
1	1700	**	0.08	**	**	**	**
2	1750	15	0.08	0.10±3.11	0.10±3.31	**	**
3	1800	15	0.08	0.10±3.60	0.10±3.64	**	**
4	1850	12	0.08	0.41±2.89	0.41±2.82	0.40±2.74	0.40±2.45
5	1900	12	0.08	0.61±1.53	2.11±1.73	4.21±1.60	4.52±1.52
6	1950	20	0.08	0.32±1.64	0.31±1.75	0.35±1.78	0.29±1.62
7	2000	**	0.08	**	**	**	**

The BM media was supplemented with different concentration of $MgSO_4$ for callus initiation in leaf. As can be seen from (Table 21) lower concentration of $MgSO_4$ (<370 mg/l) showed no callus growth, while higher concentration (>410mg/l) delayed callus initiation and no further growth. The highest callus growth was observed in the range of 370-390 mg/l with maximum fresh weight. $MgSO_4$ did not have significant effect on initiation of callus.

The BM media was supplemented with different concentration of KH_2PO_4, for callus initiation from leaf. By increasing and decreasing the concentration of KH_2PO_4 callus growth was adversely affected (Table 22). However, the changes

in the concentration of KH_2PO_4 did not significantly effect the callus initiation.

The BM media was supplemented with different concentration of $CaCl_{2.}$ for callus growth from leaf (Table 23). Callus failed to grow in the lower (<420 mg/l) as well as higher(>460 mg/l) concentration of $CaCl_{2.}$

B. Effect of macro nutrient on the callus induction from inflorescence.

The BM media was supplemented with different concentration of NH_4NO_3 (Table 24). By increasing the concentration of NH_4NO_3 >1850 to 1900 mg/l, it was found that the callus initiation enhanced by few days i.e 8^{TH} day instead of 10 days of inoculation. While lower concentration (>1950mg/l) could just initiate the callus of leaf and no further growth or no increase in fresh weight (3.2 g in 98 days) was observed. Both in lower and higher concentrations of KNO_3 failed to grow callus (Table 25).

Table-21: Effect of MgSO4 on callus growth of callus from leaf.

Sr. No	$MgSO_4$ mg/l	Callus initiation (days)	Initial approx wt. of leaf (g)	Mean wt after 24days and S.E S1 (g)	Mean wt after 48 days and S.E S2 (g)	Mean tafter72 days and S.E S3 (g)	Mean wt after 98 days and S.E S4 (g)
1	330	24	0.08	Dried			
2	350	24	0.08	Just callus initiation			
3	370	12	0.08	0.61+1.52	2.15+1.54	4.23+1.62	2.31+1.72
4	390	12	0.08	0.31+1.62	1.62+1.71	1.75+1.69	1.32+1.60
5	410	20	0.08	Just callus initiation			
6	420	20	0.08	Dried			

Table- 22: Effect of KH2PO4 on growth of callus from leaf.

Sr. NO.	KH_2PO_4 mg/l	Callus initiation (days)	Initial approx wt. of leaf (g)	Mean wt after 24 days and S.E (g)	Mean wt after 48 days and S.E (g)	Mean wt after 72 days and S.E (g)	Mean wt after 98 days and S.E (g)
1	160	24	0.08	Just initiated			
2	165	15	0.08	0.16+2.58	Dried		
3	170	12	0.08	0.61+1.52	2.17+1.57	4.25+1.56	4.32+1.52
4	175	12	0.08	0.65+2.81	2.35+2.85	3.51+2.90	2.35+2.87
5	180	10	0.08	0.12+2.71	0.15+2.76	Dried	
6	185	10	0.08	No callus			

Table -23: Effect of $CaCl_2$ on growth of callus form leaf.

Sr. NO.	CaCl2 mg/l	Callus initiation (days)	Initial approx wt. of leaf (g)	Mean wt after 24 days and S.E (g)	Mean wt after 48 days and S.E (g)	Mean wt after 72 days and S.E (g)	Mean wt after 98 days and S.E (g)
1	400	24	0.06	Just initiated			
2	420	15	0.07	0.52±1.32	0.98 ± 0.35	1.92 ±1.23	1.90±1.22
3	440	12	0.08	0.61±1.24	2.11±1.55	4.22 ±1.43	4.31±1.27
4	460	12	0.06	0.51±3.57	0.82±4.24	2.61 ±4.64	2.34±4.72
5	480	15	0.05	**Dried**			
6	500	24	0.04	Dried			

Table -24. Effect of NH_4NO_3 on growth callus from floral stem.

Sr. No.	NH_4NO_3 mg/l	Callus initiation (days)	Initial approx wt. of floral stem (g)	Mean wt after 24days and S.E (g)	Mean wt after 48 days and S.E (g)	Mean wt after 72 days and S.E (g)	Mean wt after 98 days and S.E (g)
1	1550	**	0.04	**	**	**	**
2	1650	15	0.06	0.41±2.38	1.57±1.23	2.6±1.24	3.2±1.26
3	1700	15	0.05	0.44±3.45	1.57±2.43	2.4±2.45	3.2±1.67
4	1750	10	0.06	0.46±3.41	1.55±1.63	2.5±2.34	3.2±1.87
5	1800	8	0.05	0.45±341	1.59±1.89	2.4±2.67	3.0±2.84
6	1850	8	0.06	0.47±3.84	1.69±1.53	2.7±2.48	3.0±1.53
7	1900	10	0.05	0.44±	1.48±2.34	2.7±1.89	2.8±1.89
8	1950	30	0.03	Just initiated			
9	2000	30	0.05	dried			

The lower concentration of $MgSO_4$ i.e. below 350 mg/l showed no callus initiation (Table 26), while higher concentration i.e. above 350 mg /l delayed callus initiation and callus growth was very slow (0.1 g with in 98 days). Same results were obtained for KH_2Po_4 & $CaCl_2$ (Table 27 and 28).

C. Effect of macro nutrient on the callus induction from stem.

Higher concentration of NH_4No_3 showed good callus initiation but enhanced exponential phase(Table 29). Lower & higher concentrations of KNO_3, $MgSO_4$, KH_2Po_4 and $CaCl_2$slow down the callus growth(Table 30,31,32 and 33).

D. Effect of macro nutrient on growth of callus of flower.

Changes were made the concentration of NH_4NO_3 (Table 34), KNO_3 (Table 35), $MgSO_4$ (Table 36), KH_2Po_4 (Table-

37)and $CaCl_2$ (Table-38) in BM medium. Both lower and Higher concentrations of all the elements were found unfavorable for callus initiation and growth.

6. Biochemical changes during organogenesis

Proteins, DNA, RNA and enzyme activities were studied during the callus growth and during the process its differentiation/organogenesis.

The protein value of embryonic and non embryonic callus was calculated by Lowery's method (1951). Significant differences were observed in the protein values between the embryonic and non-embryonic callus as the protein value of non embryonic callus varied from 247—379μg/fresh weight. Where as the protein value of regenerating callus was 456μg/fresh weight. The DNA and RNA in the non embryonic callus varied from 52—69μg/fresh wt and 257μg/fresh weight respectively while DNA and RNA in the regenerating callus ranged from 98μm/fresh weight and 351μg/fresh weight receptively. The α -amylase activity of non embryonic callus culture was 4.3 maltose released mg/gm/fresh wt/15 min. while the α -amylase activity of regenerating callus was higher i.e. 6.3 maltose released mg/gm/fresh wt/15 min. Electrophoretic analysis of *Suaeda nudiflora* proteins during morphogenesis showed protein bands at 43 KD while in non embryo in non embryonic callus it was observed at 26.7 KD.

Table – 25 : Effect of KNO3 on growth of callus form floral stem.

Sr. No.	KNO_3 mg/l	Callus initiation (days)	Initial approx wt. inflorescence of (g)	Mean wt after 24days and S.E (g)	Mean wt after 48 days and S.E (g)	Mean wt after 72 days and S.E (g)	Mean wt after 98 days and S.E (g)
1	1700	30	0.04	0.09±2.89	dried		
2	1750	15	0.06	0.20±2.71	0.20	dried	
3	1800	15	0.07	0.45±2.45	1.57±1.69	2.0±2.98	1.9±2.56
4	1850	10	0.08	0.45±1.89	1.56±1.89	2.9±1.84	3.2±1.68
5	1900	10	0.06	0.45±1.65	1.49±1.75	2.9±1.74	3.2±1.63
6	1950	20	0.08	0.45±1.53	1.52±1.69	2.0±1.63	1.9±1.43
7	2000	30	0.03	dried			

Table - 26: Effect of MgSO4 on growth of callus of floral stem.

Sr. No	$MgSO_4$ mg/l	Callus initia-tion (days)	Initial approx wt. of inflorescence (mg)	Mean of wt after24 days and (g)	Mean of wt after 48 days and (g)	Mean of wt after 72 days (g)	Mean wt after 98 days and S.E (g)
1	330	35	0.03	dried			
2	350	15	0.06	**0.45±1.35**	**1.57±1.87**	**2.9±1.93**	**3.2±1.62**
3	370	15	0.05	**0.45±1.63**	**1.57±1.23**	**2.9±1.32**	**3.2±1.32**
4	390	20	0.07	0.08±1.25	0.10±1.75	0.12±1.65	0.12±1.24
5	410	20	0.05	0.08±1.73	0.10±3.25	0.10±1.31	0.10±1.25
6	420	30	0.04	dried			

Table -27: Effect of KH_2PO_4 on growth of callus from stem.

Sr. No.	KH_2PO_4 Mg/l	Callus initiation (days)	Initial approx wt. of stem (mg)	Mean wt after 24 days and S.E (g)	Mean wt after 48 days and S.E (g)	Mean wt after 72 days and S.E (g)	Mean wt after 98 days and S.E (g)
1	160	24	0.02	Just initiated			
2	165	15	0.04	0.32±1.56	0.58±1.37	0.54±1.44	0.41±1.37
3	170	12	0.07	0.55±1.67	2.28±1.83	2.41±1.72	2.34±1.61
4	175	12	0.05	0.31±1.45	0.38±1.63	0.30±1.95	0.28±1.89
5	180	10	0.03	0.08±2.68	dried		
6	185	10	0.05	No callus			

Table – 28 : Effect of $CaCl_2$ on growth of callus of floral stem.

Sr. NO.	$CaCl_2$ mg/l	Callus initia -tion (days)	Initial approx wt. of inflore-scence (g)	Mean wt after 24 days and S.E (g)	Mean wt after 48 days and S.E (g)	Mean wt after 72 days and S.E (g)	Mean wt after 98 days and S.E (g)
1	400	**	0.03	**	**	**	**
2	420	15	0.05	0.45±1.57	1.53±1.89	1.70±1.62	1.92±1.36
3	440	15	0.07	0.48±1.89	1.57±1.62	2.9±1.34	3.2±1.47
4	460	15	0.08	0.08±2.78	Dried		
5	480	15	0.04	0.05±3.25			
6	500	30	0.02	Dried			

Table- 29 : Effect of Ammonium nitrate on growth of callus from stem

Sr. No.	NH_4NO_3 mg/l	Callus initia-tion (days)	Initial approx wt. of stem (g)	Mean of wt after 24 days &S.E (g)	Mean of wt after 48 days & S.E (g)	Mean of wt after72 days & S.E (g)	Mean of wt after 98 days & S.E (g)
1.	1550	15	0.05	0.30±2.43	0.38±3.13	0.65±2.67	0.65±1.32
2.	1650	12	0.04	0.50±2.38	2.00±2.89	2.65±2.43	2.75±2.23
3.	1700	12	0.06	0.51±2.53	2.50±2.62	2.64±1.89	2.75±1.59
4.	1750	10	0.08	0.55±1.49	2.30±1.86	2.52±1.68	2.35±1.45
5.	1800	10	0.05	0.57±1.63	2.08±1.57	2.42±1.63	2.33±1.57
6.	1850	8	0.09	0.53±1.48	2.18±1.53	2.42±1.35	2.33±1.52
7	1900	8	0.07	0.55±1.58	2.28±1.58	2.42±1.57	2.31±1.63
8	1950	9	0.04	0.42±1.68	2.32±1.74	2.42±1.69	2.31±1.47
	2000	25	0.02	0.25±1.28	0.28±1.63	0.11±1.53	0.10±1.31

Table - 30: Effect of Potassium Nitrate on growth of callus from stem

Sr. NO.	KNO_3 Mg/l	Callus initiation (days)	Initial approx wt. of stem (g)	Mean of wt after 24 days and S.E (g)	Mean wt of after 48 days and S.E (g)	Mean wt of after 72 days and S.E (g)	Mean wt of after 98 days and S.E (g)
1	1700	**	0.02	**	**	**	**
2	1750	15	0.04	0.31±2.67	0.62±2.54	0.51±2.42	0.51±2.37
3	1800	15	0.03	0.52±2.85	2.81±2.74	2.20±2.52	2.10±2.53
4	1850	12	0.05	0.55±1.36	2.21±1.53	2.38±1.68	2.21±1.63
5	1900	12	0.07	0.55±1.43	2.28±1.15	2.41±1.22	2.34±1.36
6	1950	20	0.04	0.61±1.53	2.30±1.74	3.89±1.38	3.80±1.64
7	2000	**	0.06	0.38±1.43	2.00±1.87	2.80±1.63	2.70±1.85

Table-31: Effect of $MgSO_4$ on callus growth of callus derived from stem.

Sr. NO.	Mg SO_4 (mg/l)	Callus initia -tion (days)	Initial approx wt. of stem (g)	Mean wt after 24days and S.E (g)	Mean wt after 48 days and S.E (g)	Mean tafter72 days and S.E (g)	Mean wt after 98 days and S.E (g)
1	330	24	0.03	Dried			
2	350	24	0.02	Just callus initiation			
3	370	12	0.05	0.55±1.54	2.22±1.34	2.45±1.23	2.36±1.54
4	390	12	0.06	0.52±1.51	2.18±1.42	2.32±1.45	2.25±1.48
5	410	15	0.05	0.51±1.89	0.98±1.67	0.99±1.52	0.89±1.89
6	420	20	0.04	0.08±2.14	0.08±2.82	Dried	

Table -32: Effect of KH_2PO_4 on growth of callus from stem.

Sr. No.	KH_2PO_4 Mg/l	Callus initiation (days)	Initial approx wt. of stem (mg)	Mean wt after 24 days and S.E (g)	Mean wt after 48 days and S.E (g)	Mean wt after 72 days and S.E (g)	Mean wt after 98 days and S.E (g)
1	160	24	0.02	Just initiated			
2	165	15	0.04	0.32±1.56	0.58±1.37	0.54±1.44	0.41±1.37
3	170	12	0.07	0.55±1.67	2.28±1.83	2.41±1.72	2.34±1.61
4	175	12	0.05	0.31±1.45	0.38±1.63	0.30±1.95	0.28±1.89
5	180	10	0.03	0.08±2.68		dried	
6	185	10	0.05	No callus			

Table – 33: Effect of $CaCl_2$ on growth of callus from stem.

Sr. NO.	$CaCl_2$ mg/l	Callus initiation (days)	Initial approx wt. of stem (g)	Mean wt after 24 days and S.E (g)	Mean wt after 48 days and S.E (g)	Mean wt after 72 days and S.E (g)	Mean wt after 98 days and S.E (g)
1	400	24	0.04	Just initiated			
2	420	15	0.05	0.58 ±1.82	0.96±1.95	1.95±1.93	1.98±1.82
3	440	12	0.08	0.55±1.92	2.28±1.96	2.41±1.89	2.34±1.97
4	460	12	0.06	0.50±3.58	0.86±4.54	2.69±4.97	2.37±4.81
5	480	15	0.05	Dried			
6	500	24	0.08	Dried			

Table - 34 : Effect of Ammonium nitrate on growth of callus from flower

Sr. NO.	NH_4NO_3 Mg/l	Callus initiation (days)	Initial approx wt. Of flower (g)	Mean wt after 48 days & S.E (g)	Mean wt after 72days & S.E (g)	Mean wt after 98 days & S.E (g)	Mean wt after 120 days & S.E (g)
1.	1550	24	0.03	Dried	Dried	Dried	Dried
2.	1650	20	0.08	0.52±2.64	1.62±2.32	2.32±1.93	2.22±1.56
3.	1700	20	0.05	0.53±1.89	1.65±2.53	2.34±1.37	2.22±1.59
4.	1750	20	0.07	0.53±1.38	1.65±1.52	2.34±1.67	2.26±1.35
5.	1800	20	0.06	0.62±1.67	1.68±1.48	2.35±1.73	2.26±1.39
6.	1850	19	0.07	0.64±1.83	1.72±1.63	2.36±1.28	2.28±1.46
7	1900	19	0.02	0.66±1.65	1.75±1.73	2.39±1.85	2.26±1.82
8	1950	20	0.02	0.40±1.62	0.84±1.37	2.28±1.53	2.11±1.74
9	2000	25	0.02	0.22±1.81	0.23±1.74	0.21±1.96	0.08±1.63

Table - 35: Effect of Potassium Nitrate on growth of callus from flower

Sr. NO.	KNO_3 mg/l	Callus initiation (days)	Initial approx wt. Of Flower (g)	Mean of wt after 48days and S.E (g)	Mean of wt after 72 days and S.E (g)	Mean of wt after 98 days and S.E (g)	Mean of wt after 120 days and S.E (g)
1	1700	--	0.02	Dried	Dried	Dried	Dried
2	1750	--	0.02	Dried	Dried	Dried	Dried
3	1800	15	0.02	0.08	0.02	Dried	Dried
4	1850	12	0.02	0.08	0.10	0.10	0.06
5	1900	12	0.02	0.52	1.63	2.0	2.9
6	1950	--	0.02	Dried	Dried	Dried	Dried
7	2000	--	0.02	Dried	Dried	Dried	Dried

Table- 36 : Effect of MgSO4 on callus growth of callus from flower

Sr No	Mg SO_4 (mg /l)	Callus initiation (days)	Initial approx wt. of flower (g)	Mean of wt after 48days and S.E (g)	Mean of wt after 72 days and S.E (g)	Mean of wt after98 days and S.E (g)	Mean of wt after 98 days and S.E (g)
1	330	24	0.02	Dried			
2	350	24	0.04	Just callus initiation			
3	370	12	0.06	0.55±1.56	2.22±1.72	2.45±1.65	2.36±1.45
4	390	12	0.05	0.52±1.38	2.18±1.45	2.32±1.38	2.25±1.26
5	410	15	0.02	0.51±1.56	0.98±1.37	0.99±1.54	0.89±1.67
6	420	20	0.03	0.08±1.45	0.08±1.56	Dried	

Table - 37: Effect of KH_2PO_4 on growth of callus from flower.

Sr. No.	KH_2PO_4 mg/l	Callus initiation (days)	Initial approx wt. Of flower (mg)	Mean of wt after 24 days and S.E (g)	Mean of wt after 48 days and S.E (g)	Mean of wt after 72 days and S.E (g)	Mean of wt after 98 days and S.E (g)
1	160	24	0.02	Just initiated			
2	165	15	0.04	0.32±1.67	0.58±1.53	0.54±1.53	0.41±1.67
3	170	12	0.03	0.55±1.56	2.28±1.64	2.41±1.42	2.34±1.53
4	175	12	0.05	0.31±1.38	0.38±1.63	0.30±1.28	0.28±1.28
5	180	10	0.04	0.08±2.32	dried		
6	185	10	0.02	No callus			

Table – 38 : Effect of $CaCl_2$ on growth of callus derived from flower.

Sr. NO.	$CaCl_2$ mg/l	Callus initiation (days)	Initial approx wt. of flower (mg)	Mean of wt after 24 days and S.E (g)	Mean of wt after 48 days and S.E (g)	Mean of wt after 72 days and S.E (g)	Mean of wt after 98 days and S.E (g)
1	400	24	0.02	Just initiated			
2	420	15	0.03	0.58 ±1.82	0.96 ±1.95	1.93 ±1.93	1.98 ±1.82
3	440	12	0.05	0.55 ±1.92	2.28 ±1.96	2.41 ±1.89	2.34 ±1.97
4	460	12	0.04	0.50 ±3.58	0.86 ±4.54	2.69 ±4.97	2.37 ±1.81
5	480	15	0.02	Dried			
6	500	24	0.04	Dried			

7. Encapsulation or shoot tips or Synthetic seeds

Shoot tips were coated with different concentration of sodium alginate(2-4%). Best results were obtained in 2.5% Sodium alginate it was found to be the optimum concentration for encapsulation.. Initially the shoot tips were encapsulated in gel matrix made either with distilled water or MS medium alone (Fig.23). Later activated charcoal was also used as it helps in the adsorption and deadsorption to control release of nutrient in the production of synthetic seeds (Fig.24) .

Plantlet development on different nutrient media. The encapsulated shoot tips were cultured on MS medium and MS 1/2 media (no hormone), MS 1/2 +IBA (0.5mg/l) +NAA (0.2mg\l) + IPA (0.1mg/l) for germination.(Table – 39, 40) Development of roots were observed after 3 days of culture while few roots protruded out from the gel matrix after about a week Among the different concentrations of MS media tested, shoot tips placed on MS medium (with hormones) showed few plant lets formation. MS (1/2) gave highest rate (90%) of plant development within a week and transplantable plant lets could be obtained in four weeks.

Plantlet development on different substrate. Encapsulated shoot tips were also cultured on different substrate

such as filter paper moistened with distill water and with Ms !/2 and autoclaved soil rite supplemented with MS 1/2 in a petridish. Among these substrates, the percentage of plant let formation was 90% on filter paper moistened with MS 1/2 while 70% plant let formation was observed on filter paper supplemented with distill water while only 10% plant let formation could be obtained on soilrite.

Plantlet formation and their establishment in soil.

I) The plantlets formed in MS medium were carefully removed from culture vessels. After thoroughly washing the roots in tap water the rooted plantlets were placed in pot containing sand and farm manure (1:1)

II) The plantlet formed on filter paper supplemented distill water and supplemented with MS 1/2 were placed in pot containing sand.

The plant was harden in a green house for a month before field planting.

8. Influence of amiolerating chemicals on salinity tolerance of *Suaeda nudiflora* under suspension callus culture.

Stationary suspension culture were initiated by pouring 25 ml liquid MS- media in flask of 125 ml. As the callus growth was seen without the use of shaker, Approx. 100 mg stock

callus generated from stem was gently inoculated using forceps. The suspension culture was added with NaCl 10ppm, 20ppm, 40ppm, 60 ppm, 80 ppm, 100 ppm, 120 ppm, 140 ppm (Table. 41-42 - 43). It was found that the cells could tolerate NaCl up to 20ppm. In the suspension cultures different concentrations of NAA and KN were also used individually as well as in combination. It was observed that by addition of NAA, cells could tolerate salinity up to 80ppm while with KN these could tolerate up to 70ppm. Their combination lead the cells to tolerate up to 140ppm NaCl. The protein value decreased as the concentration of NaCl increased while proline value increased with increase in NaCl concentration

Table –39: Plantlet development (%) from encapsulated shoot tips on different materials & media.

Encapsulation matrix 3% sodium alginate	MEDIA		
	MS	MS 1/2	MS+IBA+ NAA+IPA
Distill water	0	5	20
MS	0	10	20
MS 1/2	0	0	30
MS 1/2 +IBA+NAA+ IPA	20	90	40

Table –40: Effect of different substrates on plant let development from encapsulated shoot tips.

Substrate	% of plant let development
Filter paper supplement with distilled water	90
Filter paper supplemented with MS liquid	70
Soil rite	10

Table –41 : Amiolerating effect of NAA on protein and proline content under different levels of NaCl

NAA mg	Protein/ Proline μm	NaCl ppm								
		00	10	20	30	40	50	60	70	80
0.5	Protein	226	226	182	162	52	00	--	--	--
	Proline	62	65	150	220	230	00	--	--	--
1.0	Protein	236	236	190	160	149	135	26	--	--
	Proline	25	27	10	210	250	320	330	--	--
2.0	Protein	246	246	202	186	160	148	130	120	26
	Proline	25	27	49	66	72	240	290	310	320

Table -42 : Amiolerating effect of Kinetin on protein and proline content in callus under different levels of NaCl

KN mg/l	Protein/ Proline	NaCl ppm								
		00	10	20	30	40	50	60	70	80
0.5	Protein	236	236	174	138	45	--	--	--	--
	Proline	26	26	40	59	70	--	--	--	--
1.0	Protein	297	292	246	220	170	101	39	--	--
	Proline	36	36	91	138	162	204	255	--	--
2.0	Protein	291	291	242	216	190	168	101	35	--
	Proline	37	37	110	139	167	206	258	265	--

Table - 43 : Amiolerating effect on KN &NAA on protein and proline content in callus under different levels of NaCl

NAA Mg/l	KN mg/l	Protein /Proline µm	NaCl ppm														
			00	10	20	30	40	50	60	70	80	90	100	110	120	130	160
0.5	0.5	Protein	226	226	172	162	52	--	--	--	--	--	--	--	--	--	--
		Proline	26	26	40	69	80	--	--	--	--	--	--	--	--	--	--
1.0	0.5	Protein	982	982	921	901	870	842	810	754	721	485	130	120	30	--	--
		Proline	34	38	62	67	79	86	98	118	128	156	176	208	210	--	--
2.0	1.0	Protein	1170	1170	1135	1095	1065	1025	1000	945	910	825	730	360	120	40	--
		Proline	35	37	66	70	82	90	100	120	130	160	180	210	260	265	--
1.0	2.0	Protein	450	391	285	274	262	251	110	39	--	--	---	--	--	--	--
		Proline	32	45	60	69	75	91	100	160	--	--	--	--	--	--	--

9. Effect of different chemicals on Callus of *Suaeda nudiflora* .

1. Betain :

Callus was generated from different plant parts on MS-media (enriched with 2,4-D 1.0mg/l and BAP 0.5mg/l) was also supplemented with Betain (0.5mg/l, 1.0 mg/l, 2mg/l, 3mg/l and 4mg/l)(Table. 44). Media supplemented with betain showed also callus initiation after 10 days indicating no significant effect on callus initiation up to 3mg/l where as 4mg/l caused decrease in callus growth.

2. Casein Hydrolysate :

Callus was generated from leaf on MS-media (with 2,4-D 1.0mg/l and BAP 0.5mg/l) supplemented with CH as additive in the range of 150mg/l, 200mg/l, 250mg/l and 300mg/l(Table. 45). In the absence of CH in medium, callus growth was very slow in all the plant parts and callus initiation was observed after 20 -25 days. When media was supplemented with CH 200mg/l, it showed callus initiation with in 8-10 days in leaf. Media supplemented with CH 200mg/l generate 3.60 g callus in leaf but with increase in the concentration of CH above 200mg/l, slight increase in callus weight was observed. Media supplemented with CH 250 mg/l showed callus initiation within 8-10 days in stem, flower and inflorescence. But CH 350mg/l

delayed callus initiation in all plant parts, as callus initiation started after 10-12 days. Organogenesis was observed only in media supplemented with CH 250 mg/l in stem while in remaining concentrations callus failed to regenerate.

Table -44 : Effect of betain on callus growth (fresh weight in gm.)

Hormones (mg/l) 2,4-D + BAP	Betain (mg/l) 0.0	0.5	1.0	2. 0	3.0	4.0
1.0 + 0.5 2.0 leaf	3.61g	3.60g	3.59g	3.58g	3.61g	3.48g
1.0 + 0.5 stem	2.60g	2.60g	2.61g	2.69g	2.62g	2.51g
1.0 + 0.5 flower	2.29g	2.30g	2.32g	2.31g	2.30g	2.01g
1.0 + 0.5 floral bud	2.71g	2.70g	2.71g	2.72g	2.70g	2.64g

Table -45 : Effect of Casein hydrolysate on callus growth (fresh weight in gm.).

Hormones (mg/l) 2,4-D + BAP	Casein hydrolysate (mg/l) 00	150	200	250	300	350
1.0 + 0.5 leaf	0.89g	1.65g	3.60g	3.66g	3.69g	3.40g
1.0 + 0.5 stem	0.80g	1.10g	2.26g	2.60g	2.65g	2.45g
1.0 + 0.5 flower	0.60g	1.02g	2.20g	2.30g	2.32g	2.10g
1.0 + 0.5 floral bud	0.60g	1.10g	2.35g	2.70g	2.74g	2.51g

Table -46 : Effect of Ascorbic acid on growth of callus (fresh weight in gm.)

Hormones (mg/l) 2,4-D + BAP	Ascorbic acid (mg/l) 0.0	2.5	5.0	10	20	25
1.0 + 0.5 leaf	3.61	3.60g	3.60g	3.60g	3.61g	3.48g
1.0 + 0.5 stem	2.60	2.60g	2.60g	2.69g	2.69g	2.51g
1.0 + 0.5 flower	2.29	2.30g	2.32g	2.32g	2.32g	2.01g
1.0 + 0.5 floral bud	2.71	2.70g	2.70g	2.72g	2.72g	2.64g

3. Ascorbic acid (AA) :

Callus was generated from different plant part on MS-media supplemented with 2,4-D 1mg/l and BAP 0.5 mg/l with AA as additive in the range of 2.5mg/l, 5mg/l, 10mg/l, 20mg/land 25mg/l. By addition of AA, callus initiation started after 8-10 days and no other significant change was observed indicating AA does not have any significant effect on callus initiation (Table.46). Generally callus derived from leaf is blackish in colour, AA in media reduced blackish colour of callus.

4. Silver nitrate :

Callus was generated from different plant parts on MS-media supplemented with 2,4-D 1mg/l and BAP 0.5mg/l with silver nitrate in the range of 0.5 mg/l , 1.5mg/l, 2.5mg/l, 3.5mg/l. and 4.5mg/l as an additive. No significant change was

observed on callus initiation and growth. An increase in growth of callus from leaf was found maximum (990mg) followed by stem (910 mg). The growth of callus from floral stem was found to be 390mg while growth of callus from flower was minimum (210mg). Hence silver nitrate plays important role in callus growth. Addition of silver nitrate did not improve regeneration capacity of the callus (Table 47).

Table - 47 : Effect of Silver nitrate on growth of callus(fresh weight in gm.)

Hormones (mg/l) 2,4-D + BAP	Silver nitrate (mg/l) 0.0	0.5	1.5	2..5	3.5	4.5
1.0 + 0.5 leaf	3.61	3.75	3.98	4.61	4.60	4.60
1.0 + 0.5 stem	2.60	2.74	2.96	3.50	3.51	3.51
1.0 + 0.5 flower	2.29	2.31	2.45	2.49	2.50	2.50
1.0 + 0.5 floral bud	2.71	2.78	2.88	2.95	3.10	3.10

10 . Anther culture of *Suaeda nudiflora*.

Inflorescence was collected from the field grown plant. It was surface sterilized and anther were removed aseptically from it and inoculated on MS- media supplemented with various concentrations of IAA +NAA. The callus growth was very slow . After 60 days, callus it did not survived on the same media. Therefore, different media were tried for subculture. No media was found suitable for its survival. Even changes in the

nutrient of MS- media like NH_4NO_3, KNO_3, KH_2PO_4, $MgSO_4$ & $CaCl_2$ their was no increase in callus growth. Attempts were also made to culture anthers on N6& B5 but the callus initiation was not observed.

11. Effect of 2,4-D and colchicine in cell cultures:

Effect of 2,4-D (1,2,3, and4ppm) and colchicine (0.01%, 0.02%, 0.03% and 0.04%) was studied on cell cultures of Suaeda. From the (Table 48), it can be seen that both 2,4-D and colchicine induced cell growth in the suspension cultures during initial phase. But with the increase in concentration of both 2,4-D and colchicine, mitotic index decreased. The higher concentration of both drastically affected the growth. At 25^{th} day the maximum growth was observed in 2,4-D(2.00 ppm)while minimum was observed both in 2,4-D (4.0ppm) and colchicine (0.04%). (Table. 48)

Table -48 : Effect of 2,4-D and colchicine on mitotic index in Suaeda

Treatment	Total number of cells observed	% of cells dividing on 10 day of culture	% of cells dividing on 25 day of culture
Control (MS media without hormone)	150	4.59+1.77	1.57+0.60
2,4-D 1.0 ppm	150	6.78+1.12	8.74+1.02
2,4-D 2.0ppm	150	7.39+1.86	9.15+1.19
2,4-D 3.0ppm	150	4.67+1.57	6.48+1.20
2,4-D 4.0ppm	150	4.21+0.89	4.11+1.46
Colchicine 0.01%	150	5.23+1.51	6.47+1.34
Colchicine 0.02%	150	5.65+1.32	7.30+1.56
Colchicine 0.03%	150	5.46+1.67	5.37+0.92
Colchicine 0.04%	150	4.71+0.86	4.18+1.58

When different stages of mitosis were studied, it was found that the higher concentrations induced anomalies in the cell division. The most common anomalies were chromosome clumping, chromosome scattering and chromosome bridges. No chromosome doubling was observed in the cells.

B. SALICORNIA

A. Micropropagation through cotyledonary shoot.

Different media were used for micropropagation of cotyledonary shoots. MS media was found suitable for bud induction & growth while other medium B5 & N6 did not showed any significant effect on micropropagation. The fresh weight of plant increased to 41-42 mg with in 60 days (Table 49 & 50). Whit's media was found unsuitable of axillary bud induction. The fresh weight of explant remained 35 mg even after 60 days of inoculation with or with out sea water & sucrose 20-40 g/l. MS media with NAA (1.0mg/l) & BAP (2mg/l) and sucrose 35 g/l. induced multiple shoots from explant but further growth was not observed (Fig. 27). Even media with 10ml of sea water failed to induce shoot from explant. The increase in fresh weight of explant was 68 mg where as the fresh weight of explant in MS medium with sucrose 35 g/l was 78 mg and with double MS and 35g sucrose was 80mg in 60 days. Though 2-3 shoots were seen (Fig. – 25,26,28), they shoots remained in the same condition, without any further growth for 5 months (Table- 52) .

Table –49 : Effect of Sea water and sucrose on bud induction & growth of cotyledonary shoot tips.

N6 media	Weight of cotyledonary shoot tips (mg)			
	00days	25days	50 days	75 days
Sea water 1ml/L	32	40	40	39
Sea water 5ml/L	32	41	41	40
Sea water 10ml/L	32	41	41	40
Sea water 20ml/L	32	41	41	40
Sucrose 20mg/L	32	41	42	40
Sucrose 30mg/L	32	42	42	40
Sucrose 40mg/L	32	40	40	39

Table –50 : Effect of Sea water and sucrose on bud induction & growth of cotyledonary shoot tips.

B_5 media	Weight of cotyledonary shoot tips (mg)			
	00days	25days	50 days	75 days
Sea water 1ml/L	32	42	42	39
Sea water 5ml/L	32	42	42	40
Sea water 10ml/L	32	42	42	40
Sea water 20ml/L	32	42	42	40
Sucrose 20mg/L	32	42	42	40
Sucrose 30mg/	32	42	42	40
Sucrose 40mg/	32	40	40	39

Table –51: Effect of Sea water and sucrose on bud induction & growth of cotyledonary shoot tips.

White media	Weight of cotyledonary shoot tips (in mg)			
	00days	25days	50 days	75 days
Sea water 1ml/L	32	35	35	32
Sea water 5ml/L	32	35	35	32
Sea water 10ml/L	32	35	35	32
Sea water 20ml/L	32	35	35	32
Sucrose 20mg/L	32	35	35	32
Sucrose 30mg/	32	35	35	32
Sucrose 40mg/	32	35	35	32

Table –52 : Effect of Sea water and sucrose on bud induction & growth of cotyledonary shoot tips.

MS media	Weight of cotyledonary shoot tips (in mg)			
	00days	25days	50 days	75 days
Ms media	32	50	70	70
Sea water 1ml/L	32	50	70	70
Sea water 5ml/L	32	50	70	70
Sea water 10ml/L	32	48	68	68
Sea water 20ml/L	32	48	68	68
Sucrose 20mg/L	32	50	70	70
Sucrose 30mg/L	32	52	70	70
Sucrose 35 mg/L	32	52	78	78
Sucrose 40mg/L	32	48	68	68

B. Micropropagation through stem.

MS media (double strength) shoot tips were inoculated & supplied with BAP (2.0mg/l), NAA (1.0 mg/l) induced axillary bud. Addition of sea water (1-10 ml/l) also failed to induce shoot from stem (Table-54). The increase in fresh weight was 70 mg with in 60 days & the fresh weight of segment in 20 ml/l sea water was 60 mg. This shows that being salt resistant plant addition of sea water to it was of no use & its presence adversely affected the culture in all different concentration. .

Table –53 : Effect of Sea water and sucrose on bud induction & growth of cotyledonary shoot tips.

Double MS media	Weight of cotyledonary shoot tips (mg)			
	00days	30days	60 days	90 days
Ms media	32	50	70	70
Sea water 1ml/L	32	50	70	70
Sea water 5ml/L	32	50	70	70
Sea water 10ml/L	32	48	68	68
Sea water 20ml/L	32	48	68	68
Sucrose 20mg/L	32	52	70	70
Sucrose 30mg/L	32	54	74	74
Sucrose 35 mg/L	32	58	80	80
Sucrose 40mg/L	32	50	60	60

Table –54 : Effect of Sea water and sucrose on bud induction &growth of stem explant.

MS media	Weight of explant (mg)			
	00days	30days	60 days	90 days
Ms media	45	110	150	150
Sea water 1ml/L	45	110	150	150
Sea water 5ml/L	45	110	150	150
Sea water 10ml/L	45	110	150	150
Sea water 20ml/L	45	90	100	100
Sucrose 20mg/L	45	90	120	120
Sucrose 30mg/L	45	200	250	250
Sucrose 35 mg/L	45	210	350	350
Sucrose 40mg/L	45	100	130	130

The cotyledons, stem & roots were cultured on N6, B5, White & MS medium supplemented with 2,4-D (2.0 mg/l) &

NAA (2 mg/l) individually with NH_4NO_3 (1650-3630 mg/l), callus initiation and started in cotyledon after 25 days of inoculation callus initiation & growth (fresh weight 180mg) was obtained in 2970-3330 mg/l. in other concentration initiation was delayed (Table – 55)

Table - 55 : Effect of NH_4NO_3 on initiation & growth of callus

NH_4NO_3 (mg/l)	Initial weight	Weight of explant (in mg)			
		00days	30days	60 days	90 days
1650	30	45	110	150	150
1980	30	45	111	152	150
2310	30	45	110	154	150
2640	30	45	110	180	185
2970	25	45	120	182	170
3300	25	45	120	166	160
3330	25	45	120	160	160
3630	37	45	117	154	154

KNO_3 was used in range of 1800-3900mg/l. all treatment showed callogenesis. callus initiation started after 25 days of inoculation in media supplemented with KNO_3 in range of 2000-3850 mg/l, & was found to be the optimal concentration for callus induction & growth (183mg of fresh weight) callus was obtained with in 90 days. both lower then 2000mg & higher than 3850 mg/l adversely affected the callus fresh weight was 150 mg/l with in 90 days (Table –56).

Table –56 : Effect of KNO_3 on initiation & growth of callus.

KNo_3 (mg/l)	Initial weight	KNo_3 mg/l	Weight of explant (mg)			
			00days	25days	50 days	75 days
1800	30	1800	45	110	151	152
1850	30	1850	45	110	153	153
1900	30	1900	45	110	150	150
1950	30	1950	45	110	155	151
2000	25	2000	45	120	180	176
3800	25	3800	45	130	131	120
3850	25	3850	45	130	130	130
3900	30	3900	45	117	154	154

$MgSO_4$ in the range of 570 & 670 mg/l callus initiation after 25 days and fresh weight of callus obtained was 189 mg/l with in 90 days. Below 570 & higher then 670 delayed callus initiation, callus obtained was in range of 98-12mg/l in 90 days (Table – 57).

Table -57 : Effect $MgSO_4$ on initiation and growth of callus.

$MgSO_4$ (mg/l)	Initial weight	Weight of explant (mg)			
		00days	40days	60 days	90 days
370	30	38	92	120	122
470	30	39	92	121	123
570	25	38	101	185	189
670	25	37	108	186	189
770	37	38	68	95	98

KH_2PO_4 added in range of 270-320 mg/l initiated callus within 24 days of inoculation. Fresh weight 196mg/l callus obtained with in 90 days KH_2PO_4lower than 270 & higher than 320mg/l delayed callus initiation (Table –58).

Table - 58 : Effect KH_2PO_4 on initiation and growth of callus.

KH_2PO_4 (mg/l)	Initial weight	Weight of explant (mg)			
		00days	40days	60 days	90 days
170	30	38	90	108	120
220	30	39	92	122	124
270	25	38	102	187	190
320	25	37	108	189	196
370	37	38	65	92	96

In media supplemented with 2,4-D (2.0 mg/l) and $CaCl_2$ 460 mg/l callus initiation was observed on 25 the days of inoculation & 190 mg of fresh weight of callus was obtained within 90 days. By increasing or decreasing the concentration of $CaCl_2$ callus initiation & growth was adversely affected (Table – 59).

Table – 59 : Effect $CaCl_2$ on initiation and growth of callus.

$CaCl_2$ (mg/l)	Initial weight	Weight of explant (mg)			
		00days	40days	60 days	90 days
400	30	38	92	104	106
420	30	39	94	108	110
440	30	38	100	102	104
460	25	37	107	188	190
480	27	38	98	110	115
500	37	37	67	95	98

Hence Macronutrients were used in following concentration.NH_4NO_3-3300mg/l, KNO_3- 3800 mg/l, $MgSO_4$-670 and $CaCl_2$- 460 mg/l remaining other chemicals were as per MS medium. Callus was generated from the cotyledon, showed embryonic and non-embryonic callus. (Fig- 29, 30 , 31 , 32, 35) Root failed to induce callus in any of the composition & treatments given as above.

C. Embryogenesis.

Callus generated from cotyledon was placed on modified media supplemented with 2,4-D (2 mg/l) and NAA (1.0 mg/l) showed embryo initiation & development. Embryo initiation was observed with in 60 days (Table –60)(Fig –34,36). These embryo were later placed on modified MS medium with BAP (2.0mg/l) and NAA (1.0mg/l) for germination but, instead of organogenesis callus formation was observed. Xylogenesis was observed in modified MS media supplemented with 2,4-D (2.0 mg/l) & NAA (1.0mg/l) and 35 g/l sucrose (Fig-37,38).

Table –60 : Effect of Hormones on initiation of somatic embryos

Hormones (mg/L)		% of somatic embryos developed
2-4-D	NAA	
1	0.5	10
1	1.0	12
1	2.0	12
2	0.5	10
2	1.0	20
2	2.0	Callus
3	1.5	Callus
3	1.0	--
3	2.0	--

Stem was cultured on modified MS media supplemented with 2,4-D (2mg/l). callogenesis started with in 32 days of inoculation and fresh weight of callus generated was 98mg with in 90 days (Fig.- 33). By addition of biotin (1mg/l) & glycine (1mg/l) improved callus growth. Fresh weight obtained was 120 mg/l with in 90 days. Only 20% in cultures callogenesis was observed in stem explants.

Later callus was subcultured on modified MS media supplemented with BAP (2.0mg/l) & NAA (1.0mg/l) for embryogenesis but callus dried after 10 days.

Different additives like Casein Hydrolysate (0-3609 mg/l), Biotin (0-4 mg/l), Ascorbic acid (0-25 mg/l), & Silver nitrate (0-4.5 mg/l) were added to modified MS media supplemented with 2,4-D (2.0 mg/l) to determine their effect on callus Induction & growth.

Betain when explant was placed on modified MS media supplemented with 2,4-D (2.0mg/l) and betain (0-4 mg/l), did not showed any significant increase in fresh weight in control was 210mg. Whereas, with biotin (0-3mg/l) 200mg fresh weight callus was obtained. the fresh weight in control as well as with addition of biotin remained same.

Explant were placed on modified MS media supplemented with 2,4-D (2.0mg/l)& Casein Hydrolysate (CH). (0-350mg/l) to determine its effect on callus induction & growth. in control fresh weight of the callus 250 mg with in media supplemented with CH (200-3 mg/l) & callus induction initiation was observed after 23 days of inoculation.

Ascorbic acid, Therefore, like betain, ascorbic acid also failed to show any increase in fresh weight of callus. Fresh weight of callus in control was 210mg/l and with ascorbic acid was 211 mg/l.

Silver nitrate. Modified MS media supplemented with 2,4-D (2.0mg/l) & silver nitrate failed to induce callus & explant dried in all treatment.

Result callus generated from cotyledons 25 mg placed in modified media the callus dried with in week.

So as to develop callus from anthers, they were cultured on different types of media (N6, B5 and MS) supplemented with IPA (0.5 – 2.0 mg/l), IAA (0.1 – 2.0 mg/l) and NAA (0.1 – 2.0 mg/l) . But anthers failed to respond in all the media.

D. Biochemical changes .

The protein content was 70μm in non embryonic callus and in embryonic callus was 650 μm/fresh weight. DNA content of Salicornia non embryonic calli was 61 – 72 μg/g. fresh weight where as embryonic calli found to content 102 μg. / g. fresh weight. Similarly RNA content of non embryonic calli was 262 μg./g fresh weight and embryonic calli had 368 μg./g. fresh weight. The α - amylase activity of non embryonic calli was 5.1 units and embryonic calli showed 7.2 unit. All the determinations of samples were carried out in triplicate.