Discussion

A. Suaeda

B. *1. Effect of hormones on Callus induction and growth*

Callus was generated on different media supplemented with hormones. Callus cultures are the clumps of unorganized parenchymatous tissue formed by the small explants in culture, showing no polarity. Several species respond for callus induction with ease but other only with some difficulty. Callus induction also depend on the plant genotype, the source of the origin of the explant and the physiological state if the tissue at culture (Murashige, 1974).

When leaf as explant placed on. media supplemented with 2,4-D alone failed to induce callus similarly results were reported in *Pelargonium graveolense* by Lee *et al*, (2000). In contrary direct plant regeneration was observed in *Plumbo* leaf reported by Das *et al* (2002). The leaf placed on media supplemented by 2,4-D (2.0 mg/l) and BAP (0.5 mg/l) gave good callus growth 4.2g fresh weight (Table - 4). Callus showed increase in growth up to 72 days than stationary phase starts or starts drying. The young leaf of *Suaeda nudiflora* generates callus but older leaf generate roots at different concentration of NAA & BAP. However, morphogenetic potential of leaf or any explant also depends on its age. Excised young leaves of *Echeveria elegans* (Crassulaceae) cultured in vitro grow roots sooner than they produce shoots, whereas older leaves exhibited the reverse phenomenon Raju et al (1970). In case of inflorescence BAP alone failed to induce callus but no further growth was observed. the most favorable combination was BAP (0.5mg/l) and 2,4-D

(1.0 mg/l) as 3.2 g fresh weight callus was obtained with 96 days, than stationary phase starts. 2,4-D & BAP alone failed to induce callus from flower bud 2.9 g of fresh weight of callus was obtained with in 7.2 days. Maximum growth rate was observed with in 72 days stationary phase starts.

Roots were cultured on media supplemented with 2,4-D & BAP with different concentration failed to induce callus. 2,4-D (1.0mg/l) and BAP (0.5 mg/l) induction callus in stem. When callus was placed on MS media supplemented with NAA (1.0 mg/l) & BAP (3mg/l) organogenesis was observed after 40 days. The same results were reported by Lee *et al*, (2000). In *Pelargonium graveolense* they generated callus on MS media enriched with 2,4-D & NAA. When excised callus was sub cultured on MS media enriched with 2,4-D & NAA when excised callus was sub cultured on MS media supplemented with NAA and BAP showed highest intensity of shoots formation after 5 weeks.

The similar results were also reported by Diwedi et al. in *Lycopersicon esculentum.* Shoot growth was obtained in MS media containing BAP and NAA. Where the concentration of BAP was higher than NAA. Patnaik *et al*, (2000) reported shoot formation on media supplemented with BAP in *Dalbergia sissoo Roxb* and lower concentration of BAP with NAA induces callus. Also supported by Debnath *et al*, (2001)., they found in beach pea (*Lathyrus japonicus willd*) that BA higher concentration (4.4mM) was always found more effective for induction of organogenesis than the lower concentration (1.1mM). Multiple buds were induced from calli and formed shoots when transferred to MS medium supplemented with 2.7mM NAA + 4.4mM BA. 4.4mM BA at least 87% of the explants callused regardless of the NAA

concentration. Optimal induction was achieved with 5.4 to 10.7mM NAA depending on the explants used. Nodules generated on callus of *Ananas* reported by Teng *et al,* (1997), when cultured on media supplemented with NAA 0.54um + BA 0.44um, generated maximum no of shoots from callus. On contrary callus of wing pea [*Psophocarpus tetragonolobus* (L.)DC] generated on Ms media with 2,4-D and BAP. When transferred on media supplemented with BAP and NAA somatic embryos were obtained in by Ahmed *et al,*(1996).

2. Somatic embryogenesis.

The induction of vegetative embryoids from somatic cells provide a way to establish a clone of plants from a single parent. Embryoids have now been obtained from several different species, but with few exceptions.

Among different parts leaf, stem, inflorescence and roots. Only leaf could induce somatic embryos in *Suaeda nudiflora,* when it was transferred from MS enriched with NAA (0.5mg/l) & 2,4 –D (2mg/l) to B5 and N6 media supplemented with NAA (1.0mg/l) & 2,4 –D (2mg/l). No embryos were observed on MS full strength and half strength, in *Psophocarpus tetragonplobus* embryos were observed on MS full strength by Rina Ahmed et al, (1996).

In *S. nudiflora* somatic embryos were observed after 48 - 60 days. Similarly only callus was were obtained in *alfalfa* reported by Meijer and Brown (1987), & no somatic embryos were detected on primary culture medium, The embryos on B5 media was greenish and small bulb like structure. Where as embryos on N6 media were greenish brown long and heart shaped structure. These somatic embryos were sub cultured on B5, MS full strength

& MS 1/2 strength media for germination. But they fail to grow on any of the media. On transfer to MS media none of embryo germinated, instead callus formation was observed. The results support observation of Mallikarjun *et al* (1996), they observed in *Cajanus cajan*, that none of the plant part could show complete regeneration. In *Dalbergia s*omatic embryos did not germinate in any media (Das et al, 1997) on the contrary Shoyana et al (1997) reported germination of, somatic embryos germinated on MS 1/2 strength in *Panax notoginsing*

3. Organogenesis and Regeneration

Elongated shoots were could be easily grown on media supplemented with high concentrations of BAP (3 mg/L) with KN (0.8 mg/L). approximately 4-5 shoots per culture could be obtained. Adenine (0.5 mg/L) enhanced the growth of the shoots & dark green color leaves were generated. However, higher concentrations of adenine adversely affected the shoot growth.

Earlier, shoot proliferation was obtained on Ms media + supplemented with cytokinin + auxin and adenine in *Salvadora persica* (Mathur *et al* 2002) & *Zingiber officinaleas* & Rout *et al* (2001) respectively. Plant regeneration was achieved by Das *et al*. on media enriched with adenine & auxin in *Plumbago sp*. Das *et al*. (1988). On the contrary, Noel *et al*, (2002) reported that adenine with auxin caused callogenesis in the hybrid of *Populus sp*. but also . MS-half strength and full strength both were used by Sujatha *et al*, (1997) in *Guizotia abyssinic* and found that half strength improved rooting.

MS half strength with IBA alone showed 80-90% of rooting in *A. serica* reported by Kuer et al (1992). In the present studies also MS half strength supplemented with IBA, IAA

showed basal callus but by adding IPA root primordia were observed. IBA with IPA and NAA. The result shows that NAA is responsible for increase in root length. Media supplemented with IBA, IPA, and NAA showed fibrous type of rooting with 10 to 12 roots in numbers after 9 to 11 days of inoculation. Increase in root length was 1 cm/3 days.. MS 1/4 showed only 50 % rooting in the same composition. MS-half strength & MS 1/4 MS media used for rooting by Arockiasamy et al (2000) IAA alone showed 70% of rooting in sandal wood.

The plant lets were initially irrigated for few days with MS 1/2 strength followed by tap water after 1-2 weeks. Earlier same procedure was followed by Arockiasamy et al (2000) in sandal wood. The plant lets of *Suaeda nudiflora* were covered with plastic bag and kept in hardening unit at 30^0C with high humidity to reduce desiccation for approx. 20 days. Later, these plantlets were transferred to earthen pots containing soil:sand:farmyard manure (2:1:1), irrigated with MS salts (1/2) and kept in green house (Fig.5). After 30 days hardened plants were transferred in the nursery under natural light. The plant is found to be relatively sturdy during hardening process.

Morphogenetic changes :

Media supplemented with cytokinin in conjunction with auxin, have been shown to stimulate xylogenesis in cultured tissue of Pea, Artichoke, Lettuce, carrot, Zinnia mesophyll etc. Increasing the cytokinin concentration causes stimulation in the rate of xylem formation relatively more than cell replication (Narayanaswamy, 1999) while stimulated tracheid differentiation (Fosket & Torrey, 1968). Callus cells of 10-15um of *Suaeda nudiflora* generated on MS media supplemented with BAP (0.5

mg/l) and KN (0.2mg/l), showed xylem elements (Charien, 1998). Xylogenesis is considered an out one of functional senescence in cells. Although it is difficult to relate their differentiation because of the organic bound nature of the xylem elements with adjacent cells (Bornman, 1974). The size of regenerating tissue cells found reduced due to fact that cells started orienting themselves in organs.

Anatomical changes during hardening process.

During hardening process, It was found that the number of stomata were increased with the growth in leaf size. Gradually and eventually the number of stomata become similar to occurring plant. The stomatal size under hardening conditions also remained same as in natural plant. The leaf anatomical characters like formation of thick epidermis, reduction in cell size, well differentiated palisade double layer parenchyma along with the spongy cell become similar to natural plant leaf. The number of spongy parenchyma was more than that of leaf of tissue culture plant.

The T. S of stem of naturally occurring plant & tissue culture plant established in field showed well developed cuticle layer , epidermal out growth, 3 layered epidermis with many hairs on it. The arrangement of xylem & phloem in tissue culture plant was observed similar to naturally occurring plant. Similar observation were made by Selvaraj et al.(1995 in S*alicornia europia*.

4. Effect of macro nutrient on callus induction from various plant parts

The experiments on effect of macromolecules on callus induction and growth in different plant part was initiated to establish the requirement of particular elements for different plant parts.

5. Effect of macro nutrient on callus induction of leaf.

By increasing the concentration of NH_4NO_3, the callus initiation could be enhanced While lower concentration delayed callus initiation. Higher concentration (>1950 mg/l) could just initiate the callus of leaf but further growth was not observed. By increasing or by decreasing the concentration. of KNO_3,KH_2PO_4 callus growth adversely affected.This can be used in narrow range of 170-180 mg/l. By changing the concentration of $MgSO_4$, $CaCl_2$ minor changes were observed in callus initiation and growth. Callus failed to grow in the lower and higher concentration of both the elements.

Effect of macro nutrient on callus induction of flower bud.

- The media was supplemented with different concentration of NH_4NO_3, along with 2,4-D 2.0 mg/l and BAP 0.5 mg/l.
- NH_4NO_3> 1700mg/l caused minor change in the duration of callus initiation, 1650mg/l initiated callus 2days earlier, on 18^{th} day of inoculation. NH_4NO_3>1950 mg/l could just initiate no further growth was seen. Stationary phase starts by the 96 days of inoculation.
- KNO_3 >1900 mg as well as <1800mg/l failed to induce callus. Stationary phase starts by the 96 th day of inoculation. Callus initiation starts on 20 day of inoculation at 1650mg/l

- Here also <370 mg/l or >370 mg/l could just initiate callus & was also delayed by 4 days.
- Addition of KNO_3 ,KH_3PO_4 and $MgSO_4$ along with cacl2 mainly affected the exponential and stationary phase of callus growth.

6. Biochemical changes during callogenesis.

The protein value of regenerating callus was 456μg/fresh weight, which was higher than non embryonic callus. The similar results were reported by Tatsuhito Fujimura et al (1980) high protein and DNA contents was observed in embryonic culture and also in *Calotrpis gigentia*. that high DNA, RNA and protein contents were observed before Rhizogenesis. The non embryonic callus contain RNA (257μg/fresh weight) lower than RNA (351μg/fresh weight). The DNA content (98μm/fresh weight)of embryonic callus was more than non embryonic callus (52—69μg/fresh wt). The α -amylase activity of non embryonic callus culture (4.3 maltose released mg/gm/fresh wt/15 min) lower than α -amylase activity (6.3 maltose released mg/gm/fresh wt/15 min) of regenerating callus. The similar results were observed in embryonic culture of *Daucus carrota*. Electrophoretic analysis of Suaeda nudiflora proteins during morphogenesis showed protein bands at 43 kd and was supported by Sung et al (1981) have reported that a 43 Kd polypeptide which was specific to embryonic growth. The absence of certain peptides (embryonic callus and well developed Somatic embryos) may indicate diversity in these tissue. In rice, L.J Chan et al (1984) reported that polypeptide in the range of 40--44 KD in embryo extract

where as in non embryonic calli presence of 22.7 KD polypeptide. and similar observation in the analysis of protein from embryonic and non embryonic callus were found in Sandal wood reported by Minal Mhatre et al (1991).

7. Encapsulation of shoot tips or Synthetic seeds:

During the present studies best result were obtained in 2.5% sodium alginate. However, Maruyama et. al. (1997) while conserving the germplasm of tropical forest trees reported use of 4% sodium alginate with B5 media. Ganapathi et al (1992 used 3% sodium alginate with MS in Banana .Use of activated charcoal., was first reported in carrot by Lu et al. (1990) because it helps in the adsorption and desorption to control release of nutrients in the production of synthetic seeds. But during the present studies no differences could be observed in germination with or with out it. Rao et al. (1992) reported encapsulation of shoot tips in Banana, Mulberry and sandal wood.

Plantlet development on different nutrient media.. 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) half gave highest rate (90%) of plant development within a week and transplantable plant lets could be obtained in four weeks. Plant let development on filter paper was best among all different substrate used are 50% plants established in field

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

Plants growing under salinity stress show stunted growth and altered level of enzymes of various metabolic pathways. During the present studies protein value indicate that callus could tolerate 20 ppm of NaCl only As stress increased the proline value also increased. Under stress conditions after a particular salinity protein value gradually decreases with increase in salinity. The similar result were reported by Shankerdar et al (2000) in callus of rice under salt stress were proline content increased several fold. Where as total protein content decreased markedly with increase in salt concentration.

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

No positive effect of addition of betain was observed on callus initiation or growth..

Casein Hydrolysate(CH) ;

Increase in callus weight by addition of CH in leaf, floral bud and flower. Where as absence of CH showed poor callus initiation and growth in all explants. Similar results were reported by Reda et al (2000) in *Trigonella foenum*. They reported that with the treatment of CH, both the fresh & dry weight increases with the increase in the concentration of CH.

In present studies as the concentration of AgNo3 increased from callus fresh weight increases upto certain content but slowly reduced afterwards. organogenesis was not observed in any of treatments. Similar results were reported Khayri *et al* (2000), that 75um/l AgNo3 with 2iP resulted in high callus proliferation in *Phoenix dactylifera* L. event of

enhance elongation in Lemon seedling reported by Kotrasias et al (2000).

High concentration of 2,4-d are known to induce somaclonal variations while colchicine is well known to induce polyploidy in the plants. Therefore, during the present studies the aim of mutation was to induce or increase polyploid cells in the cell cultures which could be multiplied further after isolation and eventually lead to regeneration of plants with variable chromosome number. This is because colchicine treatment to shoot tips to both Suaeda and Salicornia for production of colchiploids remained ineffective during the experiments. Earlier Sharma and Chaturvedi (1988) also attempted the production of colchiploids through treatment of in vitro generated shoots when colchicine remained ineffective to induce tetraploid shoots in field grown plants of *Dioscoria floribunda.*

B. SALICORNIA

MS media was found to be suitable among all other media for micropropagation . As the maximum fresh weight of explant (cotyledon with shoot apex) was achieved on MS media as compared to B5, N6 and white. MS media was supplemented with 2,4-D (2.0mg/) and NAA(1.0mg/l) induction axillary buds from explant, that NAA& BAP induced shoots from cotyledons explant reported by Girija et al (2000). Addition of sea water in media showed no significant effect on shoot survival or elongation. But sucrose 35g/l showed best results as compared to other treatments. 2.5-3% sucrose induced equal xylem & phloem differentiation & 4% sucrose induced only Xylem & little phloem (Gupta,1994).

1. Micropropagation

Double strength of MS media with BAP (2.0mg/l) & NAA (1.0mg/l) and sucrose 35% give rise to 2-3 multiple shoots from with out sea water. Addition of sea water fail to show bud initiation. Where as without sea water 2-3 shoots were observed after 26-28 day. 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. These shoots multiple shoots were observed from stem after 26-38 days. The beat growth of bud was obtained in MS double strength supplemented with BAP (2mg/l), NAA(1.0mg/l) & sucrose 35mg/l remaining either composition failed to respond. 2-3 roots at node were seen. The total increase in weight of explant was 350mg with in 60 days on MS full strength, MS ½ strength with or without sea water and additive like betain, silver nitrate ascorbic acid . But all different concentration as well as composition fail to elongate them further . in contrary shoot elongation was observed in lemon seedling in media supplemented with GA3 & silver nitrate reported by Kotasia et al (2000). As betain concentration in halophytes is high but its addition to media did not showed any elongation in shoot in all species of Chenopodiaceae. Addition of ascorbic acid in media does not responded positively or negatively on shoot growth or elongation.

2. Induction of Callus.

Callus; Cotyledons, roots & stem placed on MS media for callus induction. MS media supplemented with 2,4-D (2.0mg/l) induced callus from cotyledon. In contrary BA (3 mg/L) was found most effective for callus induction,

regardless of concentration of NAA in cotyledons of (*Sesamum indicum* L.) reported by Kim, Younghee (2001).

MS media supplemented with NH_4NO_3 (2970-3330 mg/l) was the most favorable range for callus initiation & growth, initiation was started with in 25 days remaining other higher & lower concentration delayed callogenesis. The minor increase in weight was observed after 60 days of inoculation.

The most suitable range for KNO_3 was (2000-3850 mg/l) for callus initiation and growth. While for $MgSO_4$ (570mg/l-670mg/l), KH_2PO_4 (270-320 mg/l) & $CaCl_2$ (460 mg/l) for callus initiation & growth. Callus initiation was observed with in 25 days of inoculation. Higher & lower concentration delayed callus initiation (30 days) and growth. Modified MS media supplemented with 2,4-D (2mg/l) induced callus from cotyledon with in 25 days. Fresh weight of callus with in 60 days was 250 mg.. which was the highest among all different concentration results shows the promotive effect of NH_4NO_3, KNO_3, $MgSO_4$, KH_2PO_4 & $CaCl_2$ at high concentration.

When 3 months old callus was transferred on modified MS media supplemented with 2,4-D (2.0mg/l) & NAA (1.0mg/l) somatic embryos were observed after 40-50 days of inoculation. Cotyledons showed high degree of embryogenesis potential in several plant species (William et al, 1992) in Soybean (Durham et al,1992), Pigeon Pea (Gorge et al 1994). These somatic embryos were transferred to modified MS & MS standard with 2,4-D (2.0mg/) NAA (1.0mg/l) for organogenesis. Instead organogenesis callus formation was observed . although, somatic embryogenesis has been obtained

in number of species low efficiency of embryo germination & their conversion to plantlets remain a major hurdle (Ammirato, 1989) perhaps due to the presence of exogenous auxins. Sankara (1996) reported that presence of auxin is required for determination of somatic embryos in Sandal wood, but once determination occurs, auxin is inhibitory for further development of embryos. It is necessary to reduce the 2,4-D from the embryo induction medium & removed from germination medium, all attempts were made for organogenesis by callogenesis occurred.

3. Biochemical changes .

DNA & RNA content of Salicornia non embryonic calli was less than embryonic calli . The α - amylase activity of non embryonic calli was low as 5.1 units while embryonic calli showed higher activity.