Literature Review

Natural salinity is a widespread phenomenon on earth, and the evolution of living organism has resulted in numerous species that show special adaptive mechanism to growth in saline environments. Salinity is an ever increasing problem in the irrigated soil and more so in marshy land of the coastal region (Carter 1975).The majority of plant are relatively salt sensitive. However, the study of the mechanisms by which many of the naturally occurring halophytes survive is now well under stood (Flowers *et al.* 1977). Halophytes are salt tolerant plants that grow in soils or water containing significant amounts of inorganic salts. They are found in coast lands, tidal swamps, and inland saline and alkaline lakes and deserts. The amount and the kind of salt that can be tolerated by these halophytes varies among species and even ecotypes.

Literally term halophyte means salt plant, but is used specifically for plants that can grow well in the presence of high concentration of sodium salts. Greenway and Munns defined as " The negative flora of saline soils" and have assumed the salt concentration with osmotic pressure of at least -3.3 bars. Because of wide range of resistance found among halophytes, they have been subdivided into the extreme euhalophyte and the moderate oligohalophyte.

Many halophytes are even able to grow normally in low or non saline environments (Unger *et al*. 1969) and are therefore called facultative halophytes. The growth of these plants is favorably affected by NaCl but Na can be substituted by K e.g., *Aster tripolium, Artimisia maritima, Plantago maritima, and Suaeda*. Some of the facultative halophytes like *Salicornia rubra* are found at the highest salinity, yet are capable of growing normally in low to non saline environments (Unger *et al*.,969). Others which can not grow without NaCl are called obligate halophytes e.g. *Salicornia herbecea*, *S. europaea, Suaeda maritima,* and *Atriplex vesicaria*. Several *Atriplex* species have long been known for their remarkably high salt resistance. Even the calli of these two plants must be supplied with NaCl for growth (Von Hedenstroem and Breckle1974).

Halophytes have thicker leaves, smaller and fewer stomata, larger cells and presence of well developed water storing tissues as adaptive features for saline environments. Halophytes e.g., *Salicornia* and *Halocnemim* show highest degree of succulence perhaps due to development of larger cells in the spongy mesophyll and presence of multilayer palisade tissue (Sharma 1986). *Salicornia herbacea* although is a halophyte, in excessive saline conditions it acts to excrete salt to avoid salt stress and such behavior can be recognized as salt tolerance. Its main stem nodes have a high salt accumulation and excrete salt to the surface of the plant tissue reported by Nettai Nogyo (1990).

Although halophytes are tolerant to salinity but require less saline to non-saline conditions for germination. Due to the fact under natural conditions, seeds of halophytes remain dormant till the onset of rains. A number of studies have shown that germination even in case of obligate halophytes takes place under conditions of reduced salinity reported by Chapman (1960).

Now, there is a need to develop salt resistant plants due to wide spread salinity ingress. The traditional methods of plant breeding are extremely labors intensive and time consuming. It also involves lengthy back cross to recover useful agronomic features together with halotolerant genes (Cuartero *et al* 1992). It is also clear that an overriding limitation to develop salt tolerant plant is the inadequate understanding of the fundamental physiological and biological complexity that are involve in salt tolerance (Greenway *et a*, 1983, Hesegawa *et al* 1994, Flower & Yeo1995, Olmos and Hellin 1996, Daven Port *et al* 1997, Ballesteros *et al* 1997).

The vast areas lying unproductive along the seacoast are mainly represented by the halophytic species like *Spartina, Salicornia*, *Suaeda, Juncus, Salvadora* and *Atriplex.* These areas can be made productive by identifying certain halophytes of economic importance and cultivating them with proper agrotechnology.

Suaeda nudiflora Moq. (Chenopodiaceae) is a succulent halophytic species and grows wildly in salt marshes, above tidal zone and inland saline soils along the seacoast of the country. In natural stands it grows luxuriantly even over 500 mol m^{-3} NaCl. The plant is highly suitable for producing high protein biomass as well as edible oil from seeds. Various species of Suaeda are being used as vegetable substitute and suggested as an alternate for traditional oil seed crops under sea water based agriculture system (Pasternak *et al* 1985).

Out of 6 species of Suaeda only 3 species viz. *Suaeda nudiflora*, *Suaeda fruticosa* and *Suaeda maritima* are available at the sea coast of the country. *S. nudiflora* is a perennial, diffused branched, woody under shrub. Stem & branches smooth., yellowish, leaves 1-1.3 x 0.9-1.3, elliptic oblong or narrow obovate, obtuse, half terete when mature glaucous-green, soon falling off, rigid, attenuated, flower hermaphrodite, up to 12 in dense, axillary, globose cluster on leafless 10-12 cm long spike. Bracteoles, membranous, ovate, acute and margins pectinate. Parianth obovoid segments oblong, obtuse, stigma 3, unilocular. Seeds shining black and lenticular.

The two species i.e. *Suaeda nudiflora* and *Suaeda meritima* can be differentiated only by the number of styles as the former has three instead of two.

The genus Salicornia also belongs to the family Chenopodiaceae and consists of about 50 species all over the world. It is native to salt marshes of Asia, Africa, Europe and North America. Only one species *S. brachiata* occurs in India. It grown mainly in the coastal regions of Tamil Nadu, Andhra Pradesh, Orissa and Gujarat. It has been seen that *S. brachiata* is much more abundant at Bhavnagar coast as compared to other areas and least of the Orissa coast. At Bhavnagar coast (21 ° 45' N, 72 ° 14' E) along the Gulf of Cambay, homogenous population of *Salicornia* is observed in the areas inundated with sea water regularly. Where as in other parts, where inundation takes place occasionally, *S. brachiata* was found growing with other species viz. *Avicennia, Aeluropus, Sesuvium* and *Suaeda*.

S. brachiata is a dichotomously branched, branches rather slender, joints 6-12mm, long flower sunk in cavities of the joints, three on each side in alternate fashion each segment fruit membranous. The inflorescence is a receme, cylindrical spike, varies from 3 to11 cm in length. The flowers are arranged cyclically. Flower is bisexual, zygomorphic and originates directly from the vascular tissue, often chloritappalous, monochydeous and no distinct representation of calyx and corolla. Flowers are hypogynous and are embedded in the cortical tissue at the young stage of the flower. Androcieum consist of single stamen, free, 2 lobbed with 4 chambers, exposed during maturation. Stamen neither shows cohesion or addition, bithecus, versatile dehiscence is longitudinal and introde. Pollen with distinct style stigma bifurcated and ornamented. Ovary is superior, unilocular, a single camphylotropus ovule & basal placentation.

Floral formula % bisexual $P_2 A_1 G_{(2)}$

Fruit is a achyne, Parianth are seen persistent with the fruit. Seed testa is hispid.

The plant is a source of Alkaline earth or Sajji used for extracting Sodium Carbonate. Sajji or barilla was formerly used in soap and glass making. Air-dried plant contains a high percentage of sodium chloride ions, which constitute 0.86 % of the total water-soluble salt. Water extractable mineral constituents in the dried plant are as follows. Chloride 10.02; sodium, 5.68; sulphate, 0.70; potassium, 1-13; carbonate, 0.72; calcium, 0.01 and magnesium, 0.02% (Parekh and Roa 1965). The plants are strongly salt in taste; the leaves and young shoot are eaten after picking. The shoots are sometimes used as pot-herb. The plants are used as camel fodder also (Mc cann 1951).Seeds contain about 25 - 30% of oil which is rich in Linoleic acid (70 to 75 %). Oil has a future as low cholesterol edible oil.

The economic potential of *S. bigelovii* by the investigations carried by Environmental Research Laboratory (ERL), Tucson, Arizona (USA) has been well established and reported that its improved strain produces seed oil to the extent of 28% which is said to be edible.

Seed germination in relation to NaCl stress.

Most of the halophytes can germinate better under non saline conditions (Ungar 1995). They reported that *Haloxylon recurvum* seeds can germinate at very high salt con.(500 mol m^{-3})

Vary few halophytes including mangroves species, have been attempted for detailed studies on biotechnological aspects. Work on in vitro culture of halophytic plant is limited and mostly restricted to suspension cultures to study cellular responses and changes in gene expression under salt stress (Zhao *et al* 1989, Warren 1985, Warren and Gould 1982).

Now these plants have attracted the attention with regard to their micropropagation. Recently protocol for vegetative propagation was standardized for some mangrove species viz. *Xylocarpus moluccensis*, *Sonneratia apetala* and *Intsia bijuga*. In vitro propagation of *Excoecaria agallocha*, a medicinally important mangrove tree sp. was reported by Rao (1998). In vitro propagation of *Atriplex nummalaria* under saline conditions was under taken through shoot tip culture (Reddy *et al*., 1996). Micropropagation of *Uraria picta*, a medicinal plant through axillary bud culture and callus regeneration (Ajith Anand *et al* 1998). Clonal propagation and artificial regeneration of *Rhizophora species* was attempted by

Eganathan *et al* (2001). Woodhead *et al*. (1998) reported micropropagation of *Ruppia maritima* Loisel a sea grass.

But not much efforts have been made for other species like *Suaeda nudiflora* and *S. brachiata.* Experiments are also underway at MSRF, Chennei to develop organogenesis/ somatic embryogenesis protocols in this species. In the case of *Salicornia brachiata* direct shoot regeneration studies were initiated using mature embryo as well as seedling explants.

Leaf is the most common plant part used to generate sterile culture in a numbers of plants. However, its morphogenetic potential depends on the age of the leaf. Excised young leaves of *Echeveria elegans* (Crassulaceae) when cultured in vitro, grow roots sooner than they produce shoots, whereas older leaves exhibited the reverse phenomenon. While Kacker & Shekhawat (1991) have successfully regenerated plant let in *Urochloa panicoides* using inflorescence as the starting material. Regeneration of plant let was also successfully obtained from flower bud of Banana by Ganpathi and Suprasanna (1992).

Kenny and Caligari reported regeneration of plant let successfully obtained from anther of *Atriplex glauca* .

Synthetic seeds: The production of synthetic seeds has been reported in a number of plants like, sandalwood (Priya *et al*. 1991), Ginger (Revendra *et al*. 1995) and Davana (*Artemisia pallens*) . But synthetic seeds are not reported in halophytes. The encapsulation of somatic embryos or vegetative parts such as shoot tips and axillary bud for the preparation of synthetic seeds have received considerable attention in recent years (Bapat and Rao.1988; Furmanova *et al*. 1991; Redenbaugh *et al*. 1986) . Encapsulation of embryo or shoot tips in Sodium alginate was successfully carried out in Cardamom (Ganpathy *et al*. 1994; Ravinder *et al*. 1996). Embryogenic tissue of *Santalum album* cultured in vitro (Bapat *et al.* 1988) axillary bud of *Morus alba* (Machii 1992) have been encapsulated in alginate or agar beads and then stored for 34-80 days at 4 0 C without loss of viability. Koinoshita and Saito reported that the encapsulated axillary bud of *Betula platyphylla* var. japonica could be stored at 4 0C for more than 80 days without loss of viability. Micro cutting of *Eucalyptus grandis + E .urohphylla*, encapsulated in alginate beads and kept on a nutrient free agar medium were stored for 10 months at 30/25°C with a plant recovery rate at least 52% after storage.

The use of culture technique has proved effective in several systems for selecting cells for salt tolerance. Cells are exposed to normally lethal levels of salts and the resistant cells are visually isolated. Zen (1974) reported the isolation of the resistant cell line from haploid cells of *Nicotiana sylvestris*. At 15 NaCl, the resistant cells grow at 50 % the rate of the control with out NaCl. No growth occurred at 1% NaCl for the non-selected cells. Dix and Street (1975) selected cell lines of *N. sylvestris* and *capsicum annum* resistant to 1 and 2% (w/v) NaCl. Stavarek *et al* (1980) regenerated hundreds of plants from the salt tolerant Alfalfa callus by developing a new media sequence.

Pollens of higher plants represent a highly reduced haploid generation, which is characterized by determinate growth pattern and designed to perform a precise function. La Rue (1954) conceived the idea of culturing pollen of higher plants in order to examine their growth potential and to explore whether they still retain the ability to proliferate as haploid tissue in culture. Pollen cultures were first reported in *Torreya nucifera.* Since the announcement of the development of embryoids and plantlets in anther culture of *Dhatura innoxia* by Guha and Maheshwari and confirmation of their origin from immature pollen grains, their has been upsurge of interest in studies concerned with pollen of angiosperm species. Halophytic floral parts have rarely been attempted for regeneration studies in tissue culture.

Hence studies were undertaken, in both the genera i. e. Suaeda and Salicornia, related to micropropagation studies. The present studies also describe effect of various elements on callus initiation, growth and dynamics of callus culture derived from various plant parts and subsequent regeneration of plant lets. Studies related to effect of salinity under cell suspension cultures were also under taken. Attempts were made to culture floral parts particularly anther of *Suaeda nudiflora*.

Soma clonal variation has been recorded for most of the species capable of plant regeneration from tissue culture and variation has been detected in all components of the plant genome. Earlier investigations of cytological variation among cultured cells and regenerated plants have been limited for a number of reasons. But now enough information is available on various aspects which helps the conducting experiments.