INTRODUCTION

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Due to population explosion 20th century has witnessed the shortage of agricultural land and subsequent reduction in crop yield. Agricultural production must continue to meet the demand of growing population. Production of food to meet the demand of an ever-increasing human population in the world is the major task and challenge to agriculture today. In the past decade, agricultural production has increased due to higher yield and bringing more land under cultivation. There is an element of urgency in enhancing world's food production including cereals to feed eleven billion inhabitants expected to be present at the end of 2030 A.D. The doubling in the population demands a doubling in the total production of food crops including wheat and rice which at present globally is 565 and 525 million tonnes per year respectively. The scarcity of productive agricultural land may force us to produce crops under harsher environments. Cereal crops demand a constant attention and monitoring especially in South Asia where the average yield of cereal production per unit area is reported to be declining for the last 15 years, which is reported to be 10 to 20 percent in spite of the larger input of fertilizer, pesticides and water (IRRI notes, March 1993). It has, therefore, become imperative now to give more importance to improve programmes and to exploit fully the powerful technology aspects because the area of agricultural land is declining year after year due to salinity, alkalinity or other environmental stresses (Islam, 1996). In the case of India the population rate is expected to cross 1000 million mark requiring about 225 million tonnes of food grains. Due to environmental degradation of the land and conversion of agricultural land for

non-farm uses, the per capita arable land has been showing a steady decline resulting in loss of 4-6.3% of the total agricultural land out-put (Garg and Gupta, 1997).

In nature, all plants are exposed to environmental conditions and biotic influences that reduce their potential growth. The impact of non-optimal growing conditions on plant is referred to as environmental stress. When a stress is imposed plants usually exhibit a cascade of responses, occuring on different time scales, that involve biochemical and morphological adjustments leading to stress tolerance or avoidance (Mooney *et. al.*, 1991). Considerable research has been devoted to understand the basic mechanisms underlying in the response of plants to stress. It is especially challenging to identify the stress response that might be exploited to improve plant production in limiting environments (Close and Bray, 1993).

Salinity is one of the major environmental problem limiting agricultural production in many areas of the world (Lutts, 1996). Increasing salinity of soil water is a serious threat to the 14 billion hectares of available agricultural land in the world, out of which only 1/4th is potentially arable, of which nearly 25% is subjected to salinity. Besides 400 million hectares of salt affected land, the major deserts and 1/3rd of the total irrigated soil is also saline in nature. In India, about 8.1% of the total geographical land is affected by salinity. Gujarat alone has around 7 lakh hectares of noncultivable land due to salinity. About 1.2 million hectares in Gujarat is salt affected and is lying either barren or covered partially with hardy species and coarse grasses. Highly saline black soils are quite prevalent in Bhal region of Gujarat state. The soils in this region are very fine in nature and their permeability is very poor resulting in waterlogging during monsoon. The

water table is very high (0.50 to 2.5 m) with salty groundwater. The use of saline soils and brackish ground water for growing plants with varying economic utilities thus assumes utmost importance in this region (Rao *et al*, 1999). In arid agricultural regions, much of the water used for irrigation evaporates off the fields, leaving behind many minerals and salts dissolved in it. One of the most pernicious salt left behind is sodium, which retards root growth, resulting in stunting and sometimes killing the crop. As much as 10% of the world's 270 million hectares of irrigated land currently suffers from extreme salt build-up. Another 20% is showing symptoms of salt damage, according to the estimates from UN Food and Agricultural Organisation (Knight, 1997).

Two major type of salt affected soils occur throughout the country, to the extent of 7 million hectares, the saline and alkaline soils. Saline soils have an excess of neutral soluble salts such as chlorides and sulphates of Na^+, Ca^{2+} and Mg^{2+}. Plant growth is adversely affected due to reduced water uptake and ionic imbalance and/or nutrient stress. The EC (Electrical Conductivity) exceeds 4 ds/m, the pH is less than 8.5 and exchangeable sodium percentage or sodium absorption ratio is less than 15. These soils usually occur in areas where annual rainfall is less than 500mm. The ground water is usually saline and the availability of fresh water is low. It is difficult to leach such soils of soluble salts which consists of sodium, calcium and also sulphates of sodium, calcium and magnesium. Therefore, with the exception of coastal saline soils which occur in areas with high rainfall, saline soils are not suitable for rice based cropping system (Swarup, 1997).

Salinity refers to the occurrence of various soluble salts in soil that affect plant growth or it is a high concentration of certain dissolved ions (Yeo, 1998). When an ion exists in the soil solution at a concentration that exeeds the amount

needed for optimum growth, it may become toxic to the plant. Different levels of ions have different toxic levels. Concentrations of chloride of up to 200 mol/m^3 or more may be tolerated by some plants while a concentration of 0.2 mol/m^3 of boron is toxic to some other plants (Tanji, 1995). Though sodium chloride (NaCl) is sometimes the most predominant salt present, the term salinity includes, sulphates and bicarbonates of sodium, magnesium and potassium (Abrol, 1986; Chapman, 1975). There are a multitude of ways by which concentration of these salts can be expressed, but the preffered expression by physiologists and soil scientists is electrical conductivity (EC) stated as decimens per meter (ds/m) or millimohos per centimeter (mmhos/cm). According to U.S. Salinity laboratory recommendations a soil with an electrical conductivity of 4 ds/m or if all the dissolved salt is sodium chloride with an ionic concentration of 44 millimohs or more can be considered as saline. Because salinity is a serious hazard to agriculture, in many areas of the world, the development of salt tolerant crops is an important task. Because of the food shortage besetting mankind in various parts of the world, the need to produce more food is continuously pushing agriculture further on to marginal lands often characterised by soils and waters with a high degree of natural salinity. Salinity tolerance would therefore be a highly desirable character to introduce into crop plants. To reduce the problem of salinity, two approaches for increasing crop production on saline soils are feasible.

1) Technological Approach - Better soils for the crop we have

2) Biological Approach - Better crops for the soil we have (Garg and Gupta, 1997)

The technological approach of compacting salinity is extremely costly, requiring large expenditure of energy to reclaim land and salt balances. It also

involves such energy intensive procedures or recontouring land by deep-ripping and land planning, the installation of agriculture drains, the pumping and conveyance of irrigation water and even the desalination of water (Downton, 1984). As the cost of energy continues to rise it is increasingly clear that other alternative must be found. Further more, to compact salinity, there is a need to reuse water with increased salt loads arising from agriculture drains, as water is a valuable resource on its own right which can be used to grow more salt tolerant crops or even biomass for energy conversion. Ultimately, the ability of the crop itself to tolerate a given level of salinity becomes paramount in the management of soil and water resources. For this reason there has been an upsurge of interest towards tailoring crop plants to suit more saline environments. The biological approach includes introgression of useful agronomic traits from the salt tolerant wild plant species into crop species. The first approach has been used successfully in the past, but with the need to utilise marginal lands and poor quality saline water, attention should also to be paid to the second approach. Many reports are available on this aspect in recent years have emphasised the need to develop improved crop varieties best suited to specific adverse soil conditions (Rana, 1986; Sharma and Gupta, 1986; Pessarakli, 1994).

Classical plant breeding approaches have had only limited success in improving salt tolerance of plants (Choudhary *et.al.*, 1994) and also, whole plant breeding system has met with limited success in improving the response of crops to saline stress (Epstein, 1980; Norlyn, 1980). Although conventional plant breeding methods have contributed considerably to the increase in productivity of modern crops, advanced technologies must be employed to complement these methods and accelerate crop improvement. Plant biotechnology, including the various tissue

culture and gene transfer methods now available can boost crop improvement, shorten breeding process, and help to overcome some of the substantial agronomic and environmental problems that have not been solved using conventional methods (Zapata *et.al.*, 1995). But these traditional methods of plant breeding are extremely costly, labour intensive and time consuming (Shannon, 1982). In addition to conventional plant breeding methods (Shannon, 1985) tissue culture techniques have been used to obtain salt tolerant genotype in several plant species. It is possible to use cell and tissue culture techniques, together with conventional breeding and genetic engineering (Serrano and Gaxiola, 1994) for the development of plants with increased tolerance to salt stress.

Advantage of tissue culture technique for the *in vitro* selection of tolerant cell lines are mainly:

1) The suitability of cell culture technique for plant improvement is because of the ease with which a mutant cell line can be obtained and regenerated to give a plant with same character as the cell line.
2) Large population are available for selection and selection pressure can be applied more effectively and can increase genetic variability (Somaclonal Variation).
3) Environment and nutrient conditions can be precisely controlled.
4) A large number of cells can be screened rapidly in a relatively small area.
5) Genetically similar material can be used (i.e. selected and unselected cells which differ in degree of tolerance) so that observations will be related to the tolerance of the cell.

6) Traits can be selected at the cell level and the salt tolerance or somaclonal variability for that trait can be evaluated in the regenerated progeny (Rains *et.al.,* 1980).

7) Isolated protoplasts, cell and callus cultures can be used to study the physiological and biochemical processes, which regulate the salt stress tolerance (Rains *et.al.,* 1980).

Thus, it is well established that tissue culture techniques can be successfully employed to develop salt tolerance in many crops as well as to study the cellular basis of salt tolerance in plants.

ISOLATION OF SALT TOLERANT CELL LINES

As salinity is a major factor limiting crop production in many areas of the world, the selection of salt tolerant lines continues to challenge plant scientists (Cano *et.al.,* 1998). The cell culture approach has been proved effective in selecting salt-tolerant cell lines of tobacco, pepper, sugar cane, alfalfa, rice etc. (Gupta, 1997). Salt tolerant cell lines were first reported by Zenk (1974). He isolated resistant cell lines from haploid cells of *Nicotinana sylvestris.* The resistant strain was able to grow in 1% NaCl at about 50% growth rate as that of the control, while no growth occurred at 1% NaCl for the non selected cells. Since then there have been numerous reports on the *in vitro* production of salt tolerant plant species. Nabors *et.al.,* (1975) obtained tobacco cell lines which showed superior tolerance to 0.16 % NaCl and subsequently to 0.52 % NaCl. The isolation of salt tolerant cell lines can be made by adopting two strategies.

1) Direct selection

In this type of selection the salt tolerant variants are selected by exposing the various explants, callus cultures, cell suspensions, protoplasts, or even microspores to a sub- lethal concentration of salt. The selected cells are exposed to the salt concentration to once or many times, either in one step or gradually. Direct selection more closely resembles with the situations in the field, where seeds are directly planted into and therefore directly encounter the saline environment (McHughen and Swartz, 1984). By gradual adaptation procedure, NaCl and mannitol tolerant callus lines were established by Gangopadhayay *et. al.* (1997) in *Brassica juncea.* These cell lines showed sustained growth and more free proline content on stressful media.

2) Indirect selection

In this type of selection the salt tolerant variants have been selected by exposing the cells to different osmotica or to stress accumulating agents such as Proline, Hydroxyproline, Quaternary ammonium compounds (QAs), Polyols, etc. so that it can counteract the adverse effects of corresponding stress factor. According to Chauhan and Prathapasenan (1999) the hydroxyproline adapted cell line in rice showed better adaptation towards NaCl stress than non adapted one. It is observed that proline over producing cell lines are more tolerant to salt stress than non producing ones. Ochatt *et.al.* (1999) selected a stable salt tolerant potato cell line able to grow 60-45 mM NaCl. The callus was grown on 120 or 150 mM NaCl showed higher fresh weights than the other treatments. The regenerated plants isolated from stable salt tolerant cell lines (with high fresh weight and dry weight) when watered with 90 mM NaCl showed good growth and produced more tubers per plant under salt stress.

The regeneration of selected cell lines is a very important factor in tissue culture studies. The regeneration of plantlets can be either through organogenesis or through somatic embryogenesis. Salt tolerant cell lines or plants have been selected by screening either callus, protoplasts, microspores or various explant tissues grown *in vitro* on NaCl supplemented medium. Rare variant cells that survive and divide under these conditions are then the candidates for further study (Dix, 1986).

ISOLATION OF SALT TOLERANT CELL LINES THROUGH ORGANOGENESIS

There are two morphogenetic pathways in plant regeneration. It can be through adventitious root and shoot formation (organogenesis) and somatic embryo formation. A reliable tissue culture system with a high capacity of plant regeneration is a pre- requisite for using it as a biotechnological tool for the use and improvement of any plant species. In most of the cases the organogenesis occurs indirectly through the callus. *In vitro* culture may generate a new pool of genetic variability useful for the development of new crop varieties. Thus, the selection criteria in *in vitro* cultures can be improved by cellular or other molecular techniques. Organs such as shoots, leaves or other parts of a plant can frequently be induced in the culture by the addition of appropriate growth regulators to form adventitious buds which give rise to shoots or roots often termed as organogenesis or morphogenesis (George and Sherrington, 1984). Using pith explants from tobacco (*Nicotiana tabacum* L.) Skoog and Miller (1957) first showed that organogenesis was governed by the balance of auxin and cytokinin in the tissue culture medium. Media with a relatively large auxin : cytokinin ratio induce roots, those with a low auxin: cytokinin ratio induce shoots, those with an

intermediate auxin : cytokinin ratio induce unorganised growth as callus (Christianson and Warnic, 1988).

ISOLATION OF SALT TOLERANT CELL LINES THROUGH SOMATIC EMBRYOGENESIS

Plant tissues can be maintained in culture and under defined conditions it is possible to demonstrate that individual cell is totipotent or has the capacity to regenerate into a new plant. This unique property of cells in culture can act as normal embryos with a defined bipolar morphology similar to zygotic embryos with coleoptile and coleorhizal regions. They germinate and give rise to plantlets just like zygotic embryos. (Bright and Jones, 1985). This unique property of plant cells was first discovered by Steward *et.al.* (1958) in suspension cultures of carrot. Later, Reinert (1958) reported embryogenesis in callus cultures of carrot on semisolid medium. Today somatic embryogenesis is an important pathway for regeneration of plants in cell cultures and also for studying the different events in the process of embryogenesis which could be difficult under *in vivo* conditions. Somatic embryogenesis is now well established for many dicotyledonous species, but monocotyledonous species are more difficult to culture and an efficient regeneration system is reported to be slower (Gartner and Lorz, 1996).

Somatic embryogenesis is defined as a process of single cell or a group of cells initiating the developmental pathway that leads to reproducible regeneration of non-zygotic embryos capable of germinating to form a complete plant, similarly as followed only by the pre-dormant embryo within the seed. Adherence to this pattern of morphogenesis depends on coordinated behaviour of a group of cells to establish and maintain gene activation sequences specific to zygotic embryos (Williams and Maheswaran, 1986; Williams, 1987).

Somatic embryogenesis is initiated by either of the two cell types pre-embryogenic determined cells (PEDCs) which are already determined for the embryogenic pathway and await only the synthesis of an inducer (or removal of an inhibitor) to resume independent mitotic divisions and express their embryogenic potential; and induced embryogenic determined cells (IEDCs) which require redetermination to the embryogenic state, generally by exposure to specific growth regulators (Sharp *et. al.*, 1980). PEDCs are found in the embryonic tissues, including the scutellum of cereals, in certain tissues of young *in vitro* grown plantlets, and the nucellus and the embryo sac within the ovules of mature plants. Somatic embryogenesis via the root of IEDCs is more difficult to induce, since the starting material consists of differentiated vegetative cells that must undergo major epigenetic changes to initiate somatic embryo production. IEDCs occur in callus cultures, particularly after treatment with powerful synthetic auxins such as 2,4-D. Once the embryogenic state has been induced, however, there appear to be no fundamental difference between IEDCs and PEDCs. Depending on the culture conditions the full embryogenic pathway may be followed to produce a complete plant directly or it may be short circuted by the escape of individual cells or small cell groups to reinitiate embryogenesis independently. Escape of cells from co-ordinated development may give further embryoids, or if occuring continuously, may give rise to nodular embryogenic callus (Williams, 1987).

Advantage of Somatic Embryogenesis

1) Somatic embryogenesis is an ideal system for investigation of plant cell totipotency and differentiation.

2) Regeneration of whole plants from somatic cells is the vital objective of *in vitro* tissue culture and is required for the application of molecular and somatic cell genetics in crop improvement (Ammirato, 1983).

3) Since both, the growth of the embryogenic cells and subsequent development of somatic embryos can be carried out in a liquid medium, it is possible to create large-scale mechanised or automated culture systems which are capable of producing propagules (somatic embryos) in thousands repetitively with low inputs.

4) Somatic embryos from IEDCs show high frequency of somaclonal variation. As the somatic single cell, the regenerant will be a complete somaclonal variant, rather than a chimeric.

5) Nucellar embryos are free of virus and can be used for raising virus free plants.

6) For many of the tree species, somatic embryogenesis from nucellar cells may offer the only rapid means of obtaining juvenile plants equivalent to seedlings with the parental genotype.

7) Somatic embryos of a selected elite parent are potentially convenient organs for cryopreservation and germplasm storage.

8) Artificial seeds, consisting of somatic embryos enclosed in a protective coating, have been proposed as a low-cost-high-volume propagation system. The advantage of artificial seed is ease in handling embryo during delivery. The objective is to produce clonal seeds at a low cost comparable to true seeds.

9) Embryogenic callus, suspension cultures and somatic embryos have been employed as a source of protoplast isolated for a range of species. Cells

and tissues in these systems have demonstrated the potentiality to regenerate in cultures and, therefore, yield protoplast that are capable of forming whole plants.

10) Repetitive somatic embryogenic system, if employed for genetic transformation via *Agrobacterium* or particle bombardment method would yield stable non chimeric transgenic plantlets. The transformed embryos can be further induced to form unlimited number of transformed somatic embryos through repetitive embryogenesis.

11) The repetitive embryogenic system is of potential use in the synthesis of metabolites in bioreactors.

12) Immature embryos of inter-specific plants from incompatible crosses (involving wild and cultivated plants to introgress resistant genes in economically important cultivars, in which post fertilization barriers may often prevent maturation of embryos) may be rescued by culturing them for secondary embryogenesis and can be used for multiplication of plants.

13) With somatic embryos, in principle, discrete propagules are produced with which the developmental programme to grow into a complete shoot is without additional shooting and/or rooting steps.

14) Labour costs can further be reduced if somatic embryogenesis is conducted in liquid medium in bioreactors.

Limitations of Somatic Embryogenesis

1) Low frequency of somatic embryo production in many of the reported system.

2) Production of malformed embryos with abnormal cotyledons in total absence of these poorly developed shoot and root apex, long hypocotyl,

which are mostly found to be suitable for germination into complete plantlets in many systems.

3) Incomplete maturation of embryos or premature germination of somatic embryos (i.e. the somatic embryos starts germination as it reaches cotyledonary stage). So many of preposed uses of somatic embryogenic systems cannot be applied.

4) Low germination and low conversion of germinants to plantlets capable of surviving transfer to *ex vitro* conditions.

Somatic embryogenesis in cereals is a difficult task. Since many years, researchers have been working on this problem (Vasil and Vasil, 1982; George and Eapen, 1988; Purohit *et.al.*, 1992). The first report on somatic embryogenesis in a cereal was in *Zea mays* (Norstog, 1970). He described the formation of typical somatic embryos on the scutellum of cultured young embryos of barely within ten days of culture. Since then there are many reports on somatic embryogenesis in cereals. In cereals the embryogenic system are mostly in callus or suspension cultures. Callus based selection has been found to be inefficient and uncertain, as all the cells in the callus piece are not uniformily exposed to the selective agent and this results in stress avoidance due to cross feeding between the cells in close contact with each other (Meredith 1984; Jain *et.al.*, 1991b). The cell suspension cultures have yielded over hundred times more stable salt tolerance than callus pieces in *Brassica juncea* (Jain *et.al.*, 1991b). The reports on the production of salt tolerant embryoids are very few (Unnikrishnan *et.al.*, 1991; Rangan and Vasil, 1983; Lebrun *et.al.*, 1985).

The understanding of plant cell and tissue culture techniques in cereal crop improvement made it possible to produce a highly regenerative culture system in

many cereals. For a good embryogenic system a high regenerative ability is needed. The most common explant source for embryogenic callus in cereals can be mature seed, root, leaves, shoots, or even young inflorescence. In rice immature embryos and young inflorescence have shown better regenerative ability compared to other explants.

In order to increase the salt tolerance of adapted embryogenic cells or embryoids various supplements (like aminoacids, polyamines etc.) have been used in the experimental medium. Polyamines (PAs) are polycationic cellular molecules that play an essential role in cell growth, development, including cell division, flower initiation, pollen tube growth and senescence and differentiation (Evans and Malmmberg, 1989; Kaur-Sawhney *et.al.*, 1982) and are also involved in the plants response to abiotic stress (Flores and Galston, 1982; Rajam, 1997). Some of their roles in stabilizing membranes, proteins, and nucleic acid may be partly explained by their unique property of serving as polycations at physiological pH (Galston and Kaur-Sawhney, 1987). Polyamines are also capable of binding to proteins (Apelbaum *et. al.*, 1988) and phenolic compounds (Burtin *et.al.*, 1989). Despite of the abundance of correlative evidence the function of polyamines in cell, tissue and organ development remain unknown. The link between somatic embryogenesis and polyamine synthesis is very well studied in carrot cell cultures and some other dicots, there are very few reports on monocots specially in relation to salinity stress. Koetje *et.al.* (1993) reported that growth and embryogenic potential of rice is associated with polyamine metabolism. Free polyamines accumulated as much as ten-fold in cells during callus induction and subsequent growth. Putrescine treatment promoted somatic embryogenesis in *Solanum melangena* (L.) and caused a remarkable increase of about six fold in the number

of somatic embryos (Yadav and Rajam, 1997) while the inhibitor of polyamine synthesis MGBG (Methylglyoxal bis guanylhydrazone) inhibited the number of somatic embryos.

In recent years there has been an increased interest in studying polyamines, in relation to stress in plants. Putrescine accumulation, especially in cereals, has been shown to occur in response to various stresses. Jarvis *et.al.* (1983) evaluated the involvement of PAs with adventitious root development in stem cuttings of mung bean. Exogenously supplied spermine concentration was most effective in inducing rooting and promoting root growth. Studies with metabolic inhibitor MGBG support to the contention that polyamine has an early involvement in adventitious rooting, alterations in polyamine synthesis and titer (Sankhla and Upadhyaya, 1988). Malfatti *et.al.* (1983) reported that in tobacco cultures, putrescine synthesis increased markedly during the formation of roots. In *Vigna* species cultures, the increase of free putrescine and spermidine titer was high in root forming callus (Kaur-Sawhney *et al.*, 1985). O'Neill *et. al.* (1996) reported MGBG improve shoot regeneration frequencies in *Brassica napus.*

Proline accumulation under salt stress is a well documented phenomenon. There are reports that exogeneously supplied proline in salt supplemented medium can improve the plant regeneration, growth and survival of unselected cells (Pandey and Ganapathy, 1985; Handa *et.al.*, 1986; van Swaaiji *et.al.*, 1986) and organised tissues (Mathur *et.al.*, 1980).

The well developed somatic embryos encapsulated in a synthetic matrix with calcium chloride and sodium alginate are known as artificial seeds or synthetic seeds. A synthetic seed is defined as a somatic embryo inside a coating and is analogous to a zygotic seed (Redenbaugh, 1993). The coating serves as

endosperm, consisting of carbon sources, nutrients, growth regulators, anti-microbial agents, etc. The potential use of artificial seeds include delivery of elite germplasm, hand pollinated hybrids with reduced seed fertility, and genetically sterile plants with sterile or unstable genotype (Janeiro *et.al.*, 1997). These synthetic seeds can be stored like that of normal seeds.

Salinity causes a number of adverse metabolic changes leading to reduced growth *in vitro*. Decrease in growth in terms of fresh weight and dry weight of non-selected cells with increasing concentration of salinity has been reported by Pua and Thorpe (1986) and Muralitharan *et.al.* (1990). Gulati and Jaiswal (1992) have also observed a reduction in dry weight with increasing concentration of salinity. Bargawa and Chandra (1989) grew calluses of Moth bean (*Vigna aconitifolia*) on MS medium with the addition of 0.5, 1.0 or 1.5 % NaCl. A decrease in growth during initial subculture was followed by a gradual recovery with 0.5 % and 1.0 % NaCl. A shift towards salt tolerance was observed in the cell line cultured in 1.0 % NaCl solution and after six subculture callus growth was very poor in the absence of NaCl. These results indicates that *in vitro* selection for salt tolerance would be effective. A general trend of decreased callus growth with increasing levels of NaCl in the medium has been reported by Bhaskaran *et.al.*, (1983), Reddy and Vaidyanath (1986) and Li (1990). Chauhan and Prathapasenan (1998) reported that exposure of the calli of two different rice varieties to 200 mM NaCl reduced their dry weight by 50 and 42% after one month in culture. Growth inhibition by saline stress is commonly accepted to be due to lowering of the water potential of growth media caused non-specifically by dissolved excess ions (Flowers *et.al.*, 1977; Greenway and Munns, 1980). Prakash and Sarin (1993)

observed a decrease in water and solute potential of cell lines of *Cajanus cajan* subjected to increasing NaCl concentrations.

Most of the salt injury causes alterations in enzymatic pathways. The activity of number of key enzymes has been shown to increase or decrease by NaCl salinity. Salinity changes the activities of proteolytic, amyloytic, nucleolytic, phospholytic and oxidative enzymes in the germinating seeds and growing plant parts (Dubey and Rani, 1990; Garg *et.al.*, 1993). Subhashini and Reddy (1990) have reported an increase in the activities of peroxidase, polyphenoloxidase, alkaline inorganic pyrophosphatases and glutamate dehydrogenase under salinity in callus cultures of tolerant and salt susceptible rice cultivars. Studies of Gossett *et.al.* (1994) with callus cultures of salt tolerant cultivar of cotton grown on control medium showed that the NaCl induced increase in the activity of these enzymes indicates that the tolerant callus has higher capacity for scavenging and dismutating superoxide, an increased ability to decompose H_2O_2, and more active ascorbate-glutathione cycle when grown on media amended with NaCl. Thus cells are protected from the cytotoxic effects of activated oxygen species which include damage to lipids. Piqueras *et. al.* (1996) reported that salt tolerant *Citrus limon* callus exhibited an increase in the activity of antioxidant enzymes involved in oxygen metabolism and the induction of new superoxide dismutase isozyme and an increase of the peroxidase activity while the catalysis activity remained unchanged.

Field crops experience a multitude of stress conditions. In order to improve the performance of crops growing under stress it is important to understand how plants adapt under such conditions. Identification and quantification of proteins is very important because it provides a correlation between the altered expression of specific genes under harsh environments. Several proteins are synthesised and

accumulated under a range of stress conditions. Such proteins are called stress proteins (Pareek *et.al.*, 1998; Singhla *et.al.*, 1997). Stress proteins play a crucial role in assisting the cells to carry out their metabolic activities during adverse conditions. In response to NaCl stress, these proteins show an increased or decreased level of synthesis. Two new protein bands 59 Kd and 90 Kd were detected in NaCl tolerant calli of *Setaria italica* (Jia *et.al.*, 1993), and NaCl tolerant cell lines of millet (Lu and Jia, 1994). *De novo* induction of three new proteins (74 Kd, 28.5Kd, 26.2 Kd) have been reported by Ramagopal (1986). Singh *et.al.* (1985) has observed that there is involvement of a major 26 Kd polypeptide in the adaptation of the cells to NaCl. In *Lathyrus sativus* several distinct changes in polypeptides patterns were observed in NaCl treated plants. Increased levels of 52, 35, 29, 24, 21 and 17.5 Kd polypeptides and reduced levels of 40 and 14 Kd were observed with increasing levels of NaCl adaptation. But at 600 mM NaCl the expression of 52 Kd polypeptide was inhibited (Sinha *et.al.*, 1999).

Many plants produce high levels of free proline in response to stress conditions. This aminoacid is widely believed to function as a protector or stabilizer enzyme for membrane structure that are sensitive to degradation or ionically induced changes. Proline synthesis has been found to be linked with oxidation of NADH to NAD in the mitochondria, which is hampered severely during salt stress and is one of the major causes of cell death during salt and mineral toxicity (Alia and Pardhasaradhi, 1993; Pardhasaradhi *et.al.*, 1993). Proline affected salt tolerance has been reported in many plant systems (Pandey and Ganapathy, 1985; Kumar and Sharma, 1989). Thus, it is well documented that proline overproducing cell line will be salt tolerant (Li, 1990; Prakash and Sarin, 1993; ShivyaKova *et al.*, 1994; VazquezFlota and Loyola-Vargas, 1994).

Rice is the world's single most important food crop and a primary food source of more than one third of the world's population (Mohanty *et.al.*, 2000). More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's people live. Rice accounts for 35 to 60% of the calories consumed by three billion Asians. Rice is planted on about 148 million hectares annually, as on 11% of the world's cultivated land. Rice is the only major cereal crop that is consumed almost exclusively by human (Khush, 1997). Considering the current and projected rice consumes, in major rice growing countries of Asia, it is estimated that we will require 60% more rice over the next decade. India being a major rice producing and consuming country we will also face the reality and must plan to meet the challenge accordingly (Barwale, 1986). Due to the various advantages of tissue culture techniques, a large number of elite varieties have been developed through these techniques. In past, most of the works on developing salt tolerant rice varieties involved direct selection in screening for mutant and variant cell lines in the culture that are able to grow on otherwise inhibitory levels of NaCl. But sexual transmission of this character *in vivo* is not always observed in the absence of the selection pressure. So the present study is focussed on the production of salt tolerant embryoids in a rice variety CSR-10 and their germination to give a stable and resistant cell lines. There are a very few reports on such studies.

Considering the above problems and the advantage of tissue culture techniques the current research is focused on the following objectives:

1) To understand the mechanism of salt tolerance in rice (*Oryza sativa* L.) var. CSR-10

2) To isolate NaCl resistant cell lines of rice.

3) To develop a complete protocol for induction of somatic embryogenesis.

4) To understand the physiological basis of salt tolerance in embryogenic and non- embryogenic calli under the influence of NaCl.

5) To increase the salt tolerance of cells using proline and hydroxyproline.

6) To understand the role of polyamines and polyamine inhibitors in somatic embryogenesis.

7) Synthetic seed production (Standardizing the conditions for storage and germination).

The results of the studies are discussed and supported with the presence of relevant literature.