DISCUSSION

Percentage of germination, extension growth, fresh and dry weight of rice seeds have considerably decreased with increasing salinity levels. Similar effects of salinity have been observed in Cicer arietinum (Singh and Singh, 1980), Casuarina obesa (Reddell et al., 1986), Hordeum spp. and Brassica spp. (Huang and Redmann, 1995). According to Sharma and Gupta (1986) and Yasseen et al. (1988) decreased growth in NaCl exposed plants is due to reduced cell division and cell enlargement.

The role of plant growth regulators in growth and development of plants is enigmatic. Hence these compounds are used to change plant growth to economic advantage. Gibberellic acid, one of the most commonly used plant hormones, play an important role in seed germination and early seedling growth (Leopold, 1975; Schuurink et al., 1992; Sanwo and De Mason, 1994). Many earlier workers have demonstated that exogenous application of GA_3 can overcome the adverse effects of saline stress in various growth and developmental processes such as germination (Kabar, 1990), leaf and shoot growth (Prakash and Prathapasenan, 1990; Jaya et al., 1990), translocation (Singh and Singh, 1980) and pollen germination (Dhingra and Varghese, 1985b). GA_3 stimulated plant growth in salinized and non-salinized plants may be due to its ability to enhance cell division (Shininger, 1975) cell elongation (Jones, 1973) or both (Scott, 1984).

Polyamines are ubiquitous in biological systems and are closely associated with growth and developmental processes (Smith, 1985). The term polyamine has

been used in literature includes putrescine (diamine), spermidine (triamine), spermine (tetramine), other amines and their various derivatives (Evans and Malmberg, 1989). Polyamines have also been implicated in a number of stress mediated responses in plants (Flores et al., 1984; Prakash and Prathapasenan, 1988a; Durusoy et al., 1995). Variety of reports indicate that polyamine levels may increase in response to plant stresses (Flores and Galston, 1982, Klinguer et al., 1986, Weinstein et al., 1986). The physiological reasons for this is still unclear. According to Slocum et al., (1984) the induced high levels of putrescine may be the cause of stress injury where as Flores and Galston (1982) find it as a result of salt injury.

Putrescine titer seems to increase in response to NaCl stress in salt resistant varieties of rice (Basu and Ghosh, 1991; Sadhana and Dubey, 1990). High level of putrescine may be rendering a protective role to the plants under stress (Hiatt and Malmberg, 1988; Galston and Kaur-Sawhney, 1990) or simply be one of the many physiological changes, without any special significance (Flores and Galston, 1982). But a decrease in level of putrescine was observed in stress susceptible genotypes of Oryza sativa (Prakash et al. 1988) and Phaseolus (Guye et al., 1986).

Application of 10^{-5}M putrescine signficantly enhanced the extension growth, fresh and dry weight of NaCl treated rice seedlings. This indicates that under NaCl stress endogenous polyamine levels may be adversely affected, which is active in cell division (Kaur-Sawhney et al., 1980 ; Walker et al., 1985), rooting

and early growth (Jarvis et al., 1985) of plants. Moreover studies on almond protoplasts by Wu and Kuniyuki (1985) emphasize that exogenous application of putrescine is very effective in inducing cell division. Very recently, it was observed that polyamines are closely associated with nuclei than the cytoplasm and that they are also localized in the nucleoli (Amarsinghe and Carlson, 1994). In light of these it seems logical to presume that the increased growth in putrescine treated NaCl stressed plants observed in the present study may be due to the enhanced level of polyamines. However, application of high concentration of 10^{-3}M putrescine reduced the growth of non-stressed seedlings which may be due to the toxic effects of putrescine at high concentration (Flores et al., 1984).

Though the grain structure of rice is known since long (Juliano and Aldama, 1937), the details of the husk architecture is not clear. Soni and Parry (1973) investigated the silicon deposition in the inflorescence bracts of rice plant using electron probe microanalyser. According to them the jagged, comb shaped epidermal cells of husk are cemented by silica. The papillae and trichomes help the seed in reducing water loss. According to Yoshida et al. (1962) the silica deposition on the lemma could be associated with cuticular transpiration. The progressive silica deposition on the husk may be helping the seed to reduce transpiration. But irrespective of surface transpiration through stomata present on the husk epidermis, silica deposition is continuous (Sony and Parry, 1973). Based on the observations on rice and oats inflorescence bracts Kaufman et al. (1981)

proposed that trichomes are the 'preferred' deposition sites for silica immediately after the rapid growth phase and sclerification. They also found that the silica deposition is more on the inner epidermis than the outer epidermis in rice. The observed deposition on the surface of salinized seeds in the present study may be due to the NaCl which is accumulated on the elevated regions of the husk including trichome. It is too preliminary to say that this accumulation has some role in imbibition of water by the seed or intake of NaCl as it requires further investigation.

Localization of elements by analytical means helps to understand the mechanism of ion movement in plants. The main advantage of EDXA is that, it can be used to trace the elements without killing the tissues. But it cannot resolve the intracellular sites of ion accumulation. However, with ion measurements at cellular level, a much closer correlation between ultrastructure and physiological function is possible.

A high level of sodium and chloride was traced in the scanned regions of NaCl exposed seeds. The disturbances resulting from the toxicity of Na^+ and Cl^- in the growth associated physiological and biochemical processes is a major problem faced by salinity exposed plants. High tissue ion concentration of Na^+ and Cl^- decreases growth and yield of rice (Flowers and Yeo, 1981; Yeo and Flowers, 1983; Krishnamurthy et al., 1987; Prakash, 1988). The sensitive rice cultivars accumulate high level of Na^+ and Cl^- in the shoot compared to the resistant

cultivars (Krishnamurthy et al., 1987). It was also observed by them that Na+ accumulation was significantly reduced with a concomitant rise in K^+, Ca^{2+} and Mg^{2+} levels in the resistant cultivars when they were exposed to NaCl salinity.

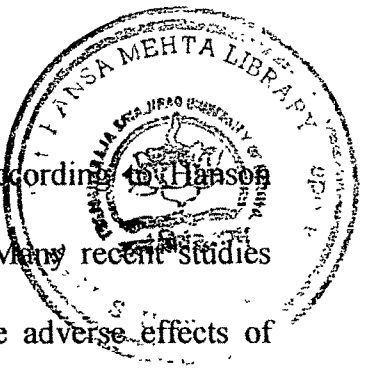
EDXA studies in Zea mays (Yeo et al., 1977) revealed that Na^+ concentration increases in the roots with an accompanying decline in the K^+ concentration. It is reverse in the case of shoot. According to them xylem parenchyma of the mature root helps in reabsorption of Na^+ from the xylem sap, which could be mitigating the adverse effect of salinity in salt sensitive plants. Investigations were also carried out to understand the cellular toxicity and nature of growth inhibition caused by NaCl salinity in rice (Yeo et al., 1985). They found that there was leaf to leaf gradient of Na^+ and maximum Na^+ was present in the oldest leaf. It was observed that net photosynthesis was decreased with an increase in Na^+ concentration in the leaf tissue. They concluded that growth inhibition in rice under NaCl stress could be due to the accumulation of Na^+ and Cl^- in shoot and root tissues to injurious levels.

Besides the toxic effects of Na^+ and Cl^-, the observed low level or absence of other inorganic elements such as potassium, magnesium, sulphur and calcium may be having a direct effect on seedling growth of rice. Plants require K^+ as an activator in number of enzymatic reactions apart from its role in protein synthesis, starch formation, stomatal movement, photosynthate partitioning and as an osmotic component (Epstein, 1972). Sharma (1986) and Begum et al. (1992) found

decreased K^+ content in the salt sensitive varieties of rice when subjected to NaCl stress. Studies conducted by Johnson (1991) in Agropyron desertorem suggest that high germination and forage production in salt resistant varieties are due to turgor maintenance, high concentration of K^+, lower Na^+/K^+ ratios and mechanisms for partial exclusion of Na^+ in leaves.

In salt stressed plants high rate of ion uptake is more in younger plant parts compared to older (Wolf et al., 1990). Based on the evaluation of salt tolerance in rice, Sharma (1986) concluded that high growth and yield of salt resistant varieties are due to better regulation over accumulation and distribution of Na^+ and K^+ in the delicate, young vital organs of the plants which are generally free from toxic levels of Na^+, besides having an assured K^+ supply under stress conditions. Endogenous hormone level decreases in presence of NaCl in plants (Prakash, 1988). Based on the studies on kinetin and IAA effects on plants Ilan (1971) suggested that endogenous auxins and cytokinins are among the factors which determine the selectivity of ion uptake by cells in the intact plants. So in the present investigation, along with other factors, NaCl induced hormonal imbalance also might have contributed to the increased accumulation of Na^+ and Cl^- as well as the K^+ reduction in salt exposed seeds.

Calcium is important for plants for its involvement in the synthesis of cell wall materials, membrane transport, besides its role as a cofactor of few enzymes. Ca^{2+} was found decreased upon salinization in the present investigation. Similar



results were obtained by Krishnamurthy et al., (1987). According to Hanson (1984), Ca^{2+} is essential for K^+/Na^+ selectivity in plants. Many recent studies suggest that exogenous application of Ca^{2+} can mitigate the adverse effects of NaCl on growth by inhibiting Na^+ uptake (Kent and Lauchli, 1985; Gong and Yang, 1994). Moreover, the findings by Hamada (1994) suggests that the reduction in the contents of soluble carbohydrates, proline and quaternary ammonium compounds by the application of Ca^{2+} in the salinized plants might be helping the plants to overcome the adverse effect of NaCl. It also affects the secretory process in plants (Steer, 1988) and plays an important role in stimulating the synthesis of α-amylase from aleurone layer (Varner and Mense, 1972). Ca^{2+} is also known to stimulate the secretion of β-amylase by the aleurone and scutellum during germination (Lauriere et al., 1992). Thus, it is conceivable that the low level of calcium in NaCl exposed tissues of seeds must be one of the factors for the observed reduction in seedling growth.

Magnesium is an activator of enzymes more than any other elements. As an activator of enzymes of phosphorylation, it has paramount importance in energy metabolism (Epstein, 1972). It is a non-dissociate constituent of chlorophyll. Krishnamurthy et al. (1987) observed that NaCl reduced the content of Mg^{2+} as much as 50% in the shoot system of rice. Prakash (1988) found a reduction in total chlorophyll content of rice leaves under the influence of salinity.

Additionally, experiments with Phaseolus vulgaris suggest that exposure to Mg^{2+} stimulates the synthesis of ∝ -amylase, ATP, GA and protein under NaCl stress (Kiss, 1979).

The sulphur content was found decreased in presence of NaCl in the present study. Sulphur is a constituent of amino acids cystine, cystein and methionine and co-enzymes thiamine, biotin and coenzyme A. The reduced sulphur content may also be responsible for the reduced vigour of rice seedlings under NaCl stress.

An inhibition of Na^+ and Cl^- accumulation and an increase in K^+ and Ca^{2+} were noticed in the GA_3 treated salt stressed plants. This may be due to the ability of GA_3 to alter the membrane permeability and to regulate the uptake and transport of ions (Wood and Paleg, 1972, 1974; De La Guardia and Benlloch, 1980). Gracia and Guardiola (1981) have also reported GA_3 induced selective ion uptake in pea plants. The enhancement of K^+ level and reduction in accumulation of Na^+ and Cl^- ions in GA_3 treated salinized plants may be due to the ability of GA_3 to alter membrane permeability (Karmokar, 1984). GA_3 stimulated K^+ uptake and decreased influx of Na^+ is also observed by Stark and Kozinska (1980) in salt stressed bean plants.

Application of putrescine reduced the influx of Na^+ and Cl^- and increased the K^+, Mg^{2+} and Ca^{2+} levels in the examined tissues of rice exposed to NaCl stress. The salinity caused membrane damage (Levitt, 1980) is altered by providing greater stability to membrane bilayers and protected from membrane

disruption by the application of polyamines (Slocum et al, 1984; Guye et al., 1986). Stabilization of cell membranes and regulation of their functions by putrescine and other polyamines were also reported by Altman (1982), Srivastava and Smith (1982) and Roberts et al. (1985). According to Riedell (1987) the role of Ca^{2+} in maintaining the membrane integrity can be substituted by polyamines. He found that the Na^{+} influx was considerably reduced when polyamines were applied to maize roots and concluded that polyamines can inhibit Na^{+} influx by influencing the membrane permeability like that of Ca^{2+}. Thus it can be suggested that the improved ionic balance in salt stressed plants treated with putrescine could be due to the inherent ability of putrescine in maintaining the structural and functional integrity of biomembranes and its ability to replace Ca^{2+} in membrane function under stress condition.

Salt tolerance of higher plants involves either salt inclusion or exclusion (Flowers, 1975; Lauchli, 1976). Most of the agricultural plants belong to the second category and they all rely on some strategy for preventing the salt build up in their tissues (Munns, 1985). NaCl is generally retained in the roots (Yeo et al., 1977). According to them, the concentration of Na^{+} is more in the xylem parenchyma tissue of root at the proximal region. The high concentration of Na^{+} and Cl^{-} in the aleurone may be because of the salt absorption by the radicle and its transportation through the scutellum. Unfortunately, the ionic transport to the endosperm and aleurone during early seedling growth did not receive much

attention. The results obtained in this study show that Na^+ and Cl^- might be transported to the endosperm from the scutellum to prevent high toxicity. The movement of Na^+ is through the plasmodesmata (Yeo et al., 1977) and it is very likely that the reduced Na^+ and Cl^- contents in the endosperm tissue might be due to the absence of plasmodesmata between endosperm and aleurone cells.

Germination of seeds involve the activation of enzyme systems as well as the mobilization of reserve foods. Although there is a good possibility that each specific cereal seed has its characteristic machinery for hydrolyzing reserve food materials, no unified mechanism has been presented (Okamoto and Akazawa, 1979). The endosperm hydrolysis during germination has been extensively studied, still there are controversies regarding the relative contribution of aleurone layer and scutellum to the production of hydrolases that mobilise endosperm reserves (Briggs, 1973; Okamoto et al., 1980; Ranki and Sopanen, 1984; Mac Gregor et al., 1984; Thevenot et al., 1991). According to Jones (1985) anatomical differences between species may be one of the reasons for varying conclusions regarding the relative roles of aleurone and scutellum in secreting the hydrolases. He also added that although the aleurone layer contribute more α- amylase for endosperm breakdown during germination, starch digestion is initiated by enzyme produced by the scutellum, which is the sole source of α -amylase during the first 24h of inbibition.

Alongwith amylases, endoproteolytic enzymes also play an important role in germination because they hydrolyse endospermic storage proteins to provide precursors for new protein synthesis (Wrobel and Jones, 1992). Ranki et al., (1994) studied the site of the synthesis of carboxy-peptidases during germination and found that 3 types of carboxypeptidases are synthesized at three different regions i.e. scutellum, starchy endosperm and aleurone layer. The increased accumulation of proteins observed in salt stressed seeds may be due to the reduced activities of aminopeptidases and carboxypeptidases and the low level of aminoacids (Dubey and Rani, 1990).

It is well known that during germination GA_3 is synthesized by the embryonic axis and gives signal to aleurone to produce the active enzymes for hydrolysis (Black, 1972). Among the various hydrolytic enzymes, ∝-amylase is the most extensively studied enzyme (Kaneko et al., 1991; Jacobsen and Knox, 1973; Jones and Jacobsen, 1991; Pereta et al., 1993). Starchy endosperm is found to regulate the GA_3 response of the aleurone layer (Schuurink et al., 1992; Skadsen, 1993). The mobilization of starch is of primary importance for rice seeds during germination as it is the main reserve food. Guglielminetti et al. (1995) and Pereta et al. (1997) studied the activity of a set of amylolytic enzymes and sucrose degradation enzymes in cereal seeds under aerobic and anoxic conditions. Pereta et al. (1997) found that rice is the only cereal which is able to degrade starch under anoxia compared to other tested cereals.

The cell wall matrix functions as a barrier to the release of hydrolytic enzymes (Benjavongkulchai and Spencer, 1989). Moreover, binding of calcium is essential for the activity and stability of ∝-amylase (Bush et al., 1989). Digestion of the cell wall matrix was observed as a result of GA_3 treatment in the present study. It is reported that the digestion of cell wall matrix is catalyzed by xylanases produced by the aleurone layer in response to GA_3 (Taiz and Honigman, 1976). The undigested cell wall and decreased Ca^{2+} may be responsible for the reduced hydrolysis of reserve foods in NaCl exposed seeds in the present study. Apart from other factors involved, high accumulation of ABA found in salinized plants are known to inhibit amylase and invertase activity (Barendse, 1984). The observed slower depletion of reserves in salt stressed endosperm might also be due to delayed activation of synthesis of hydrolases (Prakash and Prathapasenan, 1988b).

Induction of hydrolases in response to GA_3 treatment is well known (Varner and Ho, 1977). This could be due to the induction or activation of these enzymes by GA_3 (Prakash, 1988). Apart from these factors, the ability to counteract with the effect of ABA on enzyme activities (Scott, 1984) might also be responsible for improved activity of hydrolases in GA_3 treated salt-affected seeds. GA_3 treatment accelerated vacuolation in aleurone cells. Since aleurone grains are the major site of proteins storage in the aleurone, this vacuolation probably reflects mobilization of protein for de novo protein synthesis.

Putrescine treatment led to a marked increase in the digestion of reserve food materials under stressed as well as non-stressed conditions. It may be due to the direct effect of putrescine on synthesis and activity of hydrolysis (Tabor and Tabor, 1984). Besides this low levels of sodium and chloride ions and improved water content of seeds by the application might have also further increased the production or the activation of hydrolases.

Germination is characterised by the appearance of new enzyme systems and increase in activity of almost all the existing enzyme systems. They become active with the hydration of tissues. Among the various metabolic processes involved, respiration plays an important role in the germination and early seedling growth (Mayer, 1977).

Water deficits and sodium salts adversely affect many physiological activities in plants (Osmond and Greenway, 1972; Acharya et al., 1991). Germination and seedling growth requires high level of energy. The energy requirement is greater under salt stress conditions owing to compartmentalization, secretion and for repair of cellular damage caused by the presence of salt (Penning de Vries, 1975). The activity of succinate dehydrogenase was found decreased under the influence of NaCl in the present investigation. Similar results are also reported by Bharadwaj and Rao (1960) and Acharya et al., (1991). It is very likely that the osmotic stress reduces oxygen uptake in mitochondria (Schmitt and Dizengremel, 1989). Mittal and Dubey (1992b) found decreased mitochondrial

ATPase activity in both endosperms and embryo axes in salt sensitive cultivars of rice. It is also reported that water stress increase respiration (Parekh and Chatpar, 1989) or has no effect (Deng et al. 1989). This may be due to their difference in NaCl tolerance. Sadhana and Dubey (1994) suggested that NaCl inhibits the activity of malate and isocitrate dehydrogenases in growing embryo axes of salt sensitive rice cultivars, but found high activity in tolerant cultivars. The higher number of mitochondria in the NaCl exposed aleurone cells may be an essential requirement for overcoming the reduced oxygen uptake.

When the activity of SDH decreased, the G6PDH showed a sharp increase in its activity. G6PDH is a key enzyme of pentose phosphate pathway (PPP) which is active during germination. PPP provides NADPH for various reductive synthetic reactions and produces various intermediates including 5C and 4C compounds needed as building blocks for various synthetic processes (Roberts and Smith, 1977). Simmonds and Simpson (1971) suggested PPP as an alternative pathway when TCA cycle is blocked in dormant caryopsis of Avena fatua. According to Vegis (1964), under conditions of restricted oxygen supply, the oxidative breakdown of acetyl-Co A is limited and it is diverted to other pathways. Similarly the increased activity of G6PDH in the present study, might be reflecting a shift in the respiratory pathway from TCA cycle to PPP in salt stressed seeds.

The enhanced activity of SDH found in GA_3 treated stressed as well as non-stressed seedlings may be due to its ability to modulate the enzyme activity

(Acharya et al., 1991). It was reported that GA_3 treatment could induce a shift in the pattern of oxidative metabolism of water stressed embryos resulting in an increased participation of PPP (Simmonds and Simpson, 1971). They also suggested that one possible mode of GA_3 action might be the allosteric control of the pentose phosphate pathway. Hence, it can be expected that the improved G6PDH activity in the aleurone of salt stressed seeds treated with GA_3 might also be due to its functional ability to control the respiratory mechanism.

Polyamines are involved in the control of cell cycle, cell division, morphogenesis, senescense as well as in plant responses to various stress factors (Flores, 1991; Galston and Kaur-Sawhney, 1990; Zheleva et al., 1994). It was observed in the present study that the number of well differentiated mitochondria were increased upon putrescine treatment in salt exposed seeds. This might be due to a stabilization of mitochondrial membranes. Flores and Filner (1985) reported the involvement of polyamine metabolic products in the Kreb's cycle. Moreover, it was found in the tubers of Helianthus tuberosus that with polyamine treatment NADH dehydrogenase oxidase activity in the mitochondria could be increased (Rugolo et al., 1991). This suggests the likely role of polyamines in respiratory metabolism of plants.

The germination of a seed is the emergence of an embryo from rest, and reflects the developmental shift from hypometabolism to normal metabolism. The hydration during germination induces a rapid development of cell organelles

including increase in their activities and components. During germination or imbibition, the outer part of the aleurone cell layers separated leaving the inner resistant layer associated with the living protoplast. Similar observations have been made by Bacic and Stone (1981) in wheat and barley. According to him this separation may be due to the difference in nature of composition and organisation of cell wall. The ultrastructural changes of membranes and organelles during germination has been extensively studied (Doig et al., 1975; Chabot and Leopold, 1982; Jones, 1980; Thomson and Platt-Aloia, 1982).

The first phase of germination is activation of cell organelles. A significant amount of ER, mitochondria, plastids, microbodies and ribosomes (free and associated with ER) were observed in the aleurone cells of germinated seeds under non-stressed condition. The long profiles of ER in the aleurone of germinated seeds may be because of active phospholipid synthesis (Varty and Laidman, 1976) and as a result of membrane reorganisation or membrane extension (Colborne et al., 1976). The increase in number of microbodies in the aleurone cells during germination is also reported by Doig et al. (1975). According to them these are the glyoxysomes and activates β-oxidation during germination. The dictyosomes are reported to be active during germination (Yoo, 1970). The failure to find them in the present study does not prove their absense in rice aleurone cells, but implies that either they contain few dictyosomes or exist as prodictyosomes (Buttrose,

1963). The presence of numerous well differentiated mitochondria and ribosomes may reflect a high rate of respiration and protein synthesis during germination.

Exposure to saline environment leads to various morphological and anatomical changes (Poljakoff-Mayber and Gale, 1975). The cell organelles and nucleus of the aleurone cell exhibited changes in response to salinity. The chromatin in the nucleus was found to be condensed in the salt stressed aleurone cells. Similar changes in the nuclei due to NaCl salinity is also observed in the root cells of barley (Werker et al. 1983) and wheat embryos (Petruzzelli et al., 1992). According to them nucleic acid biosynthesis is suppressed in cells as a result of exposure to NaCl. The high density of mitochondria observed in the aleurone of salt stressed seeds may be a compensation for the impairment of mitochondrial function (Petruzzelli et al., 1992; Smith et al., 1982). The vacuolation elicited in some of the mitochondria of the aleurone of NaCl exposed seeds could be due to high accumulation of Na^+ and/or Cl^- differences in ion compartmentation (Smith et al. 1982). The reduced respiratory rate in the NaCl stressed seeds may be also due to changes of the structural integrity of the mitochondrial membranes which subsequently causes changes in permeability or in the transport of electrons (Ciamporova, 1980).

A reduced occurrence of ribosomes were also observed in the NaCl stressed aleurone cells. A decreased frequency of polyribosomes is considered very fast reaction due to the water stress (Ciamporova, 1980; Rhodes and Matsuda, 1976)

and probably represents the primary change leading to the growth reduction (Rhodes and Matsuda, 1976) and also to metabolic changes. The scanty and short strands may be a result of apparent loss of material from the membrane in maintaining the surface area due to NaCl stress (Einspahr et al., 1988).

There have been reports in existence of endocytosis in plant cells (Nishizawa and Mori, 1977, 1978). The process of pinocytosis has been postulated as an important factor in ion transport at the plasmalemma, ion movement in the cytoplasm and accumulation in the vacuole. Invagination of plasmalemma into the vacuoles to form endocytotic vesicles might be a means to reduce NaCl toxicity in plants. According to Nishizawa and Mori (1977) vacuoles detached from the plasmalemma after invagination could be incorporated into a vacuole. As a result, inspite of membrane incompatibility between plasmalemma and the tonoplast, a flow of exogenous material within Na^+ and Cl^- ions and their incorporation into the vacuole may be a means to prevent the cell from toxicity.

Hypersalinity conditions result in cell shrinkage and therefore directly influence the properties of the membranes (Zimmerman, 1978). Changes in membrane configuration induced by alterations in volume, alter the metabolism of inositol phospholipids which play a fundamental role in transducing the extra cellular signal (Cowan et al., 1992). Salinity is known to cause changes in membrane lipids (Giddings and Hanson, 1982) and selective permeability (Mass and Nieman, 1978). Bliss et al. (1984) reported that the plasma membrane particle

density decreases during imbibition. According to them the plasmalemma does not expand as much in salt imbibed tissues and salt promotes disaggregation of plasma membrane particles. These changes in the membrane organisation may also be responsible for transport of ions across the membrane in salt imbibed seeds.

Presence of needle like crystalline structures which store proteins have been reported in pea radicles during germination (Yoo, 1970). The needle shaped structures found in the NaCl exposed aleurone cells may be the crystals of such proteins or some crystals developed during processing. As there was an increase in the Ca^{2+} level in aleurone of NaCl exposed seeds, it can also be assumed that they are of some Ca^{2+} salts. The exact nature, composition and role of these crystals if any are to be further investigated.

Extensive studies are carried out to understand the action of GA_3 in aleurone cells of cereals (Gubler et al., 1987; Jones and Jacobsen, 1991; Gilory and Jones, 1994). The changes induced by GA_3 mainly involves aleurone grains, cell wall and ER. The digested protein bodies in the aleurone grain is used in the synthesis of ∝-amylase and other proteins (Jones and Price, 1970). The scanty spherosomes and extensive vacuole formation by the GA_3 application also indicates its role in fastening lipid digestion and energy supply for the growing embryo.

The wall system play an important role in controlling the enzyme release (Gubler et al., 1987). The inner resistant wall of aleurone is a highly specialised

wall which maintains its integrity in the presence of strong wall hydrolyzing enzymes. Their composition is different from rest of the walls and the hydrolases move through tubules similar to plasmodesmata (Taiz and Jones, 1973). According to Jacobsen et al. (1985), GA_3 stimulates the release of β-1,3-glucanase from aleurone layers, which digests the cell wall on increased exposure of these cells to GA_3. Moreover, the biochemical studies by Prakash and Prathapasenan (1990) shows that the activities of cellulase and pectin lyase are increased on exposure to GA_3. This may also be one of the reasons for increase in cell wall digestion of aleurone cells by GA_3 application.

A marked increase of ER cisternae was found in response to GA_3 treatment. The proliferation followed by vesiculation of ER on GA_3 exposure is reported in barley aleurone cells (Gubler et al., 1987). They further pointed out that RER is involved in the synthesis and intracellular transport of protein, especially the hydrolases. Thus, it is likely that the higher concentration of RER in GA_3 treated seeds is directly related to increased activity of hydrolases in these seeds during germination. Since only few free ribosomes were present in the cell, it must be concluded that most of the ribosomes associated with the new ER were also derived from the synthesis. Formation of ER and ribosomal RNA by the application of GA_3 is already reported by Jones (1969). According to Vigil and Ruddat (1973) secretion of enzymes from aleurone cells involves RER directly following distinct assembly and dispersal of vesicles as a GA_3 mediated process.

But autoradiography studies by Chen and Jones (1974) confirms that the hydrolases secreted by the ER moves outside the aleurone cell without the participation of vesicles.

The microbodies found in the aleurone of GA_3 exposed seeds may be involved in supplying the precursors for membrane synthesis or they may be the sites of metabolism. Enzyme secretion is shown to be highly energy dependent (Varner and Mense, 1972). The mitochondria of GA_3 treated aleurone cells are also reported to contain showing cytochrome oxidase (Vigil and Ruddat, 1970). The distribution of numerous mitochondria near the plasma membrane may be important for this process. Vesicles in the GA_3 treated non-stressed aleurone cells in the present investigations confirms the latter.

Though the endogenous gibberellins are secreted in the aleurone cells during germination, they respond more to exogenous application. Studies by Gilory and Jones (1994) suggests that the site of perception of GA_3 is on the external face of the plasma membrane. They further explained that the aleurone protoplasts respond more to external GA_3 with increased synthesis and secretion of $\propto$ -amylase. Vacuolation in the aleurone cells and the expression of the glucuronidase reporter gene fused to the hormone responsive elements of the $\propto$ -amylase promotor were also found increased by the exogenous application of GA_3.

GA_3 treatment enhanced the digestion of aleurone grains in the aleurone cells of NaCl stressed seeds. This may be due to the increased synthesis of hydrolases on GA_3 exposure. An increase in protein synthesis and secretion is increased in cereal aleurone on application of GA_3 (Jones, 1985). The presence of RER stacks indicate that they must be producing the hydrolases for the digestion of reserve food. The observed vesiculation of plasma membrane into the cytoplasm may be helping the seed to transport the inorganic ions into the vacuole to overcome their toxicity in the cytoplasm. There is now good circumstantial evidence that the level of sodium in the cytoplasm of halophytes is low (Hall and Flowers, 1973). This may mean that the cytoplasm is largely bypassed by sodium, perhaps by transport through microvesicles. The reduced frequency of mitochondria and retention of normal structure in the aleurone cells of GA_3 treated salt stressed seeds indicate that they are able to overcome the respiratory stress by GA_3 application.

Polyamines are known to be involved in variety of cellular, physiological and developmental processes including DNA synthesis, somatic embryogenesis and floral and fruit development (Tabor and Tabor, 1984; Flores et al., 1989). But unfortunately very little is known about the effect of polyamines on the structural changes during germination. Cytochemical localization studies in cultured cells of Picea gluaca (Amarsinghe and Carlson, 1994) indicates that polyamines are more closely associated with the nuclei and nucleoli than the cytoplasm but are excluded

from chromatin during condensation. The large well differentiated nucleus and nucleolus of the aleurone cells indicates its high activity on putrescine application. Moreover, the cationic nature of polyamines at physiological pH enables them to bind the negatively charged phosphate of DNA in the cell. The exogenous application of putrescine increase DNA synthesis (Serefini-Fracassini, 1991) and protein synthesis (Amarsinghe and Carlson, 1994) may be thereby accelerating the seedling growth. The group of free ribosomes observed in the aleurone cytoplasm of putrescine treated seeds deprives the ability of putrescine to bind with ribosomal RNA and increase their activity (Cocucci and Bagmi, 1968, Altman, 1982). This functional ability of putrescine might be responsible for the increased enzymatic activity as well as the improved growth in putrescine treated seedlings.

Polyamines are known to have immense potential in retaining the membrane integrity of plant systems under environmental stress conditions. Although it is difficult to explain a single mechanism of polyamines protective action, it is assumed that, due to their unique properties as biological polycations, they bind to the acidic sites of nucleic acids and cell membrane phospholipids, thereby stabilizing their structure and prevent the breakdown of structural macromolecules under stress conditions (Altman, 1982). Recently, polyamines have been found to be effective in retarding the loss of D1, D2 and cytochrome from thylakoid membrane as well as ribulose biphosphate carboxylase large subunits and chlorophyll from the osmotically stressed leaf tissue of oat seedlings

(Besford et al., 1993). Investigations carried out by Zheleva et al. (1994) demonstrated the protective role of polyamines against atrazine, a selective herbicide in pea leaves. The normal structure of cell organelles and membranes found in the aleurone cytoplasm of putrescine treated stressed seeds could be due to the binding of putrescine to membranes of the organelles to prevent them from alteration as a consequence of NaCl salinity.