SUMMARY AND CONCLUSION

One of the main challenges of current demographic trends is the yearly addition of over 90 million people to feed (Robert, 1994). Population growth has contributed to a per capita decline in cropland in all the regions of the world. It is projected that the world average of 0.28 hectares of cropland per capita is to drop almost by its 40 percent by 2025 A.D. (Victoria, 1994). Moreover, the quality of available cropland has reduced due to over usage and improper irrigational practices. In the recent past, 6 percent of the cropland base went out of production because of waterlogging, excessive salinity and alkalinity in India (Robert, 1994).

Among the various stress factors in agriculture, salinity of the soil or water plays an important role and thus is of major concern. Due to increased population and shrinking land resource, there is a need to practice agriculture in marginal lands afflicted by the problem of salinity.

Extensive work has been done on the adverse affects of salinity in plants (Poljakoff-Mayber and Gale; 1975, Greenway and Munns, 1980; Hampson and Simpson, 1990; Durusoy et al., 1995). Eventhough the various aspects of salt tolerance and salt induced inhibition of growth in plants are studied (Levitt, 1980; Gill and Singh, 1985; Dubey and Rani, 1990), the exact mechanism(s) of plant response to salinity is not yet clearly understood. Since it is not possible to modify the saline environment to suit the plants, an understanding of exact mechanism of growth inhibition is important in evolving some chemical treatments capable of maintaining reasonable yields of plants.

Rice is one of the most important cereals as it is the staple food of over half of the world's population. NaCl stress is known to cause impairment in normal physiological functions of rice. Growth and yield of many varieties of rice is found to be reduced due to NaCl salinity (Dhingra and Varghese, 1990; Lutts et al., 1995). However, the structure of cell organelles which are the main sites of metabolic action and their morphological changes caused due to salinity during germination received scant attention.

GA_3 is known to ameliorate the NaCl induced inhibition of seedling growth of rice (Acharya et al, 1990; Prakash, 1988). Hence the effect of GA_3 on structure of rice aleurone during germination under the influence of NaCl has been examined. The stress-mediated responses of polyamines in biological systems has drawn the attention of many scientists in the recent past (Galston and Kaur-Sawney, 1990; Zheleva et al., 1994). The ability to interact with the anionic groups of membranes, preventing leakage and causing stabilisation in stress conditionshas made them important in protecting the plants under stress (Srivastava and Smith, 1982). Keeping this in view, the role of putrescine one of the polyamines in overcoming the adverse effects of stress, its action at the structural and functional level of germinated rice under the influence of NaCl has been also examined

Germination and seedling growth of rice under the influence of NaCl salinity (0.15M) and GA_3 (10 ppm) and putrescine (10^{-5} M) has been studied after a period of 5 days. NaCl salinity reduced the percentage germination and extensive growth of seedlings by 53 and 88 percent of the control after 120h of germination. Their endosperm showed 38.5 percent more dry weight and the dry matter accumulation of the axis had reduced to about 48.5 percent than the control.

Application of GA_3 significantly increased the growth of shoot and root system of rice seedlings under saline conditions. It showed an increase of 33 and 38 percent more dry weight and extension growth respectively over the salt control. Moreover, it has brought about a 22 percent increase in the extension growth of non-salinized seedlings. Compared to the salt control 69 percent increase in the dry matter content and 23 percent decrease in the endosperm dryweight after 120h of germination were also observed.

Putrescine treatment also enhanced the percentage germination and growth of axis. After 5 days of incubation, the final percentage of germination of salt stressed seeds was increased from 72 to 91 percent as a result of putrescine treatment. It also brought about 17 percent increase in growth of embryo axis compared to salt control. An increase of 45 percent in dry matter accumulation and decrease of 20 percent in endosperm dryweight was also obtained in putrescine exposed salinized seeds compared to the salt control. Non-salinized control plants also showed a significant increase in shoot growth in response to GA_3 treatment. However, seedlings emerged from GA_3 treated seeds showed better growth than putrescine treated ones under saline as well as non-saline conditions.

The other parameters examined are:

1. Husk micromorphology.
2. Energy dispersive X-ray analysis of endosperm, aleurone layers and germ to localize the ions.
3. Histochemical localization of reserve food materials (carbohydrates, proteins and lipids) in the aleurone layers and the endosperm.
4. Localization of respiratory enzymes succinate dehydrogenase (SDH) and glucose-6-phosphate dehydrogenase in the aleurone cells.
5. Ultrastructural changes in the aleurone cells.

The husk epidermis of rice is silicious with number of projections called papillae arranged in parallel rows. The trichomes observed at regular intervals. On the either side of the papillae, granulated concentric rings are present. Stomata are distributed throughout the husk epidermis. Exposure to NaCl resulted in a thick deposition all over the surface of husk masking the stomata. The deposition was also noticed on the papillae and granules.

Among the three tissue regions of rice seed scanned after 120h of germination for detection of ions, Ca^{2+} content was found to be more in the aleurone where K^+ and S did not show much difference in the aleurone and scutellum of control seeds. Mg^{2+} showed high peak in the endosperm compared to other ions. The EDX analysis of the tissue regions of NaCl exposed seeds did not show sharp peak for K^+, Ca^{2+} , Mg^{2+} or S. A high peak of Na^+ and Cl^- was obtained in the aleurone tissue followed by the scutellum and endosperm.

GA_3, proved to be more effective than putrescine in improving the elemental balance under stress conditions except in case of Na^+. At the end of 120h, the levels of Na^+ in the scutellum, aleurone and endosperm tissues of GA_3 treated salinized seeds were 19, 26 and 9 percent where in putrescine treatment it was 21, 26 and 31 percent less compared to the salt control. A reduction in Cl level to the tune of 45, 44 and 22 percent was noticed in the scutellum, aleurone and endosperm of putrescine treated salinized seeds while it was 33, 47 and 16 percent respectively on GA_3 administration. The peak of K^+, Ca^{2+}, Mg^{2+} and S of GA_3 or putrescine treatment of salt exposed seeds were much less compared to that of control-except in the level of Mg^{2+} in the scutellum and endosperm of GA_3 treated seeds. Traces of Fe were observed in all the three regions of GA_3 treated non-salinized seeds. Peak of S was also high in aleurone and endosperm tissue of GA_3 exposed seeds compared to control.

A marked decline in the level of hydrolysis of reserve food materials especially the starch and proteins were observed in the endosperm of NaCl exposed seeds compared to the control. Their aleurone cells showed wavy outline due to lack of turgidity. They also contained cytoplasmic polysaccharides which were absent in control and GA_3 or putrescine treated seeds. Vacuolation was less in the aleurone cells of salinized seeds. On GA_3 exposure, the aleurone cells became more turgid and the cytoplasm of aleurone cells were almost digested, characterized by the presence of very big vacuoles. The protein content in the endosperm was almost nil. The starch grains in the sub-aleurone region were also much reduced. Eventhough the GA_3 treated NaCl stressed seeds showed much starch and protein in the endosperm compared to that of GA_3 treated control seeds, they were much reduced as compared to the salt control. Administration of putrescine in salt treated seeds also led to better hydrolysis of reserve food material compared to salt control. Few lipid globules were also localized in the aleurone tissue of all treatments after 120h of germination, but showed no significant change.

The aleurone cells of control seeds showed high activity of SDH compared to G6PDH and it was opposite in case of salt exposed seeds. An enhanced activity of SDH was observed in the aleurone cells of GA_3 treated control as well as salinized seeds. Incorporation of GA_3 in the salinized germination medium showed a slight increase in the activity of G6PDH. Exposure of seeds to putrescine also increase the activity of SDH in aleurone of salt stressed seeds. The increased activity of G6PDH in the salinized aleurone cells was further enhanced by putrescine.

The aleurone cells of control seeds were characterised by the presence of numerous aleurone grains surrounded by many spherosomes showing different stages of digestion. They also had other cytoplasmic organelles such as RER, plastids, ribosomes and mitochondria. The nucleus was dense with single nucleolus.

In the NaCl treated aleurone cells the cell organelles were in abundance due to less digestion. The aleurone grains were mostly in the undigested form. The chromatin in the nucleus appeared condensed in these cells. Conspicous changes were also seen in the cytoplasm involving mitochondria, ER and plasma membrane. Mitochondria were in high density. Besides the normal mitochondria, some were seen with vacuolation. ER elements were elongated, dilated and fragmented. Certain invaginations of plasmalemma into the vacuoles were observed. Other than these some crystals were also noticed in their aleurone cells.

The aleurone cell wall lost its fibrillar appearance and became thin upon GA_3 treatment. The extension and fusion of aleurone grains led to the formation of a large vacuole in the centre pushing very little cytoplasm towards the periphery. The frequency of spherosomes, mitochondria, plastids, RER and ribosomes became few but had better clarity. Aleurone cells of putrescine treated were characterised by few large vacuoles with peripheral cytoplasm. The cell organelles were less in number. Administration of GA_3 or putrescine in NaCl exposed seeds led to better digestion of aleurone grains and vacuolation compared to salt control.

The structural changes observed in the mitochondria, and the nucleus or the presence of crystals were not found upon GA_3 or putrescine treatment.

It is concluded from the present studies that NaCl inhibits the seedling growth by :

a) decreasing the contents of K^+, Mg^{2+}, Ca^{2+} and S in the aleurone, scutellum and endosperm regions of germinating seeds.

b) decreasing the hydrolysis of reserve food materials i.e., starch grains and protein bodies in the endosperm and digestion of aleurone grains in the aleurone cells.

c) decreasing the activity of SDH in aleurone cells.

d) increasing the accumulation of Na^+ and Cl^- in the aleurone, scutellum and endosperm.

e) altering the structure of mitochondria and nucleus.

Administration of GA_3 and putrescine was found to increase the seedling growth by enhancing extension growth, dry weight of shoot and root systems following salinization. This improvement could be due to :

a) improved ionic content (GA_3 and putrescine enhanced K^+, Ca^{2+}, Mg^{2+} and S in the aleurone. They also reduced the net accumulation of Na^+ and Cl^- in the aleurone, endosperm and scutellum of salt stressed seeds).

b) improved hydrolysis of reserves in the endosperm.

c) increased activity of respiratory enzymes SDH and G6PDH in the aleurone cells.

d) increased digestion of aleurone grains and by retaining the normal structure of nucleus and mitchondria.

However, all parameters showed better results under the influence of GA_3 than putrescine. Thus, administration of GA_3 and putrescine is found to ameliorate the adverse effects of NaCl to a great extend in rice seeds during germination.