INTRODUCTION

Food is a basic requirement in the hierarchical needs of man. The successful domestication of plants, animals, soil and water by settled human communities for food has hence been the starting point for evaluation of human culture. Ironically, the very centres of civilisation, where domestication of plants and animals took place in the past, are the areas which are struggling today to find an honourable equation between population growth and food supply. According to Population Research Bureau, Washington, 1994, world population is still growing at an exponential rate of 1.6 percent. Because of the rapid growth in population and consequent need of more land for domestic, industrial, communication and other purposes, land today is a shrinking resource for agriculture. Our agricultural production process is still predominantly based on the use of renewable resources but current productivity is very low. As the population has grown, the average farm size has fallen from 2.3 to 1.7 hectares per rural person between 1971 to 1986 and by late 1980s over three quarters of all land holdings became less than 2 hectares in size, and almost three fifths less than one hectare (Bowonder, 1993). As a result any future increase in global agricultural production has to be from an increased production per unit area, rather than from the expansion of arable land. But increase in production per unit area is not without problems. The total area of cultivable land is limited due to desertification, soil erosion and other environmental factors. Increased irrigation with inadequate drainage has rendered more and more areas alkaline and saline. The quality of cropland has become increasingly stressed with extensive use and improper water management (Robert, 1994). Salinity and alkalinity affect two fifth of India's irrigated soil. 40,000 square miles of the nations irrigated land is suffering from waterlogging and salinization reducing its average productivity by about one fifth (Ehrlich and Ehrlich, 1990).

Salinity refers to the occurrence of excessive amounts of salts that may interfere with the growth of plants. Excess salts, cause physical and chemical changes in soil structure which drastically change the environment of the plant (Poljakoff-Mayber and Gale, 1975). Salts build up as mineral-laden water evaporates in the root zone or on soil surface, whether drawn up from a high water table through capillary action or deposited on the surface through irrigation. The salt that accumulate in soil consist principally of various proportions of sodium, calcium and magnesium cations and chloride anions. Potassium, bicarbonate, carbonate, nitrate and borate ions occur in minor quantities (Shainberg, 1975). The U.S. Salinity Laboratory defines a saline soil as one having an electric conductivity of the saturation extract greater than 4 milliohms per centimeter (4 mmhos/cm) and an exchangeable sodium percentage (ESP) less than 15. Sodium chloride is relatively innocuous as an inorganic toxin, but it occurs commonly in the environment, and thus is one of the most troublesome salts to agriculture. Gujarat state alone has about 3,04,582 hectares of uncultivable land due to salinity (Sharma and Gupta, 1986) and sodium chloride is the most predominant salt present in the soil of Gujarat. Hence it is used to create saline condition in the present study.

Numerous reviews have been published on the effect of salinity on the growth of plant species (Levitt, 1972; Flowers et al., 1977; Greenway and Munns, 1980; Salim and Pitman, 1983; Khan and Unger, 1985; Hampson and Simpson, 1990; Aslam et al., 1993; Khatun and Flowers, 1995; Lutts et al., 1995).

Plant species differ in their tolerance to total salts and to specific ions. Also crops that are highly tolerant at one growth stage may be sensitive during another stage. A decrease in shoot and root growth and dry matter production due to sodium chloride and sodium bisulphate was reported in crop plants by Roth (1989).

Cereals are the major component of the diet of man. At world level, cereal grains contribute about 50 percent of the per capita energy intake (Duffus and Slaughter, 1980). The effect of NaCl salinity on germination and seedling growth have long been investigated especially on cereals. Francois et al. (1986) reported low grain yield and quality, reduced vegetative growth and germination in wheat as a result of sodium chloride stress. Johnson (1991) observed a reduction in establishment and productivity in wheat when subjected to salinity. NaCl influenced decrease in transpiration in rice was studied by Sugimoto et al.

(1985). According to Prakash et al. (1988), growth, dry matter accumulation and grain yield were considerably reduced when the rice plants were subjected to salt stress. Mass (1984) published a detailed list of information about relative tolerance among crop plants and percentage yield reduction as influenced by soil salinity. Salinity also affects the growth of reproductive structures (Dhingra and Varghese, 1985a; 1990). Khatun and Flowers (1995) studied the effect of salinity on pollen viability and seed set. A decrease in grain yield, seed weight and spikelet differentiation due to sodium chloride stress was observed in wheat by Mass and Poss (1989).

Generally plants are more sensitive to salinity during germination and early seedling growth (Carter, 1975). Reduction in germination and seedling vigour under saline condition has been observed in wheat (Hampson and Simpson, 1990; Petruzzelli et al., 1992; Begum et al., 1992), barley (Bliss et al., 1986; Bozcuk, 1991; Durusoy et al., 1995), maize (Lin, 1985) and rice (Babu and Ramesh Babu, 1985; Prakash and Prathapasenan, 1988b; Torres and Echevarria, 1994; Al Helal and Al Hubashi, 1995).

Rice is one of the oldest and most important cereal and it is the staple food of over half of the world's population. It is perhaps the most remarkable of cultivable crops, for although possessing the roots of a dryland crop, it flourishes in swamps or under irrigation. The crop has been grown over a very wide range of climatic and soil conditions and it is difficult to define those most suitable conditions for its development. But certain factors such as sulphides, acidity and salinity of the soil do affect the growth and development of paddy. Compared to other crops, rice is more tolerant to salinity and it can be grown in the marginal lands afflicted by the problem of salinity. Hence in the present study, rice has been chosen as the experimental system.

Varietal tolerance of rice to salinity during germination was studied by Gill and Singh (1985), Campos and Assuncao (1990), Basu and Ghosh (1991) and Torres and Echevarria (1994). Eventhough the exact inhibitory mechanism of germination and growth by NaCl salinization is still not very clear, reports are available on different aspects regarding the involvement of NaCl in impairing the normal physiological activities of rice plant. According to Gill and Singh (1985), water absorption is reduced by salinity. Panaullah et al. (1990) reported that NaCl creates water potential gradient which causes a great stress in rice during its germination. NaCl markedly reduces the α-amylase activity and mobilisation of starch in endosperm of rice (Acharya et al., 1990; Lin and Kao, 1995). Echevarria et al. (1995) observed a reduction in protease activity, proline and protein contents in the endosperm of rice under NaCl stress during germination.

Salt stress is known to cause marked and rapid alterations in the metabolic activities of plants. The specific effects of NaCl on carbohydrate metabolism was reported by Hatata and Farah (1982), Weselake et al. (1985) and

Acharya et al. (1990). Nitrogen metabolism with respect to the activity of protease enzyme was investigated by Echevarria et al. (1995). Studies by Ramagopal (1988) and Dell' Aquila and Spada (1993) showed that some 'salt stress' proteins are synthesized on subjection to NaCl during germination. Rani and Reddy (1994) observed the changes in protein profiles induced by salt stress during germination of rice. According to Dubey and Rani (1990) salt treatment sharply decreases the activity of peptidase in germinating embryos. Several studies reveal that the level of total polyamines sharply increases or decreases in rice under the influence of NaCl (Prakash and Prathapsenan, 1988b; Sadhana and Dubey, 1990; Basu and Ghosh, 1991). According to Reggiani et al. (1994) the presence of NaCl decreases the putrescine level in the roots, while that of spermidine and spermine increases in the shoot.

Many attempts have been made to understand the effect of salinity on nucleic acid metabolism and activity of associated enzymes. Mittal and Dubey (1990) studied the effect of NaCl salinity on RNA level and activity of ribonuclease. Salinity decreases RNA and DNA contents (Roy et al., 1992). The inhibitory action of NaCl on deoxyribonucleo-proteins was studied by Avilova et al. (1983) and Mohamed and Hamada (1988).

There have been many studies on key enzymes of plant metabolism under salinization. Kocacaliskan (1990), Kabar and Kocacaliskan (1990), Mittal and Dubey (1992a) observed decreased activity of polyphenol oxidase in germinating cereals with increasing salinity. The different behaviour of peroxidases in germinating seeds and seedlings of rice cultivars differing in salt tolerance was observed by Dhingra and Varghese (1990) and Mittal and Dubey (1991). The activity of phosphatases also decreases when subjected to salinity (Dubey and Sharma, 1990; Mittal and Dubey, 1992b). NaCl adversely effects the activity of ATPase (Dubey et al., 1987), alcohol dehydrogenase and ribulose, 1,5,bi phosphate carboxylase (Stiborova et al., 1987), nitrate reductase (Hsu and Sung, 1981), malate, isocitrate and glucose 6-phosphate dehydrogenases (Sadhana and Dubey 1994). According to Sheoran and Garg (1978) the effect of salinity on enzyme activity varies with the stage of plant growth, plant organ, type of salinity and the enzyme studied.

Salinity is known to alter the level of endogenous hormones in plants. A decrease in the level of auxin due to salt stress was observed by Naqvi and Ansari (1974). Boucaud and Unger (1976) reported similar decrease in cytokinin level under the influence of NaCl stress. An increase in ethylene production (Yasseen et al., 1988) and abscisic acid content (Lachno and Baker, 1986) have been found in response to salinity.

Exogenous application of growth hormones has been shown to ameliorate salt induced inhibition of plant growth. Presoaking in plant hormones is known to enhance the germination and nutrient uptake in salt treated seeds (Balki and Padole, 1982). The role of gibberellic acid (GA_3) in alleviating the adverse effects of salt on plant growth has been studied by number of workers (Levitt, 1980; Khan and Unger, 1985; Acharya et al., 1990; Lin and Kao, 1995). Kinetin is most effective in increasing the germination in dicotyledons (Bozcuk, 1981, 1990; Kabar, 1990). Supplementation of proline counteracts the effect of osmotic stress on the NaCl treated plants (Roy et al., 1993; El Sayed and El Haak, 1994). Inhibitory effect of NaCl on germination and seedling growth was mitigated also by calcium application (Zhang and Liu, 1992; Hamada, 1994).

Polyamines have been implicated in alleviating the toxic effects of NaCl salinity on plant growth and yield (Prakash and Prathapasenan, 1988a,b). Durusoy et al. (1995) reported an increased activity of α -amylase on application of spermidine in NaCl exposed germinating barley seeds.

To date, most studies have focussed on the physiological responses of plants to NaCl stress and its amelioration by plant growth regulators. However, it is not clear whether this sensitivity is exclusively due to osmotic effects or any other factors. Some attempts have been made to study the effect of NaCl on the translocation of other solutes (Gomes Filho et al., 1983; Munns, 1985; Wolf et al., 1991). The structural changes and permeability of the plasma membrane in response to salinity was studied by Bliss et al. (1984) and Cramer et al. (1985). The ultrastructural damage to mitochondria in root tips of Agrostis stolonifera was observed by Smith et al. (1982). Petruzzelli et al. (1992) compared the physiological and ultrastructural changes in isolated wheat embryos germinated in the presence of NaCl. However, structural and histochemical changes in the aleurone cells and endosperm of the germinating seeds under the influence of NaCl and growth hormones received less attention. Keeping this in view, the present work was taken up to get some insight into the cytochemistry of germinating rice as affected by NaCl, GA_3 and putrescine. The following aspects have been examined in this investigation.

a. Scanning of the husk.

b. Energy dispersive X-ray ion analysis of the endosperm, scutellum and aleurone layers.

c. Histochemical localization of reserve food materials in the aleurone layers and endosperm.

d. Localization of respiratory enzymes succinate dehydrogenase (SDH) and glucose-6-phosphate dehydrogenase (G6PDH) in the aleurone cells.

e. Ultrastructural changes in the aleurone cells.

Results of these studies are discussed in the light of relevant literature and are presented in this thesis.