INTRODUCTION

The 20th century has witnessed a dramatic change in the rate of growth of world demand for food due to population explosion. According to the current United Nations Projections, by the turn of the century the world population will be 6.5 billion and it will reach 8.0 billion by 2010. But food production growth rates are barely sufficient to keep pace with population growth especially in the poor populous countries. (Barr, 1981; Hopper, 1981; Lever, 1982). To meet this rising demand for food production, there has to be an increase in cropped area as well as increasing pressure to improve yields per unit area. As the area of cultivable land is more or less fixed, to increase the cropped area, it is highly essential to push agriculture farther onto marginal lands lying under uncultivable conditions due to soil salinity, alkalinity or other environmental stresses. Of the earth's surface about 400 million hectares of land are affected by salinity (Massoud, 1974) and the problem is increasing day by day due to increased irrigation networks and inadequate drainage facilities. In India it is estimated that about 12 million hectares of marginal land have been afflicted by the problem of soil salinity or alkalinity (Sharma and Gupta, 1986). Gujarat State alone has about 3,04582 hectares of land which lie under uncultivable condition due to salinity and sodium chloride has been reported as the major salt present in such soils (Sharma and Gupta 1986). Hence in the present study sodium chloride was used to create saline condition.

Salinity refers to the occurrence of various soluble salts in soil or water in concentrations that may interfere with the growth of plants. Though sodium chloride is sometimes the most predominant salt present, the term salinity includes chlorides, sulphates and bicarbonates of sodium, calcium, magnesium and potassium (Chapman, 1975; Abrol,1986). A multitude of ways by which concentrations of these salts can be expressed, but the preferred expression by physiologists and soil scientists is electrical conductivity (EC) stated as decisiemens per meter (dS/m) or millimhos 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 millimol or more can be considered as saline.

Salinity is known to affect many aspects of plant metabolism and to induce changes in their anatomy and morphology. The literature on salinity and the response of plants to saline environment has been reivewed by Bernstein and Hayward (1958), Strogonov (1962), Murthy and Janardhan (1971), Rains (1972), Waisel (1972), Poljakoff-Mayber and Gale (1975), Jennings (1976), Flowers et al. (1977), Ungar (1978), Greenway and Munns (1980), Poljakoff-Mayber (1982), Yeo (1983), Downton (1984) and Yeo and Flowers (1986).

Salinity adversely affects almost all growth and developmental processes of plants studied todate. A

reduction in germination under saline condition was observed in soybean by Abel and Mackenzie (1964), sunflower by Karami (1974), wheat by Kaufmann and Ross (1970), lettuce by Odegbaro and Smith (1969) and tomato, barley and cotton by Bozeuk (1981). Kaddah (1963) and Sarin and Narayanan (1968) found a delay in germination of rice seeds by salt and they observed that young rice seedlings were highly sensitive to salt. Pearson et al. (1966) studied the relative salt tolerance of rice during germination and early seedling development and noted that rice is least tolerant to salinity during seedling stage and that all varieties are not equally salt tolerant. Varietal tolerance of rice seeds during germination to different salt concentrations was also evaluated by Rao et al. (1973) and Gill and Singh (1985).

Though the exact mechanism of NaCl induced inhibition of germination and seedling growth is still obscure, there are reports about the involvement of NaCl in inhibiting radicle emergence by impairing the process of water absorption (Prisco and O'Leary, 1970; Gill and Singh, 1985) and mobilization of reserve food materials from storage organs (Prisco and Vieira, 1976; Gomes Filho et al. 1983). Studies with wheat (Kaufmann and Ross, 1970) lettuce (Odegbaro and Smith, 1969; Kaufmann and Ross, 1970), tobacco (Benzioni et al. 1967) and tomato, barley and cotton (Bozcuk, 1981) indicate that endogenous level of growth substances will be a limiting factor under stress condition.

One of the most common and conspicuous effects of salinity is the suppression of growth (Nieman, 1962; Greenway, 1973; Sharma and Gupta, 1986). Salinity has been shown to affect the size of the plant, branching, leaf area and overall plant anatomy (Poljakoff-Mayber, 1975). In contrast, halophytes, although able to grow in a non-saline substrate, will usually grow better in presence of salt. (Eshel, 1985; Cheeseman and Wickens, 1986). However, experiments with Atriplex halimus grown in culture media salinized with different levels of NaCl, under two different air humidities, suggested that growth of halophytes will also be adversely affected by salinity, depending upon the prevalence of other environmental conditions (Gale et al. 1970). After a series of studies with different plant species Ungar (1978) concluded that all vascular plants investigated display both delay in time of germination and reduction in seedling growth under high salinity levels.

Solov'ev (1969) studied the effect of NaCl salinization on the growth of pumpkin in relation to osmotic stress and mineral element supply and found that the main cause of growth inhibition was poor availability of mineral nutrients. Strogonov (1962) reported 50% inhibition of growth in tomatoes grown in soil containing 0.1 % (of dry weight) chloride. Weight of fruit per plant was reduced by 90 %. Growth of corn (Siegel et al. 1980), chick pea (Singh and Singh, 1980), wheat (Kingsbury and Epstein, 1986) and cow pea and mung beans

(Balasubramaniam and Sinha, 1976)was also found decreased when grown in saline condition. After extensive specific ion toxicity studies Kingsbury and Epstein (1986) concluded that there is a definite specific ion effect which is related to salt sensitivity in wheat. Their results also suggest that superior compartmentation of toxic ions, principally Na^+, may be a mechanism of salt resistance in wheat. In general shoot system is most affected by salinity and Greenway (1973) is of the opinion that the energy expenditure during osmotic adjustment to salinity is one of the main factors reducing growth. The effects of NaCl salinity on higher plant growth and the causes of growth inhibition are reviewed by Jennings (1976) and Yeo (1983).

Like vegetative parts growth and development of reproductive structures are also highly susceptible to salinity (Abdullah et al.1978; Dhingra and Varghese,1985a). Korkor and Abdel-Aal (1974) studied the effects of total salinity as well as specific ion toxicity of NaCl, $CaCl_2$ and $Na_2 SO_4$ on growth and yield of rice and found that increase in salinity decreased both vegetative growth and grain yield. Tillering, like other attributes of vegetative growth, is known to be affected by salinity in growth medium. Under severe salinity very few tillers are produced in barley and wheat and they die before they are able to grow and bear ears (Sharma and Gupta, 1986). As a consequence, yields are generally reduced in proportion to the decrease in tiller growth particularly in crops where grain yield is strongly

linked with vegetative growth. According to Maas and Hoffman (1977), crop yield decreases markedly with increase in salt concentration, but the threshold concentration and rate of yield decrease vary with the species. A detailed list giving information about relative tolerance among crop plants and percentage yield reduction above thershold soil salinity was published recently by Maas (1984).

Salinity affects all stages of development and, for most of the crops sensitivity varies from one growth stage to another. Some of the crop species at germination stage are not as salt tolerant as at later stages of development (Levitt, 1980). Sugarbeet, barley and cotton, for instance, are among the most tolerant agricultural crops but all are relatively sensitive during either germination or early seedling growth. Rice, on the other hand, is highly sensitive during both seedling and flowering stages (Pearson et al. 1966, Ponnamperuma, 1984).

The microscopic and submicroscopic changes occuring in response to salinity vary in different plant species. The available evidence clearly indicate that soil salinity markedly alter the anatomy of leaves (Udovenko et al. 1970; Wignarajah et al. 1975a, Harvey and Thorpe, 1986), stem (Poljakoff-Mayber, 1975) and roots (Udovenko et al. 1970; Smith et al. 1983; Hodson and Mayer, 1987). Submicroscopic studies revealed that the fine structure of cell membranes and cell organells (Smith et al. 1983; Werker et al. 1983;

Harvey et al. 1985; Hodson and Mayer, 1987) are considerably altered by salinity.

The morphological and anatomical anomalies occurring are correlated with different metabolic changes in salt-stressed plants (Wignarajah et al. 1975b; Ramana and Rama Das, 1978; Sheoran and Garg, 1978). There are several reports of a general reduction in photosynthesis in plants induced by salinity (Gale et al. 1967; Udovenko et al. 1971; Lapina and Bikmukhametova, 1972; Downton, 1977; Ball and Farquhar, 1984; Yeo et al. 1985). An exception to this, however, was found in halophytes where low concentration of salt enhanced photosynthesis (Gale and Poljakoff-Mayber, 1970).

A decrease in respiration rate in response to salinity has been reported in many plants (Bharadwaj and Rao, 1960; Levitt, 1980). However, in some cases, for example in beans (Nieman, 1962) and in pea seedlings (Livne and Levin, 1967) an increase in respiration rate has been reported as a result of salinization. Porath and Poljakoff-Mayber (1965) however found a progressive inhibition of respiration in pea by increasing salt concentration.

Carbohydrate metabolism is affected depending on the severity and the type of salinity. Sarin and Narayanan (1968) observed a decline in amylase activity in germinating wheat seeds under high levels of salinity. On the other

hand stimulation of amylase activity under salinity has been reported by El Fouly and Jung (1972) in wheat seedlings. Studies by Gauch and Eaton (1942) showed that in barley grown in sand culture flushed with saline water, during a diurnal cycle, leaves were generally 20-30% higher in starch and 30-70% higher in sugars than in controls. They pointed out that increase in starch implied a lack of utilization and concluded that salinity infact affected cellular elaboration rather than photosynthesis. Munns et al.(1982) after a detailed study reported that, carbohydrate status of the elongating leaf tissues of a salt tolerant variety of barley (cv. Beacher) support the idea that growth at high NaCl salinity was not limited by supply of carbohydrates to the growing region.

Salinity reduces the synthesis of nucleic acids in many plants (Levitt, 1980), but in Phaseolus vulgaris Nieman (1965) had found no effect of NaCl on DNA synthesis. Protein metabolism has also been reported to be disturbed as a result of salinization and both an increase (Singh and Vijayakumar, 1974; Kalir and Poljakoff-Mayber, 1981; Solomon et al. 1987) and a decrease (Kahane and Poljakoff-Mayber, 1968; Langdale et al. 1973) in protein synthesis have been observed. Salinity has been shown to interfere with the uptake of inorganic ^{15}N into young barley plants, whereas the incorporation of ^{15}N into protein was not

affected or was even stimulated (Hellal et al.1975). On the other hand, salinity was shown to inhibit the uptake of externally supplied amino acid and their incorporation into protein (Kahane and Poljakoff-Mayber, 1968).

The most commonly reported effect of salinization on amino acid metabolism is an accumulation of proline, both in glycophytes and halophytes (Greenway and Munns, 1980; Levitt, 1980). Besides proline, the levels of other amino acids such as arginine, serine and glutamic acid in the leaves of Phaseolus aconitifolius have also been enhanced by NaCl (Huber et al. 1977). Accumulation of ammonium compounds like glycine betaine (Storey and Wyn Jones, 1975; Goas et al. 1982; Diggelen et al. 1986), β -homobetaine (Larher and Hamelin, 1975) due to salt stress, has also been recorded. Even though an osmoregulatory role for proline and glycine betaine has been suggested by Stewart and Lee (1974) and Storey and Wyn Jones (1975) respectively, a precise physiological or adaptive function has not been assigned to these compounds.

Correlation between salinity resistance and membrane lipid content as well as composition have been found in various investigations (Twersky and Felhendler, 1973; Stuiver et al. 1978; Lynch et al. 1987). Changes in lipid metabolism may be attributed to stress-induced degradation reactions and enzymes such as phospholipase and lipoxygenase

(Kuiper, 1985) may be involved in such degradative processes. The mechanisms by which salt inhibits growth are not known, but there is good reason to suspect that membranes are the sites for primary salt effects (Leopold and Willing, 1984; Cramer et al. 1985). Sodium chloride interferes with a wide variety of membrane functions, including permeability (Cheeseman,1985; Eshel, 1985; Yeo, 1983; Yeo et al. 1985; Taleisnik-Gertel and Tal, 1986), transport of both organic and inorganic solutes (Prisco and Vieira,1976; Gomes Filho et al. 1983; Munns, 1985) and secretion (Kylin and Quatrano, 1975) in many plants.

The activity of a number of key enzymes in plants has been shown to be increased (El-Fouly and Jung, 1972; Sheoran and Garg, 1978; Kalir and Poljakoff-Mayber, 1981; Murumkar and Chavan, 1987) or decreased (Hanson-Porath and Poljakoff-Mayber, 1969; Osmond and Greenway, 1972; Plaut, 1974; Kalir et al. 1984, Gill and Singh, 1985; Murumkar and Chavan, 1987) or unaffected (Weimberg, 1970; Greenway and Osmond, 1972) by NaCl salinity. An inhibition of RNase activity in cotyledons and roots and its stimulation in embryo-axis and leaves of mung bean as a result of salinity have been observed by Sheoran and Garg (1978). From these results and observations of other workers they have concluded that the effect of salinity on enzyme activity varies with the stage of plant growth, the organ of the

plant, the type of salinity and the enzyme studied. Therefore most of the controversies on this subject seems to be due to the usage of different plant parts or plant species of different age and type of salinity.

Salt stress is known to cause marked and often rapid alterations in endogenous hormone levels in plants (Wright, 1978). Generally the content of growth promoters decreases while that of inhibitors increases and such modifications of hormone content, in many instances, are considered as a strategy which may enable the plant to cope up with the various environmental stresses. A decrease in the level of diffusible auxin (Naqvi and Ansari, 1974), cotykinins (Itai et al. 1968; Mizrrahi et al. 1971; Boucand and Ungar, 1976a) and an increase in abscisic acid content (Mizhrahi et al. 1971; Tal, 1977; Downton and Loveys, 1981; Yeo et al. 1985; Lachno and Baker, 1986) have been reported in response to salinity. Though the ecological implications of the hormonal changes induced by salt stress have been discussed in detail (Wright, 1978), regulatory mechanism(s) controlling hormone levels and its action under stress conditions is not yet well understood.

Some of the studies, however, proved the effectiveness of growth hormones treatment in ameliorating stress injuries caused by salinity. Successful employment of gibberellic acid in overcoming seed dormancy resulting from diverse

factors including salinity was demonstrated by many workers (Levitt, 1980). Khan and Tao (1977) reported that gibberellin was able to overcome the osmotic inhibition of lettuce seed germination. Interactive effects of gibberellic acid and salinity in increasing stem growth of beans was reported by Nieman and Bernstein (1959). Studies of Khan and Ungar (1985) and Boucaud and Ungar (1976a) also show that GA_3 can stimulate seed germination under saline condition in halophytes as well.

Interaction between indole acetic acid and salinity on plant growth has been investigated by Sarin (1962). He found that treatments with 5 ppm IAA substantially increased vegetative growth and yield. Odegbaro and Smith (1969) found that treating Lactuca sativa seeds with kinetin caused increase in germination in NaCl treated seeds. Kinetin induced stimulation of germination was also observed in tomato, barley and cotton seeds exposed to NaCl (Bozcuk,1981). Thus most of the studies are restricted to germination stage and detailed investigations are not carried out to understand the mechanism (s) by which these compounds alleviate the stress injury.

Recently a number of studies have demonstrated that hormonal action in plants is mediated through polyamines (Bernal-Lugo, 1983; Smith et al. 1983; Lin, 1984) and that certain endogenous level of polyamines must be maintained

for the full expression of hormone action (Lin, 1984). Polyamines (putrescine, spermidine and spermine) are ubiquitously distributed in animals and plants (Tabor and Tabor, 1984; Smith, 1985). These naturally occurring compounds are synthesised in plants either from arginine or ornithine or from both (Slocum et al. 1984). Putrescine may be formed by the direct decarboxylation of ornithine or indirectly, through a series of intermediates, following arginine decarboxylation. Spermidine and spermine are synthesised from putrescine by subsequent addition of aminopropyl groups donated by decarboxylated S-adenosyl methionine (Slocum et al. 1984). The aminopropyl group additions are catalysed by specific aminopropyl transferases, commonly known as spermidine and spermine synthases (Baxter and Coscia, 1973; Tabor and Tabor, 1984). Degradation of these compounds in plants is carried out by diamine and polyamine oxidases (Smith, 1985).

Very little is known about the subcellular compartmentation of polyamines and polyamine metabolism in plants and other eukaryotic systems. Preliminary studies suggest that ornithine decarboxylase (ODC) is associated with nuclear chromatin in barley seedlings, although cytoplasmic ODC activity was also detected, as in most plant species (Slocum et al. 1984). Cell fractionation studies generally support a cytoplasmic location for arginine decarboxylase

(ADC), while spermidine synthase activity has been localized in purified chloroplast preparations (Cohen et al. 1981). Polyamine oxidase activity was, however, reported to be associated with cell wall (Kaur-Sawhney et al. 1981).

Biological functions of polyamines appear to be attributable to the polycationic nature of these molecules and their electrostatic interactions with biomembranes and macromolecules in the cell (Slocum et al.1984). Polyamines which are considered as one of the important factors involved in growth and its regulation are playing a key role in regulating membrane functions by binding to the negatively charged phospholipid head groups or other anionic sites on membranes (Naik and Srivastava, 1978; Srivastava and Smith, 1982).

Possible involvement of polyamines in the regulation of structure, function and synthesis of nucleic acids has been suggested by many researchers (Bagni et al.1971; Serafini-Fracassini et al. 1980; Kaur-Sawhney et al. 1980; Bagni et al.1981). Polyamines are also known to regulate enzyme activity by increasing the synthesis of enzymes, covalent binding or by various types of ionic interactions (Slocum et al.1984; Tabor and Tabor, 1984) with the enzyme protein.

Polyamines have long been known to stimulate protein synthesis (Tabor and Tabor, 1984). Cocucci and Bagni (1968) have correlated stimulation of protein synthesis with increased polyamine synthesis following auxin-induced activation of dormant Helianthus tissue. In wheat germ system spermidine enhances peptide synthesis by increasing the rates of both peptide chain initiation and elongation (Takemoto et al.1983). Chin and Sung (1972) and Wickner et al. (1973) have shown that the activities of several enzymes regulating nucleic acid synthesis and repair are affected by polyamine availability.

The absolute requirement of polyamines for growth was first demonstrated by Herbst and Snell (1948) in Hemophilus parainfluensa and later it was confirmed in many other systems (Sneath, 1955; Bagni et al. 1981; Tabor, 1981). Putrescine and other polyamines are now known to control a number of growth and developmental processes in plants. These include cell division (Bagni, 1966; Kaur-Sawhney et al.1980; Huhtinen et al. 1982; Costa et al. 1984), seed germination (Nezovorova and Borisova,1967; Villanueva et al. 1978); seed viability (Mukhopadhyay et al.1983; Mukhopadhyay and Ghosh, 1986), tuber dormancy (Bagni et al.1980), hypocotyl growth (Cho,1983), root formation (Friedman et al.1982; Jarvis et al.1983; Kakkar and Rai, 1987), embryogenesis (Bradley et al.1984; Feirer et al.1984), senescence (Cohen et al. 1979; Altman, 1982; Shih et al.1982); tumor growth (Bagni and Serafini-

Fracassini,1979; Kulpa et al.1985), bud formation (Torrigiani et al. 1987),cell differentiation (Heby,1981; Chriqui et al. 1986), growth of internode (Smith et al.1985),development of ovaries (Cohen et al.1982; Slocum and Galston,1985), pollen germination and tube growth (Bagni et al.1981; Prakash et al. 1988) and fruit set (Costa and Bagni, 1983; Costa et al.1984). Modulation of polyamine biosynthesis by plant growth regulators is also well documented (Smith, 1985).

Recently there is an increasing interest in studying the role of polyamines in various stress-induced responses of plants (Smith, 1985). The level of these compounds, depending on the species and type of stress, may either increase (Flores et al.1984; Mc Donald and Kushad,1986; Turner and Stewart,1986) or decrease (Priebe and Jager, 1978; Guye et al.1986; Turner and Stewart, 1986) and the increase in polyamine levels as suggested by Slocum et al. (1984) may be of a protective nature conferring selective advantage to the stressed cells. Many investigations have shown that polyamines can protect the structure and functions of biomembranes and various cellular processes during induced senescence (Cohen et al.1979; Altman 1982; Shih et al.1982) and high temperature treatment (Nezgovorova and Borisova, 1967). A patent has already been awarded for protection of crops by diamines against frost damage, air pollution, loss of chlorophyll and wilting (Okii et al.1980). Keeping this background in mind the present work was take up with a view to achieve a deeper

insight into the mechanism(s) of inhibition of growth of rice by NaCl salinity and its amelioration by putrescine and GA_3. The following parameters have been examined; (a) linear growth, fresh and dry weights of shoot and root systems; (b) leaf area; (c) the content of total chlorophyll, Na^+,Cl^-,K^+, proline, total quaternary ammonium compounds, IAA, GA-like substances, ABA, polyamines and total protein in shoot and root systems during different stages of growth; (d) activity of IAA oxidase, total amylase, invertase, proline oxidase and agmatine deiminase in shoot and root systems during different stages of growth; (e) linear growth, activity of cellulase and pectin lyase and the contents of total chlorophyll and IAA during leaf growth and (f) yield parameters viz. i) total number of filled and unfilled seeds per plant, (ii) total weight of filled seeds per plant and (iii) weight of 1000 seeds. Results of these studies are discussed in the light of relevant literature.