DISCUSSION

Extension growth, fresh weight and dry matter accumulation of shoot and root systems were considerably decreased when rice plants were subjected to salt stress. Similar effects of salinity have been reported in Phaseolus vulgaris (Wignarajah et al. 1975a and b), Vitis vinifera (Downton, 1977), Capsicum annum (Walker et al. 1980) Cicer arietinum (Singh and Singh, 1980) and Casurania obesa (Reddell et al. 1986). The fresh and dry weights of shoot and root systems continued to increase even after the cessation of linear growth. This increased production of fresh and dry weights of shoot and root systems has been found due to the growth of tillers.

Administration of putrescine significantly enhanced the growth of shoot and root systems under saline condition. This indirectly suggests that in rice, the endogenous level of putrescine may be adversely affected by salinization which is needed for active cell division and growth (Slocum et al. 1984; Tabor and Tabor, 1984; Smith, 1985). Reduction in plant growth as well as the level of putrescine and other polyamines under various stress conditions including salinity has been reported in many plants (Priebe and Jager, 1978; Smith, 1984; Turner and Stewart, 1986). A marked inhibition of putrescine production by NaCl salinity was observed in the present investigation as well. Application of putrescine at high concentration (10^{-3}M) was, however, found inhibitory to growth of plants. Reduction in plant

growth observed in non-stressed plants at 10^{-3}M putrescine treatment may be due to its toxic effects (Flores et al. 1984; Prakash et al. 1988).

It is generally accepted that interactions of growth regulators are involved in the regulation of plant growth and development (Wareing, 1982). Hence these compounds are in use to modify crop growth to economic advantage (Weaver, 1972; McLaren, 1982; Thomas, 1982). Gibberellic acid, one of the commonly used hormones, when applied externally has been found to improve growth of rice better than putrescine under saline condition. Many earlier workers have demonstrated that exogenous application of GA_3 can counteract the deleterious effects of salt stress on various growth and developmental processes such as seed germination (Sarin and Narayanan, 1968; Boucaud and Ungar, 1976a; Khan and Ungar, 1985; Kuhad et al., 1987), photosynthesis, translocation and growth (Strack et al. 1975; Singh and Singh, 1980) and pollen germination (Dhingra and Varghese, 1985a; Pharis and King, 1985). With the observation of Nieman (1962) and later by the studies of many other workers (Wignarajah et al. 1975a; Sharma and Gupta, 1986; Yasseen et al. 1987) it became increasingly apparent that NaCl inhibits growth by reducing cell division as well as cell enlargement. The growth stimulation obtained under stressed as well as non-stressed conditions by GA_3 may be due to its ability to promote cell division (Sachs and Lang, 1957; Sachs et al. 1959; Bernier

et al. 1964; Shininger, 1975; Scott, 1984), cell elongation (Feucht and Watson, 1958; Kaufman, 1965; Jones, 1973; Low, 1975; Scott, 1984) or both.

The total plant biomass production depends on the accumulation of photosynthate and this in turn is determined by the rate of carbon fixation, area of leaf surface available for photosynthesis and the chlorophyll content of the photosynthetic tissue. Like other morphological parameters, extension growth and the area of rice leaves were considerably decreased by NaCl salinity and this would be one of the reasons for the restricted growth of plants grown under saline condition.

Many factors such as decreased stomatal conductance (Downton et al. 1985), lowered invertase activity (Hawker and Walker, 1978; Hawker, 1980), high contents of Na^+ and Cl^- and low level of K^+, Mg^{2+} and total amino acids (Hawker and Walker, 1978; Gorham et al. 1985) are all correlated with the reduction in leaf growth. In the present investigation changes in the activity of two cell wall loosening enzymes namely, cellulase and pectinlyase were studied in relation to leaf growth. The activity of cellulase was measurably diminished while pectin lyase registered no significant alteration in its activity in response to salinization. Again, a sizeable reduction in the IAA content during the active phase of growth was recorded in the leaves of salt-stressed plants. Besides other factors induced by salinity, the low level

of auxin and decreased cellulase activity during the active phase of growth might also have contributed to the suppression of leaf growth in salt-stressed plants. Increased activity of cellulase and high content of auxin are implicated in active cell growth in a number of plant systems (Wareing and Philips, 1982; Baker et al. 1985).

Based on the informations obtained about the changes in the DNA content Nieman (1965) concluded that cell division is inhibited during leaf growth of beans under saline condition while Meiri and Poljakoff-Mayber (1967, 1969) provided evidence for reduction in cell expansion in the developing leaves following salinization. However, Wignarajah et al. (1975 a and b) later showed that, as with other plant parts, both a reduction in cell division as well as cell enlargement are involved in the suppression of leaf growth in Phaseolus vulgaris and a similar result was obtained with barley (Yasseen et al. 1987) also.

Application of putrescine significantly increased the linear growth as well as the area of rice leaves under stress condition. This might be due to its ability to hormonize various growth processes (Slocum et al. 1984; Tabor and Tabor, 1984; Smith, 1985) distorted by salinity as evidenced by the enhanced level of auxin and the high activity of cellulase in the leaves of putrescine - treated salt-stressed plants. Again, it must be noted here that putrescine has been shown to induce cell division (Kaur-Sawhney et al. 1980; Huhtinem et al. 1982; Costa et al. 1984) and, recently its requirement

for active cell division and growth in both plants and animals has also been established (Slocum et al. 1984; Tabor and Tabor, 1984).

Application of GA_3 resulted in a more pronounced stimulation of linear growth and leaf area under saline condition than that obtained with putrescine treatment. Even in control plants gibberellic acid administration brought about a marked increase in leaf length and leaf area. Further, unlike in putrescine, cellulase and pectinlyase activities were found to increase in the leaves of stressed as well as non-stressed plants in response to GA_3 treatment. It is now unequivocally proved that gibberellic acid modulates growth by influencing a wide array of cellular processes. From the results of the present study it can be suggested that, GA_3 may be stimulating the leaf growth by increasing the activity of cellulase and pectin lyase as well as the endogenous level of auxin. GA_3 enhancement of cellulase activity associated with cell wall growth (Ratner et al. 1969; Lewis and Varner, 1970) and auxin content (Scott, 1984) are demonstrated earlier. Again, it must be noted that GA_3 can also stimulate cell elongation independently of auxin (Kazama and Katsumi, 1974; Cleland et al. 1968; Kaufman et al. 1969). (Possible explanation of how putrescine and gibberellic acid alter the enzyme activity and hormonal level will be given in the later part of the discussion).

Salt stress considerably reduced the content of chlorophyll and advanced the process of senescence in rice. The reduction in the content of total chlorophyll could be due to the decreased pigment synthesis or high chlorophyll degradation (Dostanova,1966; Sivtsev, 1973; Yeo and Flowers, 1983; Ball and Farquhar,1984; Downton et al.1985; Krishnamurthy et al.1987a). Reddy and Vora (1986) in their studies with wheat showed that the reduction in total chlorophyll content can be attributed to the destruction of chlorophyll due to high activity of chlorophyllase. Moreover, they observed that 'chlorophyll a' is more sensitive to salinity than 'chlorophyll b'. Rao and Rao (1981) also reported increased activity of chlorophyllase and low levels of photosynthetic pigments in Cajanus indicus and Sesamum grown under saline condition. Krishnamurthy et al.(1987b) have observed a reduction in the Mg^{2+} content of rice varieties on exposure to NaCl salinity. Possibly, the decreased Mg^{2+} level under saline condition also might have been responsible for the low level of chlorophyll in salinized plants.

Both putrescine and GA_3 treatments were found to increase the chlorophyll content significantly under saline condition. However, it is not known precisely how putrescine and GA_3 maintain high level of chlorophyll under adverse conditions. Recent evidence (Woolhouse,1984) suggest that a substantial degree of thylakoid breakdown is necessary to expose the

chlorophyll molecule for degradative action. Popovic et al. (1979) have reported that putrescine and other polyamines stablize thylakoid membrane systems in chloroplast of barely leaves and protect chlorophyll from degradation during stress-induced senescence. These authors (Cohen et al.1979) also suggested that polyamines may be exerting their capabilities in preserving thylakoid morphology and chlorophyll pigment through interacting with the membranes thus making it less accessible to hydrolytic activities associated with senescence. The enhanced chlorophyll level in putrescine-treated salt-stressed plants thus could be due to the activity of putrescine in protecting chlorophyll against degradation. A number of studies later confirmed this contention (Kaur-Sawhney and Galston, 1979; Altman, 1982; Kaur-Sawhney et al. 1982).

Like polyamines, gibberellic acid is also known to retain chlorophyll and delay senescence in many plants (Fletcher, 1975; Sabater, 1984). Fletcher and Osborne (1965a) found that treatment of dandelion (Taraxacum officinale) leaves with gibberellic acid delays decline in the levels of chlorophyll, RNA and protein. Based upon their studies on protein and RNA synthesis during senescence, Fletcher and Osborne (1965b) proposed that retarding action of GA_3 on chlorophyll loss and leaf senescence could be mediated through regulation of RNA synthesis. Chin and Beevers (1970) showed that chlorophyll pigment decreased parallel to the

decline of endogenous gibberllin content in Tropeolum leaves in which chlorophyll loss was effectively controlled by exogenously supplied gibberellin. It must be noted that a reduction of gibberellin - like substances under saline condition was noticed in rice plants also. The deferral of senescence with hormone treatments results in an improvement of protein and RNA contents, whether it is done through gibberellins (Fletcher and Osborne, 1965b; Fletcher,1975; Sebater, 1984) cytokinins (Osborne,1962; Sugiura et al.1962) or by auxin (Osborne and Halloway,1964). Thus,the observed increase in the chlorophyll content in GA_3 treated salt-stressed plants could be due to the antisenescence property of GA_3.

The analysis of shoot and root tissues showed that Na^+ and Cl^- were freely absorbed by the roots and transported to the shoot system at harmful levels. On the other hand K^+ concentration was markedly depleted in both shoot and root systems upon salinization. Increased accumulation of Na^+ and Cl^- (Jeschke, 1982; Yeo and Flowers, 1982; Yeo,1983; Eshel,1985; Yeo et al.1985; Cheesman and Wickens,1986; Waisel et al.1986; Matoh et al.1987) with a concomitant drop in the K^+ content under saline condition (Wignarajah et al.1975b; Rush and Epstein,1981; Weimberg,1986; Kirshnamurthy, et al.1987c) was reported in a number of plants including many varieties of rice.

A major problem facing plants exposed to salinity is the disturbances resulting from toxic levels of Na^+ and Cl^- on the physiological and biochemical processes associated with growth. Many authors correlated high tissue concentration of Na^+ and Cl^- with decreased growth and yield of rice (Pearson,1959; Paricha et al.1975; Flowers and Yeo, 1981; Yeo and Flowers,1983; Krishnamurthy et al.1987b). Krishnamurthy et al.(1987b) studied the growth and yield performance of different rice cultivars under NaCl salinity in pot culture experiments and found that sensitive cultivars accumulated higher levels of Na^+ and Cl^- in the shoot when compared with control. They also observed that the increase in internal Na^+ concentration was significantly reduced with a concomitant rise in K^+, Ca^{2+} and Mg^{2+} levels in resistant cultivars.

Investigations carried out by Yeo et al.(1985) to understand the nature of growth inhibition and cellular toxicity induced by NaCl salinity revealed that there exists a leaf to leaf gradient of Na^+ and the maximum Na^+ was present in the oldest leaf. Further, analysis of the course of events in leaves following salinization showed that net photosynthesis was inversely correlated with the Na^+ concentration in leaf tissue and declined linearly with increasing Na^+ concentration. They concluded that growth inhibition observed in rice under saline condition could be in part due to the adverse effects brought about by the

accumulation of Na^+ and Cl^- in shoot and root tissues to injurious levels.

It seems logical to presume that besides the toxic effects caused by the accumulated Na^+ and Cl^-, the low level of K^+ in salt-stressed plants will be having a direct bearing on growth and yield reduction. Plants generally require K^+ for the normal metabolic processes. Apart from its role as an osmotic component, K^+ is essential for the formation of starch, protein synthesis, photosynthate partitioning, stomatal functions and above all as an activator of a number of monovalent cation requiring enzymes (Epstein, 1972). While evaluating the salt tolerance mechanism in rice varieties Sharma (1986) found that K^+ content was very much depleted in the sensitive variety and he concluded that higher growth and yield of resistant variety is due to better regulation over accumulation and distribution of Na^+ and K^+ in the plants i.e. the delicate and vital organs like young and photosynthetically active leaves as well as the reproductive structures kept relatively free to toxic level of Na^+, besides having an assured K^+ supply under stress condition. The decreased growth of Phaseolus vulgaris, Pisum sativum and Citrus aurantium due to salinization has also been explained by a suppression of nutrient absorption particularly K^+, and this inhibition was overcome by exogenously supplied K^+ (Giorgi et al. 1967). Potassium content was also found decreased in peanut, pigeon pea

and gingelly exposed to salt stress and foliar application of K^+ partially alleviated the adverse effects of salinity on growth and yield of those crop plants (Mohan et al.1986).

The precise mechanism(s) by which NaCl influences transport of organic and inorganic solutes across membranes is not known. However, it is convincingly demonstrated that salt interferes with a wide array of membrane functions and alters the structure and composition of biomembranes (Levitt, 1980; Leopold and Willing,1984). A number of studies showed that hormonal regulation is involved in the control of membrane permeability and water relations in plants (Ilan, 1971; Collins and Kerrigan,1974; Van Steveninck,1976; Wright, 1978; Karmoker,1984). Indole acetic acid was reported to stimulate K^+ uptake by sunflower hypocotyl segments (Ilan, 1973), Minium leaves (Luttge et al.1972), pea internode segments (Lado et al.1976) and in barley coleptile segments (Kholdebarin,1981). Besides the above reports, Ilan et al. (1971), after studying the specific effects of kinetin and IAA on monovalent cation uptake suggested that endogenous auxins and cytokinins are among the factors which determine the selectivity of ion uptake by cells in the intact plants. Kinetin exerted opposing effects on the uptake of K^+ and Na^+ by leaf discs of Helianthus annus; the absorption of K^+ was stimulated and that of Na^+ was inhibited (Ilan,1971). Differential effects of IAA on the absorption of various cations were also reported by Bode (1959), who found that

treatment of tomato plants with IAA brought about an increase in the level of K^+, but not Na^+ in the leaves. In contrast to auxin and cytokinins, abscisic acid was reported to increase the accumulation of Na^+ and Cl^- and inhibit both uptake and transport of K^+ in detached plant parts as well as in intact plants (Karmoker and Van Steveninck,1978; Karmoker and Van Steveninck,1979; Karmoker,1984). At this juncture it is interesting to point out that a rapid and massive accumulation of abscisic acid, Na^+ and Cl^- and a considerable fall in the contents of IAA and K^+ were noticed in both shoot and root tissues of salt-stressed plants. From these results it can be deduced that apart from other factors affecting the structure and function of membrane due to salinization, NaCl-induced hormonal imbalance also might have contributed to the increased accumulation of Na^+ and Cl^- as well as the reduction in the content of K^+.

Administration of putrescine significantly reduced the net accumulation of Na^+ and considerably increased the K^+ level in shoot and root systems. Chloride level was also appreciably decreased in the shoot system of putrescine-treated salt-stressed plants compared with control. There is now ample evidence to suggest that salinity causes considerable damage to membranes systems (Levitt,1980). Polyamines have been shown to improve growth of intact plants in conditions of environmental stress likely to cause membrane disruption (Smith, 1982). It is suggested that polyamines, which are

highly protonated polycations at physiological pH interact with negatively charged phospholipid head group or other anionic sites on membranes conferring a greater stability to membrane bilayers and protect it from disruption under stress condition (Slocum et al.1984; Guye et al.1986). Further, the potential of polyamines in reversing the effects of membrane disruption caused by environmental stresses and chemicals in higher plants was showed recently by Okii et al.(1980) and Srivastava and Smith (1982). Regulation of membrane structure and functions by putrescine and other polyamines was also reported by Cohen et al.(1979) Popovic et al.(1979), Altman (1982) and Srivastava and Smith (1982) in a number of higher plants. Thus it can be suggested that inhibition of Na^+ and Cl^- accumulation and increased K^+ content in putrescine - treated salt-stressed plants could be due to the inherent ability of putrescine to maintain the structural and functional integrity of biomembranes under stress condition.

Calcium which is an important factor in the maintenance of membrane integrity and ion transport regulation (Hanson,1984; Hepler and Wayne,1985) was found decreased in rice plants upon salinization (Krishnamurthy et al.1987b). It has been shown that Ca^{2+} is essential for K^+/Na^+ selectivity in plants (Epstein,1961; Lauchli and Epstein, 1970; Hanson,1984). Many recent studies indicate that depletion of Ca^{2+} by NaCl disrupts membrane integrity as well as selectivity and

elevated Ca^{2+} concentration in the nutrient medium can mitigate the adverse effects of NaCl on growth by inhibiting the Na^+ uptake (LaHaye and Epstein,1969; Gerard,1971; Cramer et al. 1985; Kent and Lauchli,1985) and minimizing the leakage of cytosolic K^+ (Leopold and Willing,1984; Cramer et al.1985). Very recently Riedell (1987) has demonstrated that the role of Ca^{2+} in maintaining membrane integrity can be substituted by polyamines. He found a significant reduction in Na^+ influx when polyamines were supplied to the tissue and concluded that polyamines inhibit Na^+ influx by influencing membrane permeability in a manner similar to that of Ca^{2+}. Hence, it can be expected that the improved ionic balance in salt-stressed plants treated with putrescine might be also due to its functional ability to replace Ca^{2+} in membrane function.

The result obtained in this study show that putrescine can modulate endogenons hormone levels perturbed by salt stress. It is thus very likely that, besides the direct effect of putrescine on membrane structure and functions, the improved hormonal balance in the putrescine-treated salinized plants also might have contributed to the observed alterations in the ionic status of the salt-stressed plants.

A significant change in the ionic content (an inhibition of Na^+ and Cl^- accumulation and an increase in K^+ level) was noticed in the GA_3-treated salt-stressed plants.

Unfortunately gibberellins did not receive much attention as far as their effects on ion uptake and transport are concerned. However there are some reports to indicate that GA_3 alters the membrane permeability and regulates uptake and transport of ions (Artimonova and Artimonov, 1971; Wood and Paleg, 1972, 1974; De La Guardia and Benlloch, 1980). The inhibition of Na^+ and Cl^- accumulation and the enhancement of K^+ level observed in GA_3-treated salinized plants might be due to the ability of GA_3 to alter the membrane permeability (Karmoker, 1984). Starck and Kozinska (1980) also reported that GA_3 stimulates K^+ uptake and decreases the influx of Na^+ in salt-stressed bean plants. Further, they observed an increased Ca^{2+} and K^+ content in metabolically active organs in GA_3-treated NaCl- stressed plants compared with control. Ion uptake selectivity by gibberellic acid was also reported in pea plants (Gracia and Guardiola, 1981).

The polyamine levels diminished by saline stress was considerably recovered by GA_3 treatment. Moreover, GA_3 application greatly inhibited ABA build up and appreciably increased the auxin concentration in salt-stressed plants. As noted earlier, the decreased net accumulation of Na^+ and Cl^- and high level of K^+ in GA_3-treated salt-stressed plant could also be due to the increased production of polyamines and auxin as well as the diminution of ABA content in response to GA_3 treatment. Again, it must be pointed that, besides inhibiting the ABA level, GA_3 might have also

improved the ionic balance by counteracting the effect of ABA on uptake and transport of ions (Marre, 1977).

High levels of free proline were recorded in shoot and root tissues of rice plants exposed to salt stress. The accumulation of proline in response to salt stress has been documented in both glycophytes and halophytes (Chu et al. 1976; Aspinall and Paleg, 1981; Goas et al. 1982; Bühl and Stewart, 1983; Voetberg and Stewart, 1984; Treichel, 1986) and it is suggested that proline may acts as a storage compound (Barnett and Naylor, 1966; Morris et al. 1969), cytoplasmic osmoticum (Stewart and Lee, 1974) or as a protective agent of macromolecules (Schobert, 1977; Aspinall and Paleg, 1981) under stress conditions. However the mechanism of controlling proline accumulation under stress condition is poorly understood.

After a series of experiments using barley leaves (Stewart et al. 1977; Stewart and Boggess, 1978; Stewart, 1980; Buhl and Stewart, 1983) Stewart and his co-workers proposed that drought, ABA and salt-induced proline accumulation in barley leaves all result from common effects. Tracer experiments using radioactive glutamate established that the accumulation is due to an increase in proline synthesis from glutamate in NaCl-stressed barley leaves. Though the exact mechanism of proline accumulation under stress condition is unknown, it appears that NaCl-induced

build up of proline could be, at least partly, resulting from the increased synthesis due to high concentration of ABA (Aspinall et al. 1973; Rajagopal and Anderson, 1978; Stewart, 1980) and/or Na^+ (Treichel, 1975; Neales and Sharkey, 1981; Voetberg and Stewart, 1984; Pesci and Beffagna, 1986) in salt-affected plants. However, Stewart and Voetberg (1985) recently demonstrated that proline accumulates in salt-stressed barley leaves without a preceeding ABA accumulation and they suggested that NaCl induced accumulation of proline might be due to some other, yet unknown, cellular process. Increased synthesis of proline in response to salinity is also evident from the work of Krishnamurthy et al.(1987c) who observed a tremendous increase in the content of free proline without affecting protein-bound proline content in rice under saline condition.

Investigation by Boggess et al. (1978) and Sells and Koeppe (1981) revealed that oxidation of proline could be a mechanism in the regulation of cellular proline pool. Further, a decrease in proline oxidation has been shown to contribute proline accumulation in spinach, barley and many other plants under stress conditions (Stewart et al.1977; Stewart and Boggess, 1978; Huang and Cavalieri, 1979; Naik and Joshi, 1986). The elevated proline content discerned in salt-stressed plants thus could be a reflection of NaCl-induced inhibition of proline oxidase activity as well.

Gibberellic acid administration measurably reduced the content of proline whereas putrescine treatment only slightly lessened its accumulation in the shoot system of salt-affected plants. At the moment no convincing evidence is available to state that gibberellins and polyamines regulate proline metabolism in plants. However, from the results obtained with proline oxidase, it can be suggested that the diminution of proline level in putrescine or GA_3-treated salt-stressed plants would be, at least partly, a consequence of increased activity of proline oxidase occurring in response to growth regulator application. The enhanced activity of proline oxidase could be an outcome of the inhibition of Na^+ and Cl^- accumulation (Sharma and Gupta, 1986) in the shoot system in response to putrescine or GA_3 administration. Additionally, the decline in the ABA content in the shoot tissues of putrescine or GA_3-treated salinized plants also might have definitely decreased the synthesis of proline (Stewart, 1980). The reduction in the proline level, owing to its utilization for protein synthesis also cannot be ruled out for, the salinized plants treated with both the growth regulators exhibited a considerably higher value of protein content compared with salt control.

Putrescine treatment almost totally failed to alter the proline level in the root system of salt-stressed plants. GA_3 treatment, nevertheless, brought about a small decrease in the proline content in the root system of salt-affected

plants. This difference in response with respect to proline content between shoot and root tissues of salinized plants treated with putrescine or GA_3 can be conceivable from the fact that the root system contained much higher concentration of Na^+ and Cl^- than the shoot. A linear relationship between the rate of proline accumulation and Na^+ content is already established (Storey and Wyn Jones, 1978; Neales and Sharkey, 1981; Voetberg and Stewart, 1984). The involvement of other factors, if any, for this pattern of proline accumulation is not evident from this investigation.

It is now well recognized that just like proline, quaternary ammonium compounds (QAC) also accumulate in plants under various environmental stress conditions including salinity (Hitz and Hanson, 1980; Wyn Jones and Storey, 1981; Grieve and Maas, 1984; Diggelen et al. 1986, Matoh et al. 1987). Although a precise physiological or adaptive role has not been assigned to these compounds under stress condition, there is a wide spread conjuncture that QAC may function as a cytoplasmic osmoticum (Wyn Jones and Storey, 1981; Grieve and Maas, 1984) and protect the enzymes from electrolytes (Pollard and Wyn Jones, 1979). As reported in many other graminaceous plants grown under stress conditions (Hitz and Hanson, 1980; Wyn Jones and Storey, 1981; Grieve and Maas, 1984) a great increase in the content of QAC was noticed in salt-affected rice too. This increment in the

content of QAC was not significantly affected by putrescine treatment, whereas gibberellic acid notably lowered the QAC content in the shoot system and slightly decreased in root system of salt-stressed plants. The reason for the failure of putrescine to bring down the QAC content is obscure at the moment. Again, it is also difficult to advance a precise explanation for the GA_3-induced reduction in the level of QAC in salt-affected plants as there is no definite information available regarding the control mechanism(s) of QAC level in plants. From the circumstantial evidence it can only be speculated that the decreased content of QAC could be a repercussion of the considerable relief of stress by GA_3 on various cellular processes. It should be noted that Na^+, Cl^- and ABA levels were comparatively much less in salt-exposed plants treated with GA_3 than the one administered with putrescine.

It is argued that the disruption of plant growth and development by salinity is mainly due to the hormonal imbalance (Wright, 1978; Levitt, 1980; Sharma and Gupta, 1986). This belief was supported by the fact that exogenously supplied IAA (Sarin, 1962; Darra and Saxena, 1973) gibberellic acid (Huber and Sankhla, 1973; Huber et al. 1974; Strack et al. 1975; Singh and Singh, 1980; Khan and Ungar, 1985) and cytokinins (Benzioni et al. 1974; Singh and Singh, 1980, Dhingra and Varghese, 1985b) could counteract the negative

effects of salinization on various growth and developmental processes in many plants. When rice plants were subjected to NaCl salinization a considerable fall in the content of IAA was detected in both shoot and root systems. This decline in the IAA content coincided with the appearence of high activity of IAA oxidase suggesting that one of the mechanisms by which NaCl reduces IAA level could be by enhancing the activity of IAA oxidase. An inverse relationship between endogenous IAA levels and IAA oxidase activity has been demonstrated by Shaw and Hawkins (1958), Jain et al.(1969) and more recently by Jasrai et al. (1988).

Unfortunately very little is known about the regulatory role of various stress components on auxin metabolism. On the basis of the evidence obtained from the studies with pea and tomato, Darbyshire (1971) suggested that reduced water potential under osmotic stress condition increases the activity of IAA oxidase and may provide the plants with a stress adaption mechanism. It is well recognized that IAA oxidase regulates growth by limiting the concentration of IAA (Scott, 1984) and the increased IAA oxidase activity observed in salt-stressed plants possibly results from the accumulation of high levels of Na^+ and Cl^- which alter the water potential. A massive accumulation of free ABA is one of the earlier events in response to salinization (Wright, 1978) and a high concentration of free ABA was

recorded in rice plants in the present study also. From this observation it appears that the decreased IAA content in salinized plants may also be due to an indirect effect of ABA accumulation, for, ABA can inhibit auxin biosynthesis as well as its degradation (MacMillan, 1980; Scott, 1984). There is ample evidence to show that ABA can interfere with growth and developmental processes by inhibiting the biosynthesis of auxin (Anker, 1975) and enhancing its degradation (Milborrow, 1966).

Application of putrescine significantly increased the growth and auxin content of salt-affected plants. Although a number of studies are carried out on polyamine modification of endogenous ethylene production (Slocum et al.1984; Smith, 1985) virtually no information is available on the effect of polyamines on the biosynthesis and degradation of other growth hormones. Nevertheless from the results of ion concentration, activity of IAA oxidase and ABA content obtained from putrescine - treated salt-stressed plants it can be assumed that putrescine may be augmenting the IAA level by suppressing the destruction of auxin. It is also likely that besides the direct effect of ABA on auxin degradation (Milborrow, 1974; Anker, 1975) high level of ABA in salinized plants will lead to increased production of ethylene (Mayak and Dilley, 1976; Pharis and Reid, 1985) which in turn could enhances the oxidation of auxin (Scott, 1984). Thus the stimulation of

IAA oxidase activity and the reduction in the IAA content in salt-stressed plants could also be due to the increased synthesis of ABA as well as ethylene under stress condition. Again, this proposition gains support from the observations of Vioque et al.(1981) and Shimokawa (1983) who observed that one of the enzyme systems of ethylene biosynthesis is functioning as IAA oxidase. In this context it must be noted that the decline in the IAA oxidase activity in putrescine-treated salinized plants may be due to the effect of putrescine on ethylene formation for, putrescine and other polyamines are known as potential inhibitors of ethylene production (Apelbaum et al.1981; Suttle, 1981; Shih et al.1982). The putrescine-induced diminution of IAA - oxidase activity would be, at least partly, responsible for the higher level of IAA in putrescine-treated salt-stressed plants compared with control.

The growth and content of IAA was considerably more in GA_3- treated salt-stressed plants than in putrescine-treated salinized plants. There is extensive evidence to show that gibberellic acid increases auxin level either by enhancing auxin biosynthesis (Sastry and Muir,1965; Jindal and Hemberg, 1976) or by checking auxin destruction (Kogl and Elema 1960). Now there is convincing evidence available to show that GA_3 can stimulate elongation growth independently of auxin too (Cleland et al.1968; Kazma and Katsumi,1974). The improved ionic balance and a marked reduction in the abscisic acid content of GA_3-treated salt-stressed plants might have also

reduced the auxin destruction as evidenced by the low activity of IAA oxidase in GA_3-treated salt-stressed plants compared with salt control. Suppression of IAA oxidase activity in response to gibberellin treatment was reported earlier by Pilet (1957). Again it is also conceivable that the low build up of ABA in GA_3-treated salinized plants certainly abates the inhibition of auxin biosynthesis imposed by ABA (Anker, 1975), which further facilitates the auxin accumulation.

One of the most clearly defined effects of environmental stress conditions on hormones is the enhancement of ABA biosynthesis (Wright and Hiron,1972; Wright,1978; Levitt,1980). As noticed in many other plants a massive accumulation of ABA was found in rice plants upon salinization. Although the exact role(s) of this large accumulation of ABA under stress conditions is not fully understood, it is known that ABA induces the closure of stomata and thereby prevents excessive water loss (Davis et al.1980), increases the hydraulic conductivity of the root to maintain plant turgor (Glinka and Reinhold, 1971; Glinka, 1973; Lachno and Baker, 1986) and decreases the growth resulting in dwarfism of the plants. It is now convincingly demonstrated that chloroplast is the principal site of ABA synthesis which under normal conditions controls the entry of ABA into the cytoplasm and regulates its production by end product inhibition (Loveys,1977). Many workers have concluded that the accumulation of Na^+ and

Cl^- disrupts the structure and function of membrane systems of chloroplasts (Sharma and Gupta,1986) which results in the release of ABA into the cytoplasm and consequently depose the barrier of ABA production (Loveys,1977; Wright,1978).

It is well recognized that ABA accumulating under salt-stress condition is mainly because of the altered water potential due to the excess of Na^+ and Cl^- in the tissues (Wright,1978). Putrescine as well as gibberellin-treatments measurably diminished the accumulation of ABA in salt-stressed plants which could be a consequence of the improved water relations and reduced ion toxicity. Again, it should be remembered that, putrescine and other polyamines are known for their ability to maintain the structural and functional integrity of biomembranes and Cohen et al. (1979) and Popovic et al.(1979) have shown that these naturally occurring compounds preserve the integrity of chloroplast envelop under unfavourable conditions. Thus the regulation of ABA production by putrescine at the site of synthesis also cannot be discounted.

Unlike polyamines there is some evidence to show that gibberellins suppress the accumulation of abscisic acid (Scott, 1984). Chin and Beevers (1970) reported that the level of inhibitors like ABA decreased considerably in nasturtium leaves after gibberellin application. The suppression of ABA content by GA_3 can thus be attributable to its ability to control the ABA build up. In addition to that the efficacy of GA_3 in

inhibiting the influx of Na^+ and Cl^- into the cells also might have potentiated the effect of GA_3 in regulating the ABA accumulation under stress conditions.

There is very little information available on the effect of salinity stress on gibberellin metabolism even though a number of attempts were made to ameliorate salt stress injury by exogenous application of gibberellins (Huber and Sankhla,1973; Huber et al.1974; Strack et al.1975; Singh and Singh,1980; Khan and Ungar,1985). Upon salinization GA - like substances were found decreased in both shoot and root systems of rice plants. No substantive evidence is however available at present to advance a precise explanation for the stress-induced inhibition of GA-like substances. Nonetheless, it is quiet probable that the decreased GA-like substances in salt-affected plants could be an outcome of the quick and massive accumulation of ABA for, ABA has been shown to reduce the gibberellin levels in many plants (Thomas et al.1965; Wareing et al.1968). A slight increment in the content of GA-like substances was discernible in putrescine-treated salinized plants. At present no explanation can be given for the observed rise in the endogenous level of GA-like substances. The only information available regarding the effect of putrescine on the level of endogenous growth substance is with respect to ethylene. In the case of ethylene, putrescine has been shown to decrease its production (Apelbaum et al.1981; Suttle,1981; Shih et al.1982).

Compared to auxin the reduction in gibberellin-like substances was relatively less in salt-stressed plants. However, salinized plants exhibited a very high degree of dwarfism, despite the fact that gibberellins can induce extension growth independently of auxin (Cleland et al.1968; Kaufman,et al.1969; Kazama and Katsumi,1974). A possible explanation for the low expression of gibberellin activity could be the presence of high concentration of ABA in salt-affected plants. There are numerous demonstrations that ABA can act as antagonists of gibberellin action (Kaufman and Jones,1974; Kaufman,1984). Moreover, recently it has been suggested that certain amount of polyamines is essential for full expression of the gibberellin action (Smith et al.1983; Lin, 1984; Slocum et al.1984). The reduction in the polyamine content in salinized plants might have imposed more constraints to gibberellin action and thereby the reduced growth of the plant. Exogenous supply of putrescine might have thus relieved the inhibition of gibberellin action to some extent and that could be, at least partly, responsible for the improved growth in putrescine-treated salt-stressed plants.

Contrary to IAA oxidase the activity of invertase and amylase were found decreased upon salinization. The protein content also showed a reduction compared to the salt control. Considering the changes in the protein content the decreased activity of the enzyme upon salinization could be due to the degradation and/or low synthesis of enzyme protein. But the

activity of IAA oxidase was preferentially stimulated by NaCl despite the fact that it decreased the total protein content substantially, At present it is difficult to provide a clear interpretation of this preferential stimulation or suppression of enzyme activity by NaCl. However, it is reported that NaCl can increase or decrease the production of some enzymes by either increasing or decreasing the rate of transcription or translation (Kahane and Poljakoff Mayber 1968; Filho et al.1983; Ostrem et al.1987) as well as the turn over rates of enzymes (Cooke et al.1973; Karlekar et al.1985). The above proposition on differential gene expression is supported by the recent studies of Ramagopal (1986, 1987a, 1987b) who observed three major kinds of changes with respect to protein synthesis during salinity stress in barley: i) proteins whose accumulation was repressed, ii) proteins whose accumulation was enhanced, iii) proteins that were newly synthesised. Further, his findings suggest that during salinity stress, both transcriptional and post-transcriptional mechanisms regulate gene expression in barley genotypes. (Ramagopal, 1987a and c). It is again suggested that the ionic disturbance and cell dehydration due to salt stress may possibly be altering the conformation of enzyme protein either at the active site or changing the tertiary and quaternary structure of the protein, so as to make the enzyme in a more active or inactive form (Heuer and Plaut, 1982; Filho et al. 1983; Kalir et al. 1984).

Thus the changes in the enzyme activity observed in the present study could be accounted for some or all of the above factors induced by salinity.

An important observation in the present investigation is the modulation of enzyme activity by putrescine under saline condition. Alteration (either increase or decrease) of the activity of enzyme by polyamines was reported in both animal and plant systems (Eichberg et al. 1981; Slocum et al. 1984; Tabor and Tabor, 1984, Srivastava and Rajbabu, 1985; Kauss and Jeblick, 1986; Narindrasorasak and Sanwal, 1986). However the mechanism by which polyamines alter the enzyme activity is not completely understood. The enzymatic activity of many proteins are thought to be regulated through reversible phosphorylation (Cohen, 1980). Accordingly, polyamine modulation of protein phosphorylation may indirectly result in the regulation of the activity of enzymes (Datta et al. 1986, 1987).

A number of investigations (Wignarajah, 1975b; Krishnamurthy et al. 1987b; Prakash and Prathapasenan, 1988) showed that, upon salinization, the levels of many essential elements are decreased in both glycophytes as well as in halophytes. Krishnamurthy et al. (1987b) reported that NaCl reduced the content of Mg^{2+} as much as 50% in the shoot system of rice and the lowering of Mg^{2+} level has been shown to affect adversely the protein synthesis by disrupting the functions of ribosomes (Ts'O et al.1958). Interestingly putrescine and other polyamines are able to replace Mg^{2+} (Takeda and Ohnishi,

1975, Cohen and Zalik, 1978) in polypeptide synthesis. This functional ability of putrescine might be responsible for the increased level of protein, high activity of enzymes (except IAA oxidase) as well as the improved growth observed in putrescine-treated salt-stressed plants. Again, it must be noted that putrescine, besides stimulating protein and nucleic acid synthesis, is reported to protect RNA and protein by inhibiting the activity of RNAase and protease, which might have further contributed to the increased protein content (Altman, 1982; Shih et al. 1982).

The present knowledge about polyamines is insufficient to give a satisfactory explanation for the preferential activation or suppression of enzyme activity by putrescine. Nonetheless several lines of evidence indicate that putrescine and other polyamines, depending on the environmental conditions, stimulate or inhibit the synthesis as well as functioning of macromolecules and modulate growth in a number of biological systems (Slocum et al. 1984; Tabor and Tabor, 1984; Smith, 1985). Thus, a similar role of putrescine can be envisaged in the enzyme systems of rice too.

The activity of amylase and invertase were much influenced by GA_3 rather than by putrescine. It is well known that enzyme biosynthesis as well as their activity are greatly influenced by hormones (Varner and Ho, 1977; Barendse, 1984). Apart from other factors involved, the high accumulation of ABA might have also exerted strong inhibition on amylase and

invertase activity in salinized plants. ABA has been found to depress the activity of amylase (Chrispeels and Varner, 1966; Barendse, 1984) and invertase (Saunders and Poulson, 1968; Huber and Sankhla,1974). Many demonstrations showed that GA_3 counteracted the effect of ABA on enzyme activities as well as the growth (Wright,1968; Huber and Sankhla,1974 and 1974a; Sankhla and Huber,1974; Scott, 1984). The improved enzyme activity observed in salt-affected plants could be, to a certain extent, due to the inherent ability of gibberellic acid in negating the effects of abscisic acid.

Induction of hydrolytic enzymes, especially amylase and invertase, in response to gibberellic acid treatment is a common phenomenon (Varner and Ho,1977). The increased enzyme activity noticed in unstreased plants treated with gibberellic acid could be due to induction or activation of the enzymes by gibberellic acid. In vitro activation of purified enzymes by gibberellic acid was also reported recently (Deshpande and Swamy, 1988; Saluja et al.1988).

As observed earlier (Priebe and Jager 1978; Guye et al. 1986) salinization resulted in a measurable reduction in the contents of polyamines and this inhibition was considerably reversed by the exogenously applied GA_3. Interestingly, in response to GA_3 treatment, polyamine synthesis was found more in non-stressed plants than in salinized ones and this finding was corroborated by the activity of agmatine deiminase, an enzyme involved in polyamine synthesis. Promotion of

polyamine synthesis and the activity of arginine decarboxylase, another enzyme of polyamine synthesis, by GA_3 in pea has been reported by Dai *et al.*(1982).

GA_3 application was found to increase another polyamine bio-synthetic enzyme ornithine decarboxylase around four-fold during germination of barley seeds (Kyriakidis,1983). Lin (1984) demonstrated that treatment of barley aleurone layers with methyl-glyoxal-bis guanyl hydrazone (MGBG), an inhibitor of polyamine biosynthesis, reduced the polyamine content and amylase formation significantly and the amylase activity could be almost fully restored on adding polyamine into the medium. After a series of experiments with different inhibitors of polyamine synthesis and gibberellic acid he concluded that certain amount of polyamines are essential for the full expression of gibberellin action.

In another study with rice seedlings total polyamine content and the activity of arginine decarboxylase were found declined on treatment with ABA whereas polyamine oxidase and diamine oxidase activity registered inverse correlation with polyamine content (Mukhopadhyay *et al.*1983). Choudhuri and Ghosh (1982) found that application of GA_3 to germinating rice seeds increased arginine decarboxylase activity to 10 fold, while ABA caused considerable inhibition. The decreased polyamine content and agmatine deiminase activity in salt-affected plants thus, could be, at least partly, due to the

high accumulation of abscisic acid apart from other likely factors such as osmotic inhibition and ion toxicity on enzymes involved in polyamine synthesis. Furthermore, stimulation of polyamine oxidase by NaCl is also reported (Smith, 1976, 1984).

Salinity decreased the grain yield considerably as it depressed the vegetative growth. Analysis of the yield data shows that, compared with control, salt stress decreased the total number and weight of filled seeds and increased the number of unfilled seeds per plant basis. Similarly it also decreased the weight of seeds compared with control. The observed yield reduction under saline condition might be due to i) poor development of reproductive structures, ii) incomplete opening of inflorescence, iii) higher concentrations of Na^+ and Cl^- and lower level of K^+, iv) hormonal imbalance, v) poor grain filling and vi) loss of viability and germinability of pollen. Salinity has been shown to adversely affect development as well as functioning of reproductive structures in many plants (Reddy and Goss, 1971; Abdulla et al.1978; Dhingra and Varghese, 1985a; Sharma and Gupta, 1986).

Salinity brought about a delay in flowering, reduction in the number of flowers and early cessation of flowering as well as decreased pollen germination and tube growth in Arachis hypogea (Mohan et al.1986). They have also reported

marked reduction in seed yield and altered composition of carbohydrates, lipids and proteins in pea nut and ground nut.

The external application of putrescine and gibberellic acid appreciably increased grain yield under stress condition. The depletion of endogenous level of polyamines under stress condition might have caused more constraints to the development of reproductive structures for, the requirement of polyamines for reproductive development has been established in many plants (Cohen et al.1982; Feirer et al. 1984; Slocum and Galston,1985). The increased yield under stress condition by putrescine treatment could be partially due to its supplementation to the low endogenous levels of polyamines under saline condition besides the improved ionic and hormonal balance resulting from putrescine treatment.

Application of gibberellic acid is known to influence virtually almost all processes associated with reproductive development in plants (Pharis and King,1985). Exogenous supply of GA_3 was found to antagonize the depressive effects of salinity on pollen germination in Zea mays L. (Dhingra and Varghese, 1985b). They observed a complete recovery of pollen germination in presence of GA_3. A substantial increase in the photosynthetic area as well as chlorophyll content in growth regulator-treated salt-stressed plants can also be attributed to their higher yield as compared

with salt control. It is also interesting to note that besides the toxic effects caused by Na^+ and Cl^- the low level of K^+ in salt-stressed plants will be having a direct bearing on yield reduction, since K^+ is required for the synthesis of starch, protein and photosynthate partitioning (Epstein, 1972). Thus it is likely that the high concentration of K^+ in putrescine as well as GA_3-treated stressed plants also might have contributed towards the increase in yield output.