Chapter 5

Discussion
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5.1. Morphological parameters

5.1.1. Germination

In the present investigation, germination percentage was found out in eight varieties of rice (MO5, MO6, MO7, MO8, MO9, MO10, MO18, and MO19) after treatment with various concentrations of NaCl (0.1, 0.3, 0.5, 0.7, and 0.9 per cent) as a pilot study for the selection of two most tolerant varieties. It was found that all the varieties respond almost in the same way that a reduction in germination percentage with respect to the increased concentration of the NaCl as mentioned by Sheoran and Gang (1978) in bean, Dubey and Pflanzen (1982) in rice, Azhar and Mc Neily (1987) in rice, Dubey and Rani (1989 b) in rice. Jones and Gorham (1990) already reported that salinity reduces germination of seeds and decreased seedling vigour and induced stunted growth. The low germination is attributed to the low osmotic potential of saline medium (Ashraf and Abu 1978). Tur (1978) suggested that salt influence the seed germination and growth by inhibiting enzymes, suppress breathing and phosphorylation process and change the content of organic phosphate, nucleic acid, protein and nitrogen. Azhar and Neily (1987) reported that salt tolerant species should also be selected on the basis of early seedling growth and germination. Akbar and Senadhira (1988) reported that tolerance in sensitive varieties increased with age.

Mishra et al., (1996) suggested that poor germination results in poor plant vigour and growth, which in turn affect the yield. Delayed germination under stress in rice was recently reported by Rahman et al., (2001). The biochemical mechanism responsible for inhibition of germination due to salinity is still not fully understood. It is agreed that salinity affects germination by creating low water potential to inhibit water uptake (osmotic effect) and or by providing conditions for the entry of ions that may be toxic to the growing embryo (Bewley and Black, 1982). All these findings support the view that germination percentage under salinity stress may be used as a preliminary clue for identifying the level of tolerance in plants. Among the different MO varieties analysed, MO18 and MO19 showed the least reduction in percentage of germination at the highest stress applied (Table 1.1).

5.1.2. Root Characteristics

Among the different morphological features, the root parameters were considered as the most significant measure of resistance. Hence the response of root parameters of both the varieties,
MO18 and MO19 to salt stress were assessed. Root characteristics studied in the present investigation include seminal root length, total number of nodal roots, total length of nodal roots, average number of root hairs per mm of nodal roots and average length of root hairs. A negative relation between salinity stress and root characteristics was noted; i.e., as the concentration of salt increases a decrease was observed in all root characteristics in the both varieties.

Root characteristics of rice and root-shoot relations of grasses in response to various stress conditions were studied and analyzed by many researchers like Thangeraju et al., (1990); Rao et al., (1992); Dutt and Bal (1993) Sharma et al., (1994). They observed decrease in weight and number of root, in response to drought in certain cultivars of rice.

Uptake rate depends on the root surface area as photosynthetic rate depends on leaf area. The diameter of the rice roots is more or less analogous to one another even though varieties are different. Therefore, the root surface area depends on the total length of root. Linear relationship was found between the shoot dry weight and the natural logarithm of root length per plant at various growth stages among different rice varieties (Chul, 1989).

Root is clearly one of the most favourable objects for the study of environmental adaptations in multicellular plants. The understanding of these processes requires their study to be pursued at the structural, physiological and biochemical levels (Street, 1966). The development of root system is of great importance in every agricultural crop. It seems, however that, in the case of cereals it has not yet seriously investigated and only very few data on varietal difference have been established (Pinthus and Eshel, 1998). The pattern of root growth has a major influence on resistance. The roots of a plant are the principal organs by which nutrients are absorbed and understanding of rooting habits and activity of roots are essential in developing an understanding of effective nutrient uptake (Raez, et al., 1964). Productivity of herbage plants is influenced by the ability of root systems to absorb nutrients and water from the soil.

The epidermis, cortex and root cap were more sensitive to salt stress than the meristem and stele (Rahman et al., 2001). Major part of cytoplasm was occupied by these vacuoles. The number of mitochondria was increased significantly by treatment with NaCl. The mitochondria were randomly distributed in control roots but in NaCl-treated roots they were aggregated in the cytoplasm in the root tip and root cap cells (Rahman et al., 2001). Mitochondria were deficient in cristae and the matrix appeared pale in the plants treated with
The present investigation gives a detailed account of the response of the root system of MO18 and MO19 varieties of rice under different salinity stress conditions which nobody has determined earlier. The change in seminal root length, nodal root length, root hair number and length are all very good morphological expressions towards salinity. SEM and Stereo microscopic studies also give a very good idea of the ultra morphological features of roots of the two different varieties of rice under salinity stress.

5.1.2.1. Seminal Root Length
Table 1.2.1 shows that the mean seminal root length gradually decreased from 6.7cms in MO18 and 6.48 cms in MO19 at control to 3.38 cms in MO18 and 2.78 cms in MO19 with increase of NaCl percentage from 0.0 to 0.9. A comparison between MO18 and MO19 seminal root length shows that the effect of NaCl concentrations was not pronounced strongly between the two, though there was reduction in both. Previous workers like (Chul, 1989 and Thangeraju et al., 1990) also observed such reductions in seminal root length due to NaCl stress.

5.1.2.2. Number of Nodal Roots
In control study, average number of nodal roots was found to be 12.6 in both cases (Table 1.2.2). Along with the increase of salinity stress from 0.0 per cent to 0.9 per cent, it was observed that there is a gradual reduction in nodal root numbers in MO18 and MO19 varieties. Among these two varieties, MO18 showed less number of nodal roots (7.2) than in MO19 (7.8) at 0.9 per cent NaCl stress. From the table it is clear that MO19 is slightly more tolerant to salt stress. Here also the reduction in nodal root number is dose dependent as mentioned by earlier researchers like Ahmed and Gupta (1991), Bandyopadhyay (1998).

5.1.2.3. Nodal Root Length
Control of MO18 and MO19 possessed a mean length of 5.54cm and 5.18cm respectively on 18th day after germination. The nodal root length was reduced from 5.54 to 2.68 in MO18 and from 5.18 to 2.28 in MO19 due to salt stress increase (Table 1.2.3). Though an increase in reduction of nodal root length is observed in both cases due to increased stress, an absence of varietal difference can be noticed since the values of both the varieties are almost similar i.e. 2.68 in MO18 and 2.28 in MO19.

5.1.2.4. Number of Root Hairs per Unit Length (per mm)
There was considerable effect of NaCl stress on root hairs of MO18 as well as MO19 varieties of rice. As in previous parameters, there occurred a decrease in number of root hairs per unit length in both the varieties as the concentration of NaCl increased from 0.0 per cent
to 0.9 per cent (Table 1.2.4). Among the two varieties examined, MO19 is more resistant to salt stress as the values are ranging from 71.8 at 0.0 per cent stress to 65.2 in 0.9 per cent stress when compared to MO18 where the numbers ranged from 58.6 at 0.0 per cent salinity to 44.2 at 0.9 per cent. Similar observations were made by earlier researchers like Raez et al., (1964) and Bohra (1991). More root hair number and length will give more resistance to stress since it provides more surface area of absorption to make resistance as found by some other researchers. Thus MO19 has more resistance to salt stress since it bears more root hairs.

5.1.2.5. Length of Root Hairs
Since root hair length plays a considerable role in the development of rice, especially in absorption, it was considered reasonable to make a comparison of this also. The control of MO18 root hair showed a length of 91.6µ while a gradual reduction in length can be observed to 76.6µ at 0.9 per cent salt stress. In case of MO19 also the reduction in root hair length varies from 166µ in control to 99.8µ at 0.9 per cent salinity. Among the two varieties of rice, highest reduction is observed in MO18 since it shows 15µ difference when compared to MO19 where the length difference is 6.2µ between control to 0.9 per cent salinity.

5.1.2.6. Stereomicroscopic Study
Stereomicroscopic observations on MO18 and MO19 revealed considerable variations among normal and stressed roots. In MO18 the root tip as well as the roots showed shrunken nature due to increase of salinity from 0.0 per cent to 0.9 per cent (Figs. 5, 7 and 9) whereas shrinkage was less in MO19 (Figs 6, 8 and 10). The number of root hairs are reduced considerably in both MO18 and MO19 as the salinity stress increases. Among the two varieties studied, MO19 has greater number of root hairs though large numbers were lost due to increase of NaCl stress as seen from figures.

5.1.2.7. Scanning Electron Microscopic Study of Root Tips
Scanning electron microscopic studies of normal root tips of both MO18 and MO19 varieties showed turgid root tips. MO18 has more pointed root tip followed by a bulged area where as MO19 carries less pointed and more or less uniform size behind the root cap region .The texture of the surface is uneven in both the cases (Fig. 11 and 12) As concentration of NaCl increased, the bulged region of MO18 reduced and the root tip became more pointed. Root tip of MO19 showed prominently, pointed end at 0.5% concentration of NaCl. Tissue exhibited more shrinkage in both verities as the concentration of salinity increased at 0.9% salinity stress; MO18 root tip not only showed shrinkage but also exhibited bending where as
MO19 exhibited only shrinkage (Fig 15 and 16). In both the cases, the surface view showed change in texture due to stress.

5.1.3. Shoot Characteristics

5.1.3.1. Length of Shoot

In rice, seedling stage is one of the critical stages for salt damage because a proper seedling development is prerequisite for flowering and grain development. Even though both MO18 and MO19 are salt resistant varieties, these two cultivars showed a decrease in the length of shoot with increasing concentrations of NaCl. Under normal conditions, MO19 was better than MO18 regarding the length of the shoot. Towards higher concentrations, both MO18 and MO19 showed decrease in shoot length (Table 1.3.1). At the highest stress, both the varieties showed around 50 per cent reduction in their shoot length. Similar results were reported by Khan et al., (1977), Prakash and Prathapasen (1988) and Gupta (1993).

Khan et al., (1977) suggested that the plant height was decreased drastically from 0.9 per cent onwards in most of the varieties, but in Pokkali, it was not much affected. Ashraf and McNieilly (1986) reported seedling growth in Sorghum under stress. Richards, (1983) suggested that seedling vigour is a suitable character for selection for tolerance to high levels of salinity. In addition to these, Prakash and Prathapasen (1988) reported that with an increasing salinity stress, the shoot length was reduced in most of the rice varieties. Growth parameters like main shoot length, relative weight etc. were adversely affected by salt stress in wheat as reported by Rashid et al., (1999).

Stress induced the production of abscisic acid (ABA), which can inhibit growth (Hall and McWha, 1981; Francois, 1986; Weimberg, 1987; Ashraf and McNieilly, 1986; Creelman, 1986; Saab et al., 1990). ABA at the cellular level inhibits all growth by affecting cell division and cell expansion (Evans, 1984; Kutschera and Schofer, 1986). But the response of stress induced ABA varies on various plants and even in same plant. In most cases it inhibits shoot growth but the response of root is variable. In the present study the shoot growth inhibition under higher salinity may be attributed to the reduction in root growth which in turn affected water transport to the shoot and severely limited the growth.

5.1.3.2. Leaf Area

Leaf area was taken as a parameter for evaluation of varietal difference in rice by several researchers like (Liu and Lin, 1985; Murthy et al., 1991). The reduction in leaf area under stress condition was attributed to the role of salinity-induced ABA which limits the plant growth at whole plant level. It is known that NaCl accumulation in the leaves is correlated
with reduced photosynthetic activity and ultra structural as well as metabolic damage (Yeo and Flowers, 1989). The results obtained in this investigation fully agree with the early findings. Both the MO18 and MO19 varieties show reduction in leaf area towards higher salinity stress. But as the result shows MO19 is having slightly better tolerance than MO18 (Table 1.3.2), since MO19 presents more leaf area at highest stress.

5.1.3.3. Biomass
Root weight is a good parameter for characterising the total mass of root in soil. Root weight (dry and fresh) should be a unit for evaluation where the productivity of underground vegetation being considered. Root weight can also be considered as a fundamental measure of photosynthetic storage in plant. Root and shoot weight decreased with salinity in the present investigation supported the findings of Gill and Singh (1988). They also reported similar types of retarded root and shoot growth under salinity stress. They also reported that the effects are irreversible. Dutt and Bal (1993) reported that as salinity increased, root volume and maximum root growth was significantly reduced; while dry root matter increased in some cultivars of rice. Relative water content showed downward trend with increase in salinity.

As reported by Cramer et al., (1995) the moisture content of the leaves was increased by salinity while that of the stem was decreased. The root biomass was considerably less affected than the shoot and fruit yield under high salinity, indicating the preference of root to the shoot growth as salinity increases (Perez et al., 1996). Sodium chloride salinity reduced the mean root dry weight of *Eucalyptus camaldulensis* at four salt concentrations viz. 40mM, 80mM, 120 mM and 160mM (Rawat and Banerjee, 1998). Root dry weight of *Dalbergia sissoo* increased significantly at the lower salinity (40mM.) and then progressively decreased with increasing salt concentrations (Rawat and Banerjee, 1998). Increase in salinity stimulated production of fresh mass. However, dry mass did not show significant enhancement. The response of both MO18 and MO19 prove that biomass at higher stress must be assessed on account of its dry weight and not fresh weight.

MO18 exhibited a gradual reduction in fresh as well as dry weight (Table 1.4). In the case of MO19 fresh weight showed an increase from 182.03 mg-H2O/g DW in control to 185.87 in 0.5 per cent NaCl and 184.24 in 0.9 per cent NaCl stress. Dry weight showed a reduction from 163.4 in control to 163.24 in 0.9 per cent NaCl medium i.e. a difference of 0.16 only. From the above data it is clear that MO19 is slightly more salt tolerant than MO18.
5.2. Physiological Parameters

5.2.1. Ions

NaCl induced accumulation of Na\(^+\) and Cl\(^-\) and a decrease in K content is observed in both MO18 and MO19. Accumulation of Na\(^+\) and Cl\(^-\) and decrease in K content at the shoot level were restricted to the oldest leaves in salt-resistant genotypes while proline accumulated in the youngest leaves in all cultivars (Lutts et al., 1996). At the whole plant level, salinity stress frequently induces an increase in NaCl content as well as a decrease in K, Ca, NO\(_3\), and Pi concentrations (Cramer et al., 1991; Grattan and Grieve, 1992; Izzo et al., 1991; Martinez and Lauchi, 1994). In glycophytes, salinity resistance is associated to a restriction of toxic ion absorption at the root level and salt-resistant rice cultivars generally accumulate less Na\(^+\) and Cl\(^-\) than salt-sensitive ones (Akita and Cabsulay, 1990; Fageria, 1985).

5.2.1.1. Sodium Ions (Na\(^+\))

In both MO18 and MO19, in shoot and root the Na\(^+\) content showed a significant increase from control to 0.5 and 0.9 per cent NaCl treated plants (Table 2.1.1). Similar results in Soyabean is reported by Absel and Mackenzia (1964) and is attributed that the Cl\(^-\) exclusion is controlled by a single gene. Tolerant cultivars accumulate less amounts of Na\(^+\) than susceptible cultivars (Kaddath et al., 1975; Flowers and Yeo 1981; Slama, 1986; Karim et al., 1992). Cheeseman (1982) reported that under moderate salinity stress plants grow without extreme root Na\(^+\) accumulation. Reduced Na\(^+\) and Cl\(^-\) levels under external NaCl stress in bean leaves was reported by Seeman and Critchley (1985). NaCl induced accumulation of Na content is observed in both MO18 and MO19. Similar results were reported by or by Weimbery (1987) in Wheat and John et al., (1997) in rice.

Rashid (1986) is of opinion that shoot Na\(^+\) was responsible for a significant proportion of the salt damage at the lower salinity level, while at the higher level both Na\(^+\) and Cl\(^-\) negatively influence the shoot weight. Sodium compartmentation, however, may have limited physiological significance because of recent reports of high cytoplasmic Na\(^+\) concentrations in salt adapted corn roots (Hajibagheri et al., 1987) and tobacco cultured cells (Binzel et al., 1988). In Scirpus a drastic reduction in total growth under salt stress observed by Cheeseman (1988). Blum (1988) reported that the excess amounts of Na\(^+\) create a toxic effect on plant metabolic process and therefore, the susceptible cultivars having high amounts of Na\(^+\) suffer more from the effect than the resistant cultivars. Sodium concentration in roots increased as salinity increased in rice (Dutt and Bal, 1993). When the roots fail to prevent the uptake of excess Na\(^+\) the plant dies due to excessive accumulation of Na\(^+\) in the shoot,
provided there is no partitioning of the offending ions into mature shoot tissues. This two-tier defence against Na⁺ toxicity in rice plants perhaps helps the distal young shoots to survive and bear seeds (Sathish et al., 1997).

Again the result showed that increase in NaCl concentration gradually increased Na⁺ accumulation and was significantly higher in root than in the shoot system (Table 2.1.1) of MO18 and MO19. Prakash and Prathapasenan (1988) suggested that NaCl salinity causes an accumulation of Na⁺ and Cl⁻ ions with a concomitant drop in K⁺ concentration. According to Akita and Cabsulay (1990), rice is unable to regulate Na⁺ absorption at root level and Na⁺ accumulation is dependent on transpiration rates. Na⁺ and K⁺ are important physiological trait for salt tolerance (Cuartero et al., 1992, Perez-Alfocea et al., 1993). In the present study Na⁺ was found to be increased but K⁺ ion content was found to be decreased as the concentration of salt increases. Naf and Ki (1992), Yeo et al., (1982) etc. were of opinion that the net transport of K⁺ and Cl⁻ need to adjust the turgor and changes the membrane permeability. It is a well known fact that tolerant varieties show lesser accumulation of sodium in their tissues. Among the two varieties compared, both the MO18 and MO19 have accumulated high amount of Na⁺ in their tissues towards higher salinity stress. Accumulation of Na⁺ in the shoot system of MO18 is significantly higher than that of MO19 at the highest stress induced. However, the roots of MO18 and MO19 kept a higher concentration of Na⁺ than shoots. Here also it can be seen that since less accumulation of Na⁺ is seen in MO19 than MO18, MO19 is more resistant to salinity.

5.2.1.2. Potassium Ions (K⁺)

The notable accumulation of sodium content in MO18 and MO19 is evident under salt stress in the present investigation. The sodium accumulation is lesser in MO19 as compared to MO18 variety. In other words, MO19 is a salt resistant variety compared to MO18. Singh et al., (2000) reported that ability of drought tolerant varieties of wheat to accumulate more potassium. The high K⁺ affinity and the maintenance of a constant K⁺ at high salinity are interpreted as requirements for a minimal cytoplasmic K⁺, possibly associated with the K⁺ requirement of protein synthesis (Jones et al., 1979). Greenway and Munns (1980) and Ashraf et al., (1986), are of opinion that the shoot tolerance could either be due to the dilution effect of more water content in the tissues of a tolerant genotype or the compartmentation of Na⁺ and Cl⁻ in vacuoles by the tolerant genotype. However, in contrast, the photosynthetic capacity of Avicennia marina was unaffected by the same range of salinity and NaCl accumulation in leaves when plants were grown such that the K⁺ concentration in
leaves maintained relatively constant (Ball, 1981). The enhanced K\(^+\) uptake is an adaptive mechanism that allows the cells to evade K\(^+\) starvation in presence of high concentrations of NaCl. Higher K\(^+\) in shoot was due to the ionization of better plant water status in relation to leaf water potential. Harun et al., (1987) is of opinion that during salt stress, the cellular K\(^+\) concentration has been implicated in cellular osmotic adjustment and growth capacity.

A decreasing tendency in K ion accumulation was observed with the increasing salinity levels except in Pokkali, which showed static concentrations of K\(^+\) at all levels of salinity. Blum (1988) and Qader (1988) suggested that under water stress as well as salt stress conditions, K\(^+\) plays an important role in osmoregulation and stress tolerant cultivars accumulate higher amounts of K than susceptible cultivars. The ability to maintain constant minimum shoot K\(^+\) concentration is regarded as crucial to tolerance at a cellular level (Groham et al., 1990; Wolf et al., 1990, 1991). This variation was attributed to the changes in membrane potential affecting passive ionic fluxes that occur in response to osmotic stress and changes in membrane permeability and proton pump activity which directly add to the transport of K\(^+\) to adjust the turgor (Grieve and Fujiyama, 1987).

Sodium ions in the soil are suspected to hinder the absorption of K\(^+\) through rice roots. A direct source to sink translocation of K\(^+\) should increase K\(^+\) utilization, rice grain yield and impact some salinity tolerance to the plant by excluding Na\(^+\) from the shoot. The ability of a cultivar to accumulate K\(^+\) in its shoot correlate well with salt tolerance (Nair et al., 1993). K\(^+\) influences the water economy and crop growth through its effect on water uptake. In the present study also the accumulation of K\(^+\) is due to the combination of the above factors along with the inherent potential of the variety towards tolerance. However the sharp decrease in K\(^+\) content in the tissues of both MO18 and MO19 (Table 2.1.2) towards salinity stress prove that both of them are saline tolerant varieties and MO19 is more salt resistant.

5.2.1.3. Sodium/Potassium (Na/K) Ratio

In the present study Na/K ratio was found to be increased under NaCl stress because the content of K decreases as the concentration of stress increases (Table 2.1.3). This increase of Na/K value is irrespective of source and variety. Na/K in the present study was found to be increased from control in plants under salt stress. The Na\(^+\)/K\(^+\) ratios increased considerably in shoot and root at moderate salinities and fluctuated at higher salinities. Na/K ratios as high as 10.0 have been reported for Chenopodiaceae (Flowers and Yeo, 1986). When taken on a whole plant basis the ratio increased at all levels of external salinity as compared to control in Suaeda nudiflora. Increase in Na\(^+\): K\(^+\) ratios in root, and especially in shoot, with increase in
salinity was also reported by Hajibagheri et al., (1987) in maize. Naidoo and Raghuraman (1990) reported a ratio of 16.6 at 400 molm-3 NaCl in Sarcocorina natalensis.

Increase in Na/K ratio under salt stress in the present investigation was attributed to the disturbed ionic balance due to the greater uptake of Na. In the present study K⁺ content was decreased in response to salt stress. Similar results were reported by Yeo and Flowers (1989). Quader (1991) and Sharma and Singh (1998) reported the decrease in K⁺ content due to the increase of Na⁺ and it was attributed to the relative tolerance in rice. Aslam et al., (1991) suggested that a K/Na discrimination mechanism similar to that observed in wheat (Gorham, 1994) could operate in salt- resistant rice cultivars also. Sharma (1996) suggested that growth reduction caused by salinity was accompanied by increased Na⁺ and Cl⁻ concentration, Na⁺/K⁺ ratio and decreased K⁺ concentration. These values obtained in this regard also suggest that saline resistance exist in both the MO18 and MO19 varieties.

5.2.1.4. Chloride Ions (Cl⁻)

A significantly increased accumulation of Cl⁻ was observed in the present study in both varieties under salt stress. Response of salinity stress on physiological traits such as K⁺, Na⁺, Cl⁻ ions, osmotic adjustments etc. were studied by Abel and Maekenzie (1964) in barley; Yeo and Flowers (1982) in rice; Kingsbury et al., (1984) in wheat; Kingsbury and Epstein (1986) in wheat; Weimbery (1987) in wheat; Yeo et al., (1990) in rice. They postulate that the physiological process involved in turgor regeneration are to a great extend function the membrane properties of the plasma membrane and to tonoplast; this in turn affect the membrane potential and the passive ionic fluxes. Turgor regulation was mainly by the changes in K⁺ and Cl⁻ as reported by Hoffman and Bission (1986).

It was observed in the present investigation that the great Cl⁻ accumulation in leaves could contribute to maintain an osmotic gradient at moderate salinity, because plants regulate their osmotic balance to maintain normal metabolism. Salinity stress induces an increase in the Na⁺ and Cl⁻ level as reported by Lutts et al., (1996). Rashid et al., (1996) suggested a similar response in tomato after salt stress Wheat posses the ability to exclude Na⁺ and Cl⁻ ions as salinity level increases and concentrate these ions at older leaves than young ones as reported by (Rashid et al., 1999).

Difference was observed in the expression of salt sensitivity in the two varieties of MO18 and MO19 in the present investigation. A significantly increased accumulation of Cl⁻ (Table 2.1.4) was observed in the present study in both varieties under salt stress. It was observed in
the present investigation that the great Cl\textsuperscript{−} accumulation in leaves could contribute to maintain an osmotic gradient at moderate salinity, because plants regulate their osmotic balance to maintain normal metabolism. Here again, comparatively MO19 was found to be more salinity resistant than MO18 as evident from the table.

5.2.1.5. Phosphorous (P)
The decreased P content in leaves of salt-sensitive cultivars of cotton was clearly related to an inhibition of P transport as reported by Martinez and Lauchli (1994). It is suggested that the metabolic requirement of P is enhanced in salt stressed plants, a decrease in the transport of this element could have deleterious effect on plant growth and this could explain the strong positive correlation between shoot P content and shoot relative growth rate (Lutts et al., 1996). The changes in P content in both MO18 and MO19 varieties agree with these previous observations. The samples under study shows decrease in P content as observed in both shoots of Mo18 and MO19. Among the two, MO19 is slightly more salt resistant, since it has less P content than MO18. In the case of roots, considerable difference occurs between MO18 and MO19, since the values of P are 346.16 and 324.18mmolkg\textsuperscript{−}\textsuperscript{1}DW respectively (Table 2.1.5).

5.2.2. Leaf Chlorophyll
One of the salt tolerance mechanisms of many plants is the accumulation of salts in the stem and prevention of their permeation in to the actively growing and photosynthesizing leaves (Waisel, 1972; Reddy et al., 1992). According to Lapina and Popov (1970); Prisco and O’Leary (1972); and Strogonov (1974), salinity in the root medium results in disarrangement in the pigment composition which induces a decreased chlorophyll content. Similar results have also been reported by Wignarajiah et al., (1975), in Phaseolus; Downton et al., (1977) in Vitis; Walker et al., (1980) in Capsicum and Delane et al., (1982) in Hordeum. The decrease in chlorophyll content of the present study can be attributed to a weakening of pigment-protein-lipid complexes as reported Strogonov. (1974).

Chlorophyll concentration of leaves declined with higher salinity as reported by earlier workers like Petolino and Leone, (1980); Grant and Sommen (1981); Chawan and Karadge, (1986); Rawat and Banerjee (1998). The role of NaCl salinity in distorted ion transport was reported by Greenway and Munns (1980) and Yeo (1983). Robinson et al., (1983) reported that leaves of salt-grown spinach plants had less chlorophyll than control plants and also a decrease in photosynthetic capacity of about 10% when expressed on leaf area basis.
Decreased chlorophyll content under saline conditions has been reported by several workers (Grant and Somers, 1981; Chavan and Karadge, 1986; Reddy et al., 1992).

The present study also agrees with the above view, which is evidenced by the decrease in total chlorophyll content with increase in external salinity. Huber (1985) reported that variation in salinity affects the foliar concentrations of $K^+$, a nutrient required in relatively high concentration for photosynthesis and other metabolic processes. Hence it can be assumed that loss of photosynthetic capacity with increase in salinity was related to metabolic requirements for $K^+$ and decreasing capacity to satisfy those needs with increasing salinity (Ball et al., 1987). Prakash and Pathaparenan (1988) were of opinion that salinity markedly reduced leaf growth and chlorophyll content. Root zone salinity also greatly reduced the photosynthetic capability of the plants (Glenn et al., 1998). During the present investigation on both MO18 and MO19 varieties of rice, a decrease in chlorophyll is observed (fig.23) which is directly proportional to the increase of salinity stress. Under 0.5 per cent NaCl stress, MO19 shows significant tolerance than MO18. In short, MO19 is more tolerant to salinity stress than MO18, since it shows more chlorophyll content in its leaf under high salinity stress.

### 5.3. Biochemical Parameters

#### 5.3.1. Starch

Effect of response of starch metabolism in relation to salinity was already studied by many investigators such as Shimose, (1963); Sarin and Narayanan, (1968); Gupta et al., (1999). They were of opinion that starch metabolism is affected by increased salinity and the type of salinity. Starch and cellulose contents in leaves and stem decreased with increasing salt concentrations and trended to be lower in plants as the NaCl content in the soil increased. Under high salinity, reduced hydrolysis of starch is reported in wheat by Sarin and Narayanan, (1968). Munns et al., (1982) were of opinion that hydrolysis of starch was one reason for increase in sugars under salinity, particularly in salt tolerant genotypes. The higher distribution of sucrose towards the root could help to explain the small growth reduction of this organ at high salinity compared to shoot. This could be considered as an adaptive response to exclude more Na$^+$ and increasing the surface for water and nutrient uptake, although to the detriment of the shoot growth and fruit yield (Perez et al., 1996).

In the present investigation starch content was found to be reduced under stress irrespective of the source and variety. This reduction may be attributed to the reduction of chlorophyll contents due to NaCl stress. There is significant difference between starch contents of shoots
and roots of MO18 and MO19 as evident from the Table 3.1. MO19 carries more starch content in normal as well as experimental conditions, hence more tolerant than MO18.

5.3.2. Protein

Protein metabolism is found greatly affected in plants growing under saline conditions. Salinity causes either decrease (Levitt, 1972) or increase (Dubey, 1982) in levels of total and/or soluble proteins depending on the plant parts studied, promotes the synthesis of salt stress-specific proteins (Hurkman and Tanaka, 1987; Ben-Hayyim et al., 1989), and leads to increased activity and synthesis of many enzymes (Dubey and Rani, 1987; Dubey and Sharma, 1990; Mittal and Dubey, 1991). Stress leads to accumulation or depletion of certain metabolites, alterations in the behaviour of many enzymes and synthesis of many proteins that are specific to the particular type of stress (Jacoben et al., 1986). It is noted that various environmental stresses cause important modifications in gene expression in plants (Vierling, 1991). The present results showed that in both MO18 and MO19, the total protein content was high in shoot and root and they respond to stress in different manner. The low levels of protein content observed may be due to the decreased synthesis of proteins as well as increased activity of protein hydrolyzing enzymes as mentioned by (Reddy and Vora, 1985; Dubey and Rani, 1990). In the present study an increased protein level is noticed under salinization, as evident from table 3.2, possibly as a result of the increased synthesis of new salt induced proteins or the decreased activity of proteolytic enzymes as reported by Dubey, (1994).

As the products of abiotic stress and ABA inducible genes are predicted to play an important role in the mechanism of salt tolerance, the expression of transcription factor that recognizes abscisic-acid-responsive element is likely to be regulated when plants are exposed to abiotic stress (Gupta, 1999). Shoots and roots of MO19 expressed more tolerance to salt stress than MO18 as it is evident from the experiment. (Table 32)

5.3.3. Amino Acid

In the present investigation amino acid content in MO18 and MO19 under salt stress was found to be increased than control (Table 3.3). Many workers demonstrated the effect of NaCl stress on amino acid content and metabolism of various plants (Wadleigh and Aiyers, 1945; Ungar, 1974; Weimberg, 1975; Grattan and Grieve, 1985; Epstein and Rains, 1987; Dubey and Rani, 1989). Flowers et al., (1977) is of opinion that amino acid functions as a compatible cytoplasmic solutes and their increased accumulation serves as a means of intracellular osmotic adjustment in order to equalize the osmotic potentials of cytoplasm in
adverse conditions of salinity. Stewart and Larher (1980) reported that the level of amino acid is a component of salt tolerance mechanism. Saline tolerant varieties are characterized by higher level of amino acids and their increased accumulation under saline conditions compared to sensitive varieties suggests the direct correlation between levels of accumulation of amino acid and degree of salt tolerance. Numerous organic solutes were found to be accumulated and help in osmotic adjustments of cytoplasm under stress condition was reported (Adams et al., 1992; Kuzuestov and Sheryakova, 1997).

In response to salinity, numerous organic solutes are presumed to accumulate in cytosol. Among these, sugars, polyols, amino acids, and quaternary ammonium compounds (betaines), have been associated with osmotic adjustment (Paul and Cockburn, 1989; McCue and Hanson, 1990; Adams et al., 1992; Kavi Kishore et al., 1995; Kuzuestov and Shevyakova, 1997). They suggested that tolerant rice were characterized by higher levels of free amino acids arginine, leucine, alanine, valine, glutamine and proline accumulation under saline conditions and direct correlation between levels of amino acids and degrees of salt tolerance. Tolerance of salinity caused marked decrease in root enzyme activity (Blake and heel, 1992). In the present study the varietal difference in salt tolerance was not much significant because there exists an inbuilt varying level of amino acid. It was confirmed from the present study that the total amino acid might be taken as an indices for tolerance level in rice. Even then MO19 was found to be more tolerant than MO18.

5.3.4. Proline

In the present study, the proline content was found to be increased in response to salt increase. Stewart and Lee (1974) reported the role of proline in halophytes and according to them the effect of increased accumulation of salt ions in the vacuoles, proline has been reported to increase in the cytoplasm, which might act as an osmoticum. He is of the opinion that proline accumulates in the cytoplasm without having any detrimental effect. The role of proline during stress is a subject of controversy and interesting because it accumulates to very high levels under adverse conditions (Tal et al., 1979; Patel and Vora, 1985).

Hanson et al., (1977) assumed that proline could act as a free radical scavenger in salt tolerance mechanism and it may be even the result of damage. Testing of high concentrations of proline and glycine betaine on isolated enzymes have shown that they protect various enzymes against a range of perturbing effects (Pollard and Wyn Jones, 1979). Cellular content of proline was shown to increase from two fold to 20 when cells were exposed to NaCl stress (Dix and Pearce, 1981; Watad et al., 1983; VanSwaaij et al., 1986; Chandler...
The accumulation of proline in response to environmental stress indicates that its synthesis is a non-specific response to decreased water potential. Watad et al., (1983) observed that tobacco cell lines which had the ability to grow and develop indefinitely in high NaCl media accumulated proline when grown in these media suggested that proline accumulation is not the visible result of damage to mitochondrial membranes, but accompanies survival and growth in a saline environment. Proline is known to play a critical role in osmoregulation in stressful environment in many plant systems (Reddy and Vaidyanath, 1986) observed that in Oryza sativa L. a concomitant increase in proline content with increase in concentration of NaCl. Increased proline synthesis was considered as an attempt to limit cytoplasm acidification in stress conditions (Venekamp, 1989). It appears that osmoregulating substances affect the enzymes, there by stabilizing the active conformation and by this way protecting enzymes against conformational disturbances caused by ions (McCue and Hanson, 1990).

Role of proline in salt resistance was already studied by Manetas, (1990); Gouzales and Labradora (1995) and Chu et al., (1996) and observed that proline content was significantly increased under salt stress in rice. They also suggested that salt tolerant varieties do not necessarily accumulate large amount of free proline in relation to sensitive varieties. Chu et al., (1996) reported that high salinity induced a proline accumulation especially in petioles, old leaves and roots. Proline accumulation is already reported in salt stressed plants (Lutts et al., 1996). Increase in proline content under moisture stress was reported by Baruah et al., (1998) in rice. These metabolites may replace water at the surface of biopolymers, stabilize macromolecular structure or act in scavenging of radicle oxygen compounds (Reddy and Iyengar, 1998). It was suggested by Lutts et al., (1996) that the salt sensitive cultivar accumulated higher levels of sodium and proline than the salt-resistant cultivar and displayed lower levels of osmotic adjustment. MO18 and MO19 shoots as well as roots showed increase of proline accumulation as the salt stress increased. MO19 expressed more accumulation of proline than MO18 (Table 3.4). Difference is not much significant.

5.3.5. Enzymes
5.3.5.1. Peroxidases (POD)
In the present study peroxidase (POD) activity decreased as a function of NaCl stress. Various researchers dealing with rice (Mittal and Dubey, 1991) and other plants (Kalir et al.,
1984, Sheoran and Garg, 1996) have also reported increase in peroxidase under salt stress in salt-sensitive cultivars. Peroxides can modify the cell wall properties (Fry, 1986), catalyses the cross linking of extensions and pectins of cell wall (Davies, 1987) and the crosslinking of extra cellular matrix proteins could be a protective mechanism in eukaryotic cells to operate in a variety of stress conditions (Fridovich, 1986).

Peroxidase often has been discussed as an indicator, for developmental changes, both as its isoenzyme pattern. Some research suggests that embryonic and non-embryonic callus contained different isoenzyme pattern and activity of peroxidases (Stewart and Larther, 1980). The enzymes have been involved in several physiological and biochemical processes such as cell growth and expansion (Wallace and Fry, 1994; Lin and Kao, 1996) differentiation and development and (Mansouri et al., 1999; Lagrimini et al., 1997) lignifications (Otter and Polle, 1997) as well as abiotic and biotic stress responses (Medina et al., 1999). Peroxidase activity is correlated with the reduction of plant growth (Mittal and Dubey, 1991). Although, the precise role that anionic POD plays in plant growth, development and stress tolerance remains uncertain. There is significant evidence that anionic POD is involved in host defence and stress-induced lignifications (Lagrimini et al., 1997). Excess uptake of toxic metals like Fe$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$ induces both quantitative and qualitative metal-specific changes in POD isoenzyme patterns in rice leaves (Wei-Ching and Huei- Ching, 2000). It is observed that the production of POD was decreased in MO19 (T Fig 24) and the decrease is a sign of salt tolerance as mentioned by earlier workers.

5.3.5.2. Superoxide Dismutase (SOD)

In the present study superoxide dismutase (SOD) activity increased as a function of salinity stress. It has been proposed, however, that physiological mechanisms underlying salt tolerance such as ion exclusion are more relevant criteria for improving salt tolerance in crops (Noble and Rogers, 1992). Because of the inherent sensitivity of rice plant to salt stress (Francois and Mass, 1986) there has been a great interest in developing varieties that are resistant to salinity. The chloroplast and mitochondria of plant cells are important intracellular generators of activated oxygen species. These cytotoxic oxygen is highly reactive and in absence of any protective mechanism they can seriously disrupt normal metabolism through oxidative damage to lipids, proteins, nucleic acids (Davies, 1987 and Fridovich, 1986). An increase in SOD production was observed (F Table 25) in MO19 which is more tolerant than MO18.
5.3.6. Electrophoretic Separation

5.3.6.1. Protein

The SDS-PAGE study was done to make a comparative assessment of various polypeptides, which could alter in response to salinity stress conditions. This can be used as a reference in distinguishing between the stress response of stress-sensitive and tolerant cultivars. The present studies revealed significant changes in the protein pattern when exposed to different salinity stress conditions. Variations in protein profiles were observed in both root and shoot in SDS PAGE studies. It is noted that various environmental stresses cause important modifications in gene expression in plants (Vierleng, 1991) and may lead to accumulation or depletion of certain metabolites, alterations in the behaviour of many enzymes and synthesis of new proteins that are specific to the particular type of stress (Jacoben et al., 1986). Under stress conditions a similar change in root proteins of Suaeda nudiflora was observed by (1998). The increased protein level is possibly as a result of the increased synthesis of new salt induced proteins or the decreased activity of proteolytic enzymes (Dubey, 1994).

Levels of a large number of proteins were altered up on exposure of seedlings of rice to different abiotic stress. The protein alterations of the cultivar – Lal nakanda ranged in molecular weights from as low as 10.2 kDa (in response to salinity stress, drought stress, low temperature stress and abscisic acid) to as high as 123 kDa (in response to salinity stress and abscisic acid) application (Pareek et al., 1997). Previously, it has been reported that tolerance to one type of stress often leads to cross-tolerance against the related stress type (Khush and Toenniessen, 1991). Apart from the proteins, which were co-triggered, several proteins were noted to be specific to a given type. In shoots, 15 and 13kDa polypeptides accumulated specifically in response to salinity stress (Pareek et al., 1997 b). More protein alterations were scored in shoots than roots for all the stresses and this differential response in shoot and root tissues reflect their relative sensitivities to stress conditions (Mishra et al., 1994). It is possible that the proteins unique to a given stress signal have roles in governing stress- specific cellular responses (Mishra et al., 1996). As evident from Fig 26 it can be said that MO19 is more salt tolerant than MO18 since additional bands of proteins are formed at maximum stress which is an indication of resistance.
5.3.6.2 Isoenzyme

Peroxidase

Peroxidase isoenzymes increased as a result of salt stress in the present study. To protect the cells under stress conditions, plant tissues contain several enzymes such as peroxidases (POD), catalases and superoxide dismutase (SOD) and a network of low molecular mass antioxidants like ascorbate, glutathione, phenolic compounds and tocopherols (Bohnert et al., 1995).

The intracellular level of H₂O₂ is regulated by a wide range of enzymes, the most important being peroxidase. CuSO₄ and ZnSO₄ were observed to induce peroxidase activity, is not specific for rice cultivars. In rice (Oryza sativa L.) it was found that excess Fe²⁺, Cu²⁺ or Zn²⁺ induced both quantitative and qualitative metal-specific changes in peroxidase isoenzyme patterns in detached rice leaves. A new peroxidase isoenzyme can induced by Fe²⁺, Cu²⁺ and Zn²⁺ in detached rice leaves (Wei-Ching and Ching, 2000).

The peroxidase pattern shows an increase with the increase in salt stress. Both shoot and root of MO18 and MO19 showed a similar pattern. Various researchers dealing with rice and other plants have reported increased peroxide activity under salt stress (Sheron and Garg, 1978; Kaliar et al., 1984; Mittal and Dubey, 1991). This could be due to increased activity of peroxidase encoding genes or increased activation of already existing enzymes.

Mittal and Dubey, (1991) suggested that salinity affects mainly the de novo synthesis of the enzyme since inhibition in vitro conditions and activation in invivo conditions were observed in their studies in salt-sensitive cultivars. Lopez et al., (1996) has shown that salt-induced increase in ascorbate peroxidase activity in radish plants was not accompanied by a corresponding increase in mRNA level, suggesting that the salt-induced ascorbate peroxidase expression is probably the consequence of post-transcriptional events. Aside from their function in the metabolism of active oxygen, peroxidases in plants are involved in the biosynthesis of cell wall including lignification and suberization (Espelie et al., 1986; Polle, 1994; Negrel and Lheruinier, 1987). As observed in Fig 29, a decrease in POD was observed.
in MO19 shoot since one isoenzyme band disappeared. Decreased activity of POD is assign of salt tolerance.

**Superoxide dismutase (SOD)**

Superoxide dismutase - isoenzymes increased as a result of salt stress. Evidence for the effects of salt stress-inducing changes in plant metabolism is well documented (Greenway and Munns, 1980). In salt tolerant plants NaCl treatment brought about an increase in mitochondrial Mn-SOD activity and increased the generation of superoxide by sub-mitochondrial particles (Hernandez et al., 1993). Increased activity of SOD would be a means of removing any excessive amount of superoxide anions generated. Salt stress, in addition to the known components of osmotic stress and ion toxicity, is manifested as an oxidative stress, and all of these contribute to its deleterious effect (Gossotian et al., 1999). However, ion content and salt tolerance are not often correlated, and several studies indicate that acquisition of salt tolerance may also be a consequence of improving resistance to oxidative stress (Hernandez et al., 1993, 1995, 1999; Gosset et al., 1996; Gulati 1989 Gomez et al., 1998).

Several isoforms of superoxide dismutase (SOD) with a high isoelectric point have been identified by isoelectric focusing chromatography in protein extracts from *Pinus silvestris* (Shu et al., 1999). Antioxidant enzymes function to eliminate reactive oxygen species (ROS) produced as a consequence of normal metabolic functions as well as environmental stress (Ruzsa et al., 1999). Superoxide dismutases (SOD) are metalloproteins that catalyse the dismutation of superoxide radicals to oxygen and hydrogen peroxide. This enzyme plays a major role in the defence against toxic reduced oxygen species that are generated in many biological oxidations (Bowler et al., 1989). MO19 showed an increase in SOD activity since it is more resistant to salt.

5.4. Anatomy

5.4.1. Shoot (Transmission Electron Microscopic Study)

The chloroplasts and mitochondria of plant cells are important intracellular generators of activated oxygen species. Therefore, these organelles seem to be damaged by oxidative reactions. NaCl salinity markedly damaged the chloroplasts and mitochondria of leaf cells in rice plants (Rahman et al., 2000).

In chloroplasts, O$_2^-$ and O$_2^-$ derived H$_2$O are mainly produced by the electron acceptor of PS I (Tabaka et al., 1987; Salin, 1991). There are reports that RuBP carboxylase may be inactivated
by oxidative damage rather than, or in addition, may direct salt stress. When the CO₂ concentration inside the chloroplasts decreases as a result of stomata closure due to salt stress, availability of NADP⁺ to accept electrons from PS I is lowered and thus O₂ reduction occurs concomitant with the generation of activated oxygen species (Halliwell, 1982), which could damage both Rubisco (and other enzymes) and cellular membranes. Hernandez et al., (1995) reported that salt induces oxidative stressing chloroplasts of pea plants and suggests that salinity induces an over production of O₂⁻ and O₂⁻ derived H₂O concentration. In the present study the disturbed chloroplast is due to the effect of salt stress.

Opik (1965, 1966) and described similar changes in mitochondria by salt stress. There are reports that leaf discs of dwarf French bean (Phaseolus vulgaris) seedlings developed larger number of mitochondria per cell when floated on solutions of 200mM NaCl than when floated on water (Siew and Klein, 1968). The decrease in electron density within the matrix of the mitochondria seems to reflect a degeneration of the mitochondria and therefore a decrease in mitochondrial activity (Borkird et al., 1991). The damage to mitochondria in NaCl treated plants could be a direct effect of sodium and chloride ions in the tissue. It could however, also be a response of water stress imposed by the NaCl present in the growth medium (Opik 1966). In the present investigation, the chloroplast of MO19 showed little expansion of thylakoids and damage of grana under the highest stress, so MO19 is tolerant than MO18.

5.4.2. Root

Palta (1990); Mansfield et al., (1988), reported that stress induced certain anatomical adaptations like development of aerenchyma in the root cortex. This increase in air spaces ensures their survival under stress condition. More air spaces are located at the root- shoot junction or root tip, as reported by Flowers and Yeo (1981) and suggested that more aerenchyma per root- shoot junction and less near the root tip were found more tolerant than the sensitive.

According to Yeo, (1991) vacuolation could be due to high accumulation of Na⁺ and or Cl⁻ differences in ion compartmentalization Cell expansion in both roots and leaves can be inhibited by salinity as reported by Katterman (1990); Cooper et al., (1987). They explain that the cell expansion depend on an inwardly directed water potential gradient which generate turgor pressure and cell water uptake – i.e. volume of cell increases. This also
correlate with hydraulic conductivity of water uptake path way, uptake of solutes to maintain 
osmotic potentials and the yielding of surrounding cell wall as suggested by Borkid et al., 

The enlargement of cells and appearance of vacuoles within the cells is a sign of disturbance 
to the cells under stress conditions. This view point was supported by the work of early 
researchers. Here, in this work also more expansion and appearance of vacuoles were 
observed in MO18 than MO19. So it is proved beyond doubt that MO19 is a salt tolerant 
variety than MO18.