Pak. 1. Agri. set. Vo!.' 39(1), 2002

# ACCUMULATION AND ROLE OF SOME ORGANIC AND INORGANIC OSMOTICA UNDER SALINITY IN PEARL MILLET

Intshar-ul-Haq Javed, Abdul Wahid, M, Irfan Anwar &Ejaz Rasul
Department of Botany, University of Agriculture, Faisalabad

Accumulation of various osmotica plays a crucial role in the maintenance of cell water balance under stressfull conditions, This study was initiated to find out the role of some osmotica in various parts of differentially salt tolerant pearl millet lines at various growth stages, The inorganic osmotica included soluble nitrate (SN) and soluble phosphate (SP), while organic ones were soluble sugars (SS) and free proline (FP), There was a greater accumulation of SS and SP in the young leaf and root of Togo and OB-5, while Ghana white behaved otherwise. On the other hand, a reverse trend of accumulation was evident for FP and SN. The role of SP and SN as osmotica was further confirmed from the ratio of SPI total P and SNI total N in various parts, SPI total P was greater for young leaf and root of Togo followed by OB-5, while that of SN/total N was greater for old leaf of these lines and young leaf of Ghana White, The establishment of various correlations between Na + and Cr content and inorganic and organic osmotica revealed that SS and SP are the real contributors to the maintenance of water balance of the . cell, while the accumulation of SN and FP appear to be the effect of ion toxicity,

Key words: growth stages, osmotica, pearl millet, salinity, soluble phosphate, soluble sugars

## INTRODUCTION

It has been documented that salinity tolerance is a cost effective phenomenon, which implies that a considerable amount of metabolic energy , in the form of certain metabolites is spent on the maintenance of growth and physiological processes (Yeo, 1983), Salinity tolerant plants d b hesi . I' h' . d o so. y synt esizmg or accum~ atl~g t e morgamc an organic compounds called osmotica m the soluble phase (Marcum, 1999). These compounds are non-toxic and play at' crucial role m the hydration of cytoplasm and reduce the toxicrty of excessive Ions m this compartment of the cell (W n Jones , 1984 ,or G ham et a I 1985) ,

The osmonca may be morganic , or organic m nature, Lauchli (1986) reported that accumulation ofcertam Ions may be the resu lt of specific elect o excess o Ions present m t e growth, medium. In other pl~n,ts such Ions may be synthesized ~o 'protect the sensttive cytopla~m from t~e excess of tOXIC Ions. In general, the accumulation/ synthesis o so.me osmot~ca me u mg ,ee pro me, nitrate, p os~ ate ~~87Is contentious (Lauchli, 1986; Moftah and Mlchel, ). I hi: d . h b d I'd h n t IS, stu y an att~mp as een ma e to e UCI ate t e synthesis, accumulation and role of soluble sugar, free proline, soluble nitrate and soluble phosphate as osmonca under mcrease d sa I" mrty con d' mon m diff I rerenna . II y sa I t tolerant pearl millet lines at three growth stages using a novel approach,

## MATERIALS AND METHODS

Plant Matena I: Three pear I ml'11et (*Penntse tum amertcanum* L) lines selected for this study were screened under increased level of NaCI salinity at various growth stages. and declared highly tolerant (Togo), moderately responsive (OB-5) and highly sensitive (Ghana White), based on $EC_{50}$ values (Javed et al., 200 I), For the elucidation of the role of soluble sugars (SS), free proline (FP), soluble nitrate (SN) and soluble phosphate (SP) as osmotica, these lines were grown under no salinity (control), 10 and 15 dS m' level of NaCI salinity, The accumulation of above mentioned osmotica was studied at seedling, tillering and 'grain filling stages in young and old leaves, and roots. The salt app~ication treatm~nt and rest of the growth details were similar to ~s d~scnbed elsewher~ by Javed et al. (200 I). For the determination of concentrations of SS, FP, SN and SP the young (p~nultimate leaf), old (second green le~f from the btaSe) and primary roots were sampled at respective growth sages,

Extraction and Determination of Osmotica: Fresh leaf and root samples (2 g each) were extracted in 50 ml of wa er m a blender (Hit ac hi mo dIVA 8906) for 5 mmu tes. This vo I ume was re d uce d t o 5 I' m usmg ro t a t ory vacuum evapora t or a t 60°C A fr ti (2 I) f th I d , ac Ion m o e samp e was use for the esnmanon of each of the above mentioned osrnotica. The SS were es t'Ima t e d usmg th e an th rone reagen t me th o d (Y os hid a et t., I 1976) w hil lee th me th o d o f B a t es t ea, I (1973) was followed for the determma tion of FP . The SN was determined using the protocol of Kowalenko and Lowe (1973) and SP was estimated using Bartens reagent method (Y os hid a et t., I 1976) T tIP ,o a was d e t ermme d f rorn th . e nitric acid: perchloric acid (3: I ratio) digested samples using Barterrs reagent while total N was determine? by micro-Kjeldahl method. The two way analysis of variance was used to find out significant differences among lines, treatments, p an parts and the ell' m teraction, usmg MSTAT -C computer software. Certain correlations were also drawn b t e ween diff I t eren varia bl es usmo th e same pac k age.

## RESULTS

Accumulation of Osmotica

Soluble Sugars (SS): Accumulation ot SS indicated slgnlfican~ (,P<?,O I~ differences among the pe~rl millet I~nes under salinity. In different plant ~arts d:ter,mmed at varIOUS ~tages ,of growth,. alon~ With SIgnificant (P<O.O I) mteracnons except mteraction ?f gr?wth stages X. plant parts (Table I). The tolerant line displayed the highest accumulation of soluble sugars in younger leaf under all salinity levels and at various growth stages (Fig. I), Togo and OB-5 indicated similar trend of SS accumulation at different growth stages, while in Ghana White, the older leaf accumulated substantially greater SS, even more than young

leaf and the root. BO-5 displayed a trend similar to Toga, but a significant varietal difference was evident for this parameter.

Table I. Statistics of some osmotic a in different parts of pearl millet lines at different growth stages under increased salinity

| S.O.V | df | Soluble sugars | Free proline | Soluble PO/ | Soluble $NO_3$ | NO)'/ Total N | PO/'/ Total P |
|---|---|---|---|---|---|---|---|
| Analysis of variance (F-values) | | | | | | | |
| Harvests (H) | 2 | 18.78** | 3.57** | 7.43** | 136.06** | 200.46** | 62.55** |
| Lines (C) | 2 | 84.21** | 362.00** | 72.93** | 79.34** | 586.71** | 82.29** |
| Salinity (S) | 2 | 117.63** | 29.43** | 440.86** | 572.37** | 3.28** | 54.58** |
| Parts (D) | 2 | 601.72** | 1294.66** | 1916.26** | 9800.47** | 4010.73** | 5077.52** |
| HxC | 4 | 22.74** | 4.30** | 10.18** | 63.42** | 77.06** | 37.42** |
| HxS | 4 | 14.31** | 2.35ns | 16.17** | 14.13** | 19.95** | 18.37** |
| CxS | 4 | 8.19** | 20.60** | 70.17** | 92.95** | 88.99** | 25.94** |
| HxCxS | 8 | 1.35** | 1,21ns | 5.61** | 18.14** | 18.78** | 9.19** |
| HxD | 4 | 0.75ns | 4.89** | 12.26** | 66.68** | 207.68** | 85.96** |
| CxD | 4 | 225.28** | 109.09** | 29.21** | 260.41** | 737.75** | 232.87** |
| IlxCxD | 8 | 8.63** | 4.28** | 17.69** | 65.83** | 48.36** | 04.14** |
| SxD | 4 | 71.58** | 18.97** | 12.75** | 129.32** | 31.95** | 16.49** |
| I-IxSxD | 8 | 7.22** | 0.80ns | 4.60** | 51.33** | 41.82** | 28.84** |
| CxSxD | 8 | 13.92** | 20.11** | 17.23** | 58.83** | 43.39** | 53.26** |
| I-IxCxSxD | 16 | 3.53** | 1,94** | 4.70** | 20.80** | 10.46** | 16.68** |

Sgnificant at *, $P<0.05$; **, $P<0.01$, ns, non-significant ($P>0.05$).

Free Proline (FP): Significant ($P<0.01$) differences were evident for the content of FP in different parts ana stages of plant growth in response to increasing root zone salinity (Table I). Individual interactions for most of the factors and overall interaction of these factors was also significant ($P<0.01$). The highest accumulation of FP was manifested by old leaf of Togo and OB-5 under all levels of salinity at all growth stages (Fig. I). Ghana White (sensitive line) indicated an increased accumulation of FP in younger leaves and roots under salinity.

Soluble Phosphate (SP): Data revealed significant ($P<0.01$) differences for this parameter in various parts of pearl millet lines at different growth stages under increased salinity levels with significant ($P<0.01$) interactions for various factors (Table I). Tolerant and medium responsive lines exhibited greater accumulation of SP in all parts and at various stages of growth under increased salinity, Toga and DB-5 indicated a trend of salt induced accumulation of SP in greater amount particularly in the young leaf and root at seedling and tillering stages, but indicated no specific trend at grain filling stage (Fig. I).

Soluble Nitrate (SN): All the pearl millet lines indicated significant ($P<0.01$) differences for the accumulation of SN at three stages of growth with significant ($P<0.01$) interactions for these factors (Table I). Soluble nitrate accumulated in varying amount up to 10 dS $m^{-1}$ in different parts of the Toga and OB-5. but a decreasing trend of this osmoticum was noted at 15 dS m'l in young leaf and root. On the contrary. SN accumulation was greater in the young leaf and root of Ghana White (Fig. I).

## DISCUSSION

Accumulation of inorganic and organic osmotica plays a key role in osmotic adjustment under saline or drought conditions (Paleg and Aspinall, 1981; Munns, 1988). Among non-toxic cytosolutes, low molecular weight sugars are important (Wyn Jones, 1984), while the role of free proline is unsettled (Moftah and Michel, 1987; Wahid et al.,1998). Studies were extended to investigate the accumulation of certain ions in young and old leaves and root. Estimation of different organic and inorganic osmotica (Fig. I) revealed a variable pattern of accumulation in three differentially salt responding lines of pearl millet. Among them SS and SP revealed an enhanced accumulation in the young leaf of the tolerant line (Togo) followed by the medium responsive (OB-5) at all growth stages. However, the level of accumulation of SS and SP was substantially lower in parts of the sensitive line (Ghana White). On the other hand, the accumulation of SN and FP was the lowest in young leaf and root of the tolerant line Togo, while the older leaf indicated greater accumulation of both of them. The accumulation of SS was observed specifically in the tolerant lines under increased salinity, which confirms their osmoregulatory role as reported for many other crops (Sacher and Staplese, 1985; Munns,1988; Wahid et at, 1998). The content of FP was the greatest in the old leaf and the lowest in the young leaf and the root. This revealed that, as far as pearl millet is concerned, the production of FP is due to ion toxicity to the tissue as has been reported for soybean (Moftah and Michel, 1987) and maize (Wahid et al., 1998).

Lauchli (1986) reported the accumulation of phosphate and nitrate due to specific effects of ions. In this study, both these ions were investigated and their osmoregulatory role was determined in terms of their content in the soluble phase versus total tissue content (i.e. *SN/* total N and SP/ total P) (Table 2). The data suggested that there was higher nitrogen content in the older leaf of the tolerant line and younger and older parts of Ghana White (data for total N and total P not presented). whereas SP/total P was greater in the young leaf and root of tolerant (logo) and moderately responsive DB-5) lines. These findings revealed that SP acts as an osmoticurn and plays its characteristic role in the salinity tolerance of millet,

Table 2. Soluble $PO_4^{3-}$/total P (SPIP) and Soluble NOy/totalN (SN/N) ratio (XIO$^{-3}$) in three parts of three millet

| Lines | Growth stages | Salt levels (ds m") | Parts (SNIN ratio) | | | Parts (SP/P ratio) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Young leaf | Old leaf | Root | Youna leaf | Old leaf | Root |
| Togo | Seedling | Control | 23.09 | 24.87 | 7,33 | 12.15 | 8.15 | 3.87 |
| | | 10 | 25.01 | 24.21 | 5.63 | 12.73 | 9.26 | 3.57 |
| | | 15 | 2344 | 28.36 | 7.7 | 14.51 | 9.6 | 2.9 |
| | Tillering | Control | 20.91 | 32.91 | 9.23 | 13,37 | 12,53 | 3.58 |
| | | 10 | 28.06 | 33.85 | 5,33 | 12.68 | 14.48 | 3.7 |
| | | '15 | 22.32 | 39.25 | 7.58 | 9.22 | 16.68 | 4.62 |
| | Grain filling | Control | 19.14 | 55.95 | 13,28 | 11,79 | 11,82 | 3.92 |
| | | 10 | 24.06 | 48.95 | 6.76 | 10.84 | 10.96 | 5.13 |
| | | 15 | 24.27 | 39.61 | 7.15 | 12,54 | 12,42 | 3.56 |
| DB-5 | Seedling | Control | 1904 | 21.29 | 10,21 | 11,47 | 10.71 | 5.06 |
| | | 10 | 18.67 | 21.98 | 7.1 | 8.99 | 8.77 | 4.64 |
| | | 15 | 25.74 | 25.18 | 9.89 | 12,56 | 10.51 | 4.7 |
| | Tillering | Control | 24.52 | 20.42 | 6.74 | 11,47 | 11.25 | 2,41 |
| | | 10 | 23.66 | 30.29 | 7.03 | 9.12 | 10.44 | 3.51 |
| | | 15 | 25.35 | 40.33 | 11,01 | 10.93 | 12.68 | 4.51 |
| | Grain filling | Control | 17.57 | 22.94 | 6.84 | 13.84 | 9.05 | 4.11 |
| | | 10 | 22.22 | 35.94 | 4.75 | 12.63 | 6.78 | 3.98 |
| | | 15 | 22.09 | 28.38 | 9.18 | 12.64 | 9.37 | 5,39 |
| Ghana White | Seedling | Control | 25.86 | 11,59 | 11.43 | 8.25 | 9.16 | 4.24 |
| | | 10 | 17,44 | 11,45 | 10.12 | 9.73 | 7.79 | 4.95 |
| | | 15 | 14.11 | 11,79 | 12.3 | 11,18 | 7.76 | 7.15 |
| | Tillering | Control | 30.23 | 16.78 | 11,69 | 9,37 | 9,38 | 4.84 |
| | | 10 | 22.95 | 19,96 | 12.01 | 9.94 | 9.1 | 6.86 |
| | | 15 | 16,4 | 17.04 | 11,74 | 10.72 | 7.02 | 8.8 |
| | Grain filling | Control | 19.79 | 18.7 | 14.08 | 10.12 | 13,37 | 6.1 |
| | | 10 | 17.02 | 16,53 | 9.45 | 12.56 | 9.9 | 4,34 |
| | | 15 | 14.85 | 13.89 | 15.02 | 8.99 | 9.76 | 6,43 |

Table 3. Correlation coefficient (r) of Na+ and Cr with some osmotica of pearl millet lines under control and 15 dS m"

| Lines | Growth Stages | Elements | Correlation coefficient | | | |
|---|---|---|---|---|---|---|
| | | | Na+ | Cl' | Na+ | er |
| | | | Control | 15 dS m"l | Control | 15 dS m"l |
| Togo | Seedling | FP | +0. 897ns | +0.983ns | +0.817ns | +0.997* |
| | Tillering | SP | +0.908ns | +0.999* | +0.848ns | +0.948ns |
| | | SNIN | +0.818ns | +0.997* | +0.818ns | +0.999* |
| | | SP/P | +0.343ns | +0.983ns | +0.34 Ins | +0.999* |
| | Grain filling | TSS | +0.603ns | +0.999* | +0.833ns | +0.988ns |
| | | SN | +0.998* | +0.97Ins | +0:946ns | +0.999* |
| DB-5 | Seedling | FP | +0.656ns | +1.000** | +0.655ns | +0.984ns |
| | | SN | +0.938ns | +0.984ns | +0.937ns | + 1,000** |
| | Tillering | TSS | +0.998** | +0.056ns | +0.886ns | -0.284ns |
| | | FP | +0.961ns | +0.885ns | +0.997* | +0.676ns |
| | | SN | +0.994* | +0.972ns | +0.953ns | +0.995ns |
| | | SP | +0.999** | +0.948ns | +0.90Ons | +1.000** |
| | | SNIN | +0.828ns | +0.953ns | +0.542ns | +0.999* |
| | | SP/P | +0.928ns | +0.999* | +0.669ns | +0.933ns |
| | Grain filling | TSS | +0.997* | +0.997* | +O.90lns | + 1,000** |
| | | SP | +0.997* | +0.998* | +0.949ns | + 1,000** |
| | | SP/P | -0. 728ns | +0.997* | +0.359ns | +0.571 ns |
| Ghana White | Seedling | FP | +0.976ns | +0.914ns | +0.822ns | +0.988ns |
| | | SP | +0.932ns | +0.951ns | +0. 726ns | +0.999* |
| | Tillering | SN | +0.11 Ins | +0.942ns | +0.997* | +0.925ns |
| | | SP | +0.283ns | +0.718ns | +0.970ns | +0.999* |
| | Grain tilling | SN | -0.278ns | +0.983ns | +0.659ns | +0.998* |
| | | SP | -O.027ns | +0.997* | +0.827ns | +0.98Ins |
| | | SP/P | -0.233ns | +0.997* | +0. 693ns | +0.975ns |

Significant at *.P<005: **, P<0.01:, ns. non-significant(P>0.05). The 'r' values have been presented for only those means which are significantly correlated at any level of salinity.

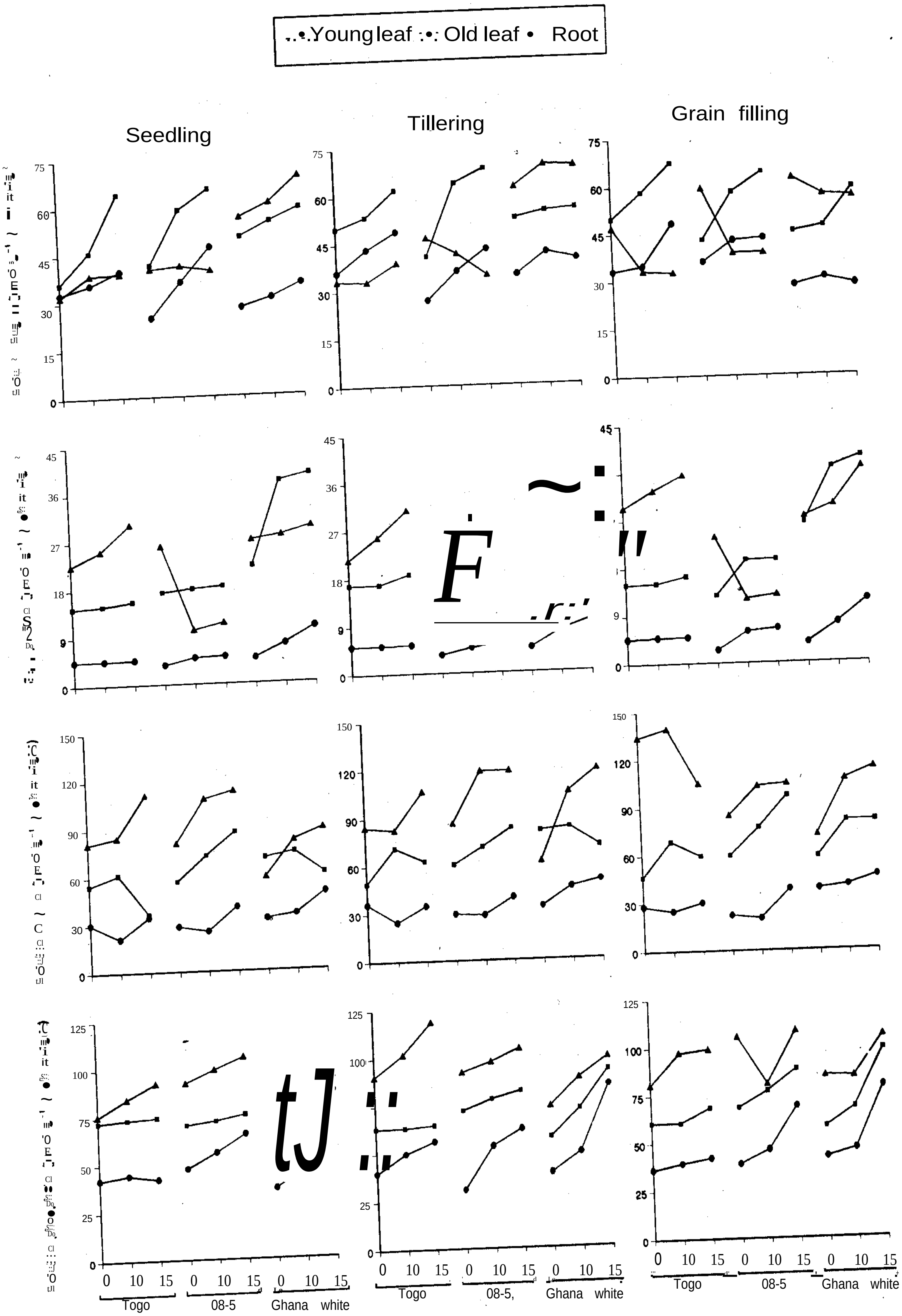


Fig.1: Soluble sugar, free proline, soluble nitrate and soluble phosphate content in young and old leaf and root of differentially salt tolerant pearl millet lines under ... ot arowth.

To substantiate the role of various osmotica, some correlations were worked out between Na+, Cl' and various osmotica, as well as SN/total Nand SP/total P (Table 3). These findings revealed that as far as tolerant and medium responsive lines are concerned, SS and SP had a major role as osmotica. On the contrary, FP and SN had a little or no role as osmotica in the salinity tolerance potential of pearl millet.. A relationship of total N content with the SN and FP appeared to be negative (Table 3). This led to the conclusion that a tolerant line can effectively utilize nitrogen even at the highest level of salinity which is a basic requirement for the growth and dry matter production (Deitz and Harris, 1997). However, enhanced content of these nitrogenous compounds in the older leaves point to the fact that older leaf being physiologically less active and containing greater amount of toxic ions (Na and Cl) caused its accumulation in the soluble phase. The other reason may be that the N-compounds already present in these parts were hydrolysed by catabolic actions of Na+ and Cr and caused an accumulation of SN in these leaves. To conclude, a greater accumulation of SS and SP in the young leaf and root of tolerant and moderately responsive lines showed their osmoregulatory role under NaCI stress. On the contrary, accumulation of SN and FP is due to Na+ and Cr toxicity.

## REFERENCES

Bates, L. S., R .P. Waldren and I. D. Teare. 1973. Rapid determination of free proline for water stress studies. Plant Soil, 39:205-207.

Dietz, K.J. and G. e.. Harris. 1997. Photosynthesis under nutrient deficiency. In Handbook of Photosynthesis (Ed. M. Pessarakli). Marcel Dekker Inc. New York. 951-975.

Gorham, J., R. G. Wyn Jones and E. McDonnel1.. 1985. Some mechanisms of salt tolerance in crop plants. Plant Soil, 89: 15-40.

Javed, l-ul-H, A. Wahid and E. Rasul. 200 I. Selection of pearl millet lines for tolerance to increased salinity. JAPS. 11:18-23.

Kowalenko, e.G. and L .E. Lowe. 1973. Determination of nitrates in soil extracts. Soil. Sci. Soc. Amer. Proc. 37:660.

Lauchli, A. 1986. Responses and adaptation of crop to salinity. Acta Hort. 190:243-246.

Marcurn, K. B. 1999. Salinity tolerance mechanisms of grasses in subfamily Chloridoideae. Crop Sci. 39: 1153-1160.

Moftah, A. E. and B. E. Miche1.I987. The effect of sodium chloride on solute potential and proline accumulation in soybean leaves. Plant Physiol.. 83:238-240.

Munns, R. 1988. Why measure osmotic adjustment? Aust. J. Plant Physiol.. 15:717-726.

Paleg, L.G. and D. Aspinall.. 1981. The Physiology and Biochemistry of Drought Resistance in Plants. Acad. Press, London.

Sacher, R.F. and R.e.. Staplese. 1985. Inositol and sugars in adaptation of tomato to salt. Plant Physiol.. 77:206-210.

Wahid, A., I-ul-H. Javed, I. Ali, A. Baig and E. Rasul. 1998. Short term incubation of sorghum caryopses in sodium chloride levels: changes in some pre- and post-germination physiological parameters. Plant Sci. 139:223-232.

Wyn Jones, R.G. 1984. Phytochemical aspects of osmotic adaptation. In Recent Advances in Phytochemistry. Vol. 3. (Eds. B.N. Timmerman, C. Steelink and FA Loewus). Plenum Press, New York. 55-78.

Yeo, A.R. and T. J. Flower. 1983. Varietal differences in the toxicity of sodium ions in rice leaves. Physiol.. Plant. 59: 189-195.

Yoshida, S., D.A.Forno, J.H. Cock and KA Gomez. 1976. Laboratory Manual for Physiological Studies of Rice. IRRI, Philippines.