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BRIEF COMMUNICATION

Short-term salinity induced changes in two wheat cultivars at different growth stages

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Abstract

Soluble sugars, proline, total chlorophyll contents and electrolyte leakage were measured in two wheat (*Triticum aestivum* L.) cultivars KRL 1-4 and HD 2009 at different growth stages [crown root initiation (CRI), flowering, and soft dough] under short term salinity (NaCl, $CaCl_2$ and Na_2SO_4). In control plants sugar contents were maximum at flowering stage. Proline and sugar concentrations increased in both cultivars under salinity with a maximum increase at CRI. Electrolyte leakage increased and chlorophyll content decreased with the plant age. A sharp increase of electrolyte leakage was noticed at salinity of 10 and 15 dS m^{-1} in HD 2009 and KRL 1-4, respectively. The short-term salinity at CRI stage proved more detrimental as compared to salinity at flowering and soft dough stages in term of all biochemical changes induced. In wheat, plant resistance to salinity increased with the age of plant. The cultivar KRL 1-4 performed better under salinity as compared to HD 2009.

Additional key words: chlorophyll, crown root initiation, electrolyte leakage, proline, soluble sugars, *Triticum aestivum* L.

Most of the crops respond to salts as typical glycophytes and show marked differences in their ability to grow under saline conditions at species as well as at cultivar levels (Maas and Hoffman 1977). Alteration of various physiological and biochemical processes of plant under salinity is well documented. Sugar metabolism in plants was found to be affected by salinity, generally sugar content increase (*e.g.*, in roots and shoots of barley, Polonenko *et al.* 1983). Proline is the most studied compound under salinity stress, content of proline usually increase under salinity (Madan *et al.*). The reduction in chlorophyll content under salinity has been reported (*e.g.*, in *Sesbania*, Chavan and Karadge 1980). Membranes are important in regulating uptake and transport of ions and differences in response to salt stress exist at plasma membrane level between the salt tolerant and salt sensitive cultivars (Bliss *et al.* 1984). Long term salinity effects are very different to those of short term and in wheat, more alteration was found at various growth stages either by long term salinity application or constant salinity overlapping two or more stages (Gill and Dutt 1987, Maas and Poss 1989). However, the variation in biochemical changes under short term salinity at different stages and their relevance with salt tolerance needs critical assessment. The aim of this experiment was to determine the pattern of changes in sugar, proline, and chlorophyll contents, and electrolyte leakage at different growth stages of two wheat cultivars (differing in their relative salt tolerance) subjected to five levels of salinity.

The present investigations were carried out in the greenhouse at Chaudhary Charan Singh Haryana Agricultural University, Hisar (India). The two cultivars of wheat (*Triticum aestivum* L.) KRL 1-4 and HD 2009 were grown in earthen pots. The Central Soil Salinity Research Institute, Karnal (India), supplied seeds of the two wheat cultivars. Each pot was filled with 5.0 kg of dry and sieved yellow dune sand (ECe 1.9 dS m^{-1}). Four plants of uniform size were maintained in each pot. Five salinity levels S0, S1, S2, S3, S4 of electrical conductivity of 0, 5, 10, 15, 20 dS m^{-1} were prepared by using a mixture of salts in Hoagland's solution. The salts used for preparing saline solutions were NaCl, $CaCl_2$ and Na_2SO_4 in a composition that the ratio of sodium to calcium (Na:Ca) and chloride to sulphate ($Cl:SO_4$) was 4:1.

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Abbreviations: CRI - crown root initiation, EC - electrical conductivity; EL - electrolyte leakage.
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The five levels of salinity were applied at three different growth stages: *1*) at crown root initiation (CRI) stage, this stage included the period of tiller initiation, *2*) the flowering stage, *3*) the soft dough stage. All plants were irrigated with non-saline nutrient solution before and after the saline treatment at all the stages. The biochemical estimations were made in third leaf from top after 4 d of salinity treatment. Total soluble sugars were extracted according to Barnett and Naylor (1966) and estimated colorimetrically according to the phenol-sulfuric acid method (Dubois *et al.* 1956). Proline was extracted in 3 % sulphosalicylic acid and was estimated colorimeterically according to the method of Bates *et al.* (1973). To determine the electrolyte leakage, leaf discs (5 mm in diameter) were placed in the test tubes containing 10 cm^3 distilled deionized water. The tubes were covered with plastic caps and placed in water bath maintained at the constant temperature of 45 °C, an initial electrical conductivity EC_a, just before transfer to the high temperature was recorded. After 30 min the electrical conductivity were again measured (EC_b). The sample were autoclaved at 100 °C for 20 min to kill the tissues and to release all electrolytes. Samples were then cooled to 25 °C and final electrical conductivity (EC_c) was recorded. The per cent electrolyte leakage (EL) was expressed following the formula:

$$EL = [(EC_b - EC_a) / EC_c] \times 100$$

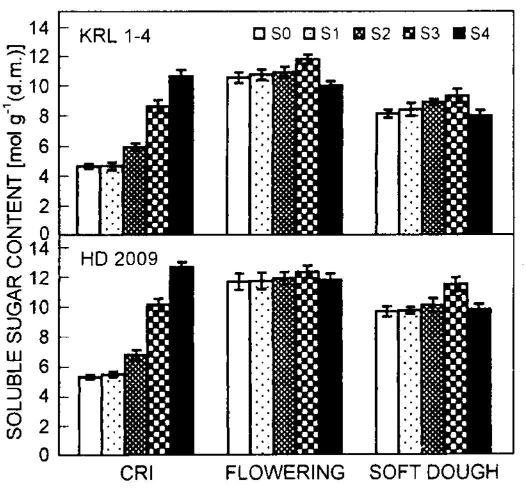

Fig. 1. Effect of salinity (0, 5, 10, 15, 20 dS m^{-1}) on soluble sugar content in leaves of two wheat cultivars at different growth stages. Means of 4 plants ± SE (*vertical bars*).

Total chlorophyll contents were estimated according to Welburn (1994). A completely randomized design with four replicates per treatment was used. Data were subjected to analysis of variance for testing significant differences between means.

At different growth stages the concentrations of total soluble sugars varied and unstressed plants was having maximum concentrations at flowering stage followed by soft dough and CRI (Fig. 1). Both cultivars KRL 1-4 as well HD 2009 showed increased content of total soluble sugars after salinization. At CRI stage this increase was maximum (56 and 57 % of control plants in KRL 1-4 and HD 2009, respectively). During salt stress, increase in sugar concentrations was reported in many species (Avice *et al.* 1998, Dubey and Singh 1999). This might be due to inhibitory effects of salinity stress on the translocation of assimilates. At the reproductive stage as the demand increased at sink site, the sugar concentrations were recorded higher than at CRI in control plants. Declined soluble sugar contents at highest EC might be due to inhibition in photosynthesis. Weimberg (1987) reported increased content of sucrose with salinity (but to lesser extent in tolerant wheat cultivar), probably due to starch hydrolysis by enhanced activity of α-amylase under salinity.

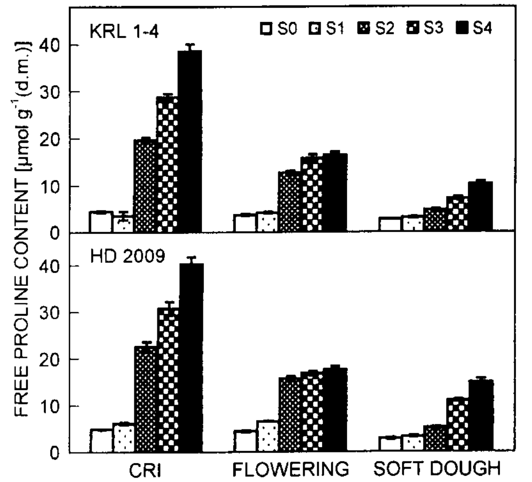

Fig. 2. Effect of salinity (0, 5, 10, 15, 20 dS m^{-1}) on proline content in leaves of two wheat cultivars at different growth stages. Means of 4 plants ± SE (*vertical bars*).

There was a conspicuous accumulation of proline under salinity. At lower level of salinity cultivar HD 2009 accumulated more proline, but the per cent increase over control plants was more in KRL 1-4 at CRI stage (Fig. 2). The maximum accumulation of proline was at CRI stage as compared to flowering and soft dough stages. The increased levels of proline, under salt stress have been reported, *e.g.*, in *Brassica* (Madan *et al.* 1994). It has been shown earlier that proline accumulation may be due to increased proteolysis or due to decreased protein synthesis. The higher concentrations of proline under stress are helpful to plant as proline participate to osmotic potential of leaf and thus to osmotic adjustment. Besides the role as osmolyte, proline can also confer enzyme protection and increase membrane stability under various condition.

The extent of membrane damage was assessed indirectly by conductometric measurements of solute leakage from cells. The increasing magnitude of salinity stress increased the amount of electrolyte leakage from the leaves of both cultivars, but the effect was more severe in HD 2009 than KRL 1-4. The leakage under salinity increased at three stages was in order CRI > flowering > soft dough. This is in support to the hypothesis that differences in response to salt stress exist at plasma membrane level between salt tolerant and salt sensitive cultivars (Bliss *et al.* 1984).

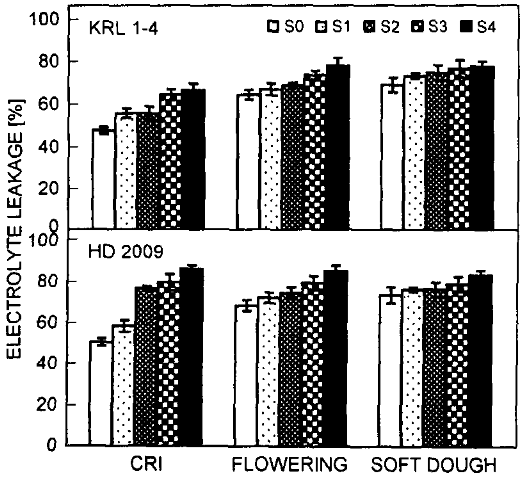

Fig. 3. Effect of salinity (0, 5, 10, 15, 20 dS m^{-1}) on electrolyte leakage from leaves of two wheat cultivars at different growth stages. Means of 4 plants ± SE (*vertical bars*).

The untreated plants at flowering stage have maximum content of chlorophyll as compared to other stages. Total chlorophyll content in both cultivars declined with increase in salinity as well as and with the age of plant (Fig. 4). Cultivar KRL 1-4 maintained higher concentration of chlorophyll at salinity as compared to cultivar HD 2009 at CRI. The reduction in chlorophyll might be due to enhancement of chlorophyllase activity at higher salinity levels or due to reduction in *de novo* chlorophyll synthesis (Sudhakar *et al.* 1991).

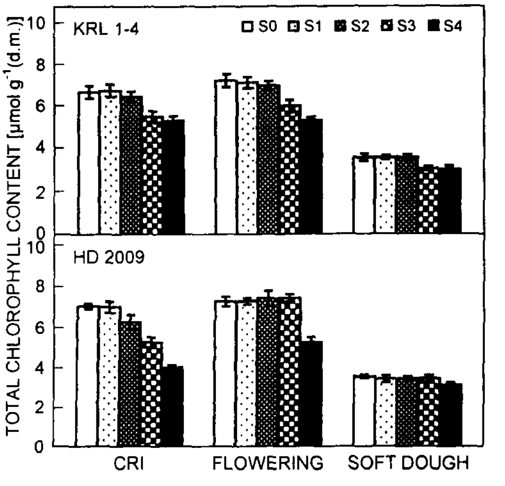

Fig. 4. Effect of salinity (0, 5, 10, 15, 20 dS m^{-1}) on chlorophyll *a+b* content in leaves of two wheat cultivars at different growth stages. Means of 4 plants ± SE (*vertical bars*).

The result of this study shows that both cultivars were found to be most sensitive to salinity during CRI stage and least at soft dough stage. It indicates that in wheat, plant resistance to salinity increase with the age. During salt stress the salt sensitive cultivar HD 2009 accumulated more proline at moderate salinity levels, had higher electrolyte leakage and more declined chlorophyll content than salt tolerant KRL 1-4. The pattern of change in total soluble sugars at higher salinity levels in both cultivars was comparable at all the three growth stages.

References

Avice, A.M., Pradier, E., Houchi, R.: Osmotic adjustment in triticales grown in presence of NaCl. - Biol. Plant. **41**: 227-234, 1998.

Barnett, N.M., Naylor, A.W.: Amino acid and protein metabolism in Bermuda grass during water stress. - Plant Physiol. **41**: 1222-1230, 1966.

Bates, L.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline for water stress studies. - Plant Soil **39**: 205-08, 1973.

Bliss, R.D., Platt-Aloia, K.A., Thompson, W.W.: Changes in plasmalemma organization in cowpea radicle during imbibition in water and NaCl solutions. - Plant Cell Environ. **7**: 606-606, 1984.

Chavan, P.D., Karadge, B.A.: Influence of salinity on mineral nutrition of peanut (*Arachis hypogea* L.). - Plant Soil **54**: 5-13, 1980.

Dubey, R.S., Singh, A.K.: Salinity induced accumulation of soluble sugars alters the acitivity of sugar metabolizing enzymes in rice plants. - Biol. Plant. **42**: 233-239, 1999.

Dubois, M., Gilles, K.A., Hamilton, J.K., Roberts, P.A., Smith, F.: A colorimetric method for determination of sugars and related substances. - Anal. Chem. **28**: 350-356, 1956.

Gill, K.S., Dutt, S.K.: Physiological aspects of salt tolerance in barley and wheat grown in pots in coastal saline conditions. - Indian J. agr. Sci. **57**: 409-415, 1987.

Maas, E.V., Hoffman, G.J.: Crop salt tolerance - current assessment. - J. Irrig. Drain Div. ASCE **103**: 115-134, 1977.

Maas, E.V., Poss, J.A.: Salt sensitivity of wheat at various growth stages. - Irrig. Sci. **10**: 29-40, 1989.

Madan, S., Nainawatee, H.S., Jain, S., Jain, R.K., Malik, M.S., Chowdhury, J.B.: Leaf position dependent changes in proline, pyroline 5-carboxylate reductase activity, water relations under salt stress in genetically stable salt tolerant somaclones of *Brassica juncea* L. - Plant Soil **163**: 151-156, 1994.

Polonenko, D.R., Dumbroff, E.B., Mayfield, C.I.: Microbial responses to salt induced osmotic stress. III. Effect of stress on metabolites in the roots, shoots and rhizosphere of barley. - Plant Soil **73**: 211-225, 1983.

Sudhakar, C., Reddy, P.S., Veeranjaneyulu, K.: Changes in respiration, its allied enzymes, pigment composition, chlorophyllase and Hill reaction activity of horsegram seedlings under salt stress. - Indian J. Plant Physiol. **34**: 171-177, 1991.

Weimberg, R.: Solute adjustment in leaves of two species of wheat at two different stages of growth in response to salinity. - Physiol. Plant. **70**: 381-388, 1987.

Welburn, R.W.: The spectral determination of chlorophyll *a* and *b* as well as total carotenoids using various solvents with spectrophotometer of different resolution. - J. Plant Physiol. **144**: 307-313, 1994.