BIOLOGIA PLANTARUM 42 (1): 113-117, 1999

BRIEF COMMUNICATION

Changes in soluble proteins in spring wheat stressed with sodium chloride

M. ASHRAF* and J.W. O'LEARY**

*Institute of Pure and Applied Biology, B.Z. University, Multan, Pakistan**
*Department of Plant Sciences, College of Agriculture, University of Arizona, Tucson, AZ 85721, USA***

Abstract

Two newly developed salt-tolerant genotypes of spring wheat, S24 and S36 and their salt-tolerant parents, LU26S (from Pakistan) and Kharchia (from India) along with a salt-sensitive cv. Potohar were grown in full strength Hoagland's nutrient solution with 0 or 125 mM NaCl. At the onset of the booting stage third leaf from top was sampled for protein analysis. Total soluble protein content increased due to salt treatment in all cultivars/lines but this increase was more marked in salt-sensitive cv. Potohar and low in salt-tolerant S24 as compared with the other lines. Patterns of labelled polypeptides in all cultivars/lines were identical; the differences were only quantitative (for instance, 29 kD and 48 kD polypeptides were reduced significantly due to NaCl treatment only in the cv. Potohar).

Additional key words: electrophoresis, polypeptides, SDS-polyacrylamide gel, salt stress, salt tolerance.

In a previous study on evaluation of some newly-developed salt-tolerant lines of spring wheat (Ashraf and O'Leary 1996) it was found that S24 was more salt-tolerant than its salt-tolerant parents cv. Kharchia (from India) and cv. LU26S (from Pakistan), whereas the other newly-developed genotype, S36 was as good as its parents. In the same study, cv. Potohar (from Pakistan) was found to be relatively salt-sensitive.

Received 4 December 1997, *accepted* 10 August 1998.
Acknowledgement: The senior author gratefully acknowledges the substantial support through Fulbright Research Award (1993-94).
*Address for correspondence: 51-C Sheikh Colony, ABC Road, Faisalabad, Pakistan; fax: (+041) 653380

In wheat, it is generally accepted that salt tolerance is related to its low uptake of Na and/or Cl (Qureshi *et al.* 1980, Wyn Jones *et al.* 1984, Wyn Jones and Gorham 1986, Ashraf and O'Leary 1996). The effects of salinity on accumulation/synthesis of different organic compounds are also well evident (El-Shourbagy and Koshk 1975, Downton 1977, Gorham *et al.* 1981, Cusido *et al.* 1987, Ashraf *et al.* 1993). During characterization of salt-induced proteins in tobacco a 26 kD protein named as osmotin was found (Singh *et al.* 1987). In barley accumulation of two 26 kD polypeptides related to germin in response to salt stress was observed (Hurkman *et al.* 1991). Conversely, a 22 kD protein was found in radish (*Raphanus sativus*) in response to salt stress or water deficit (Lopez *et al.* 1994). Other salt-induced proteins have also been characterized in rice (Claes *et al.* 1990), and tomato (Torres-Schumann *et al.* 1992). With this in mind the aim of the present study was to examine whether accumulation of soluble proteins is perturbed due to salt stress and up to what extent in some wheat lines differing in salt tolerance. Examination of some closely related wheat lines differing in salt tolerance provides a sound basis to determine if any of the polypeptides whose levels increase or decrease with salt are related genetically to salt tolerance.

Seed of the spring wheat (*Triticum aestivum* L.) salt-tolerant cultivars Kharchia and LU26S, and a salt-sensitive cv. Potohar was obtained from the University of Agriculture Faisalabad, Pakistan. Two salt-tolerant genotypes S24 and S36 were selected from the F_3 seed material derived from a cross LU26S × Kharchia (Ashraf and O'Leary 1996). All seeds were surface sterilized in 5 % sodium hypochlorite solution for 8 min. In October 1993, three hundred seeds of each line were sown about 5 mm deep in thoroughly washed sand in plastic containers (32 × 32 × 7 cm) with drainage holes in the bottom. The sand was irrigated on alternate days with Hoagland's nutrient solution prepared in tap water (ion content in tap water was 0.036 mM K^+, 1.08 mM Na^+, 0.78 mM Ca^{2+}, and 0.08 mM Mg^{2+}). The experiment was conducted in a naturally-lit glasshouse in which PAR measured at noon ranged from 450 to 1350 μmol m^{-2} s^{-1}, day/night relative humidity 60 - 80 % and temperature 21 to 2 °C. After 5 d seven seedlings were transplanted into a plastic pot (21.5 cm diameter and 21.0 cm deep) which contained 6.35 kg of well-washed dry sand. All the pots were irrigated for 14 d with full strength Hoagland's nutrient solution. Salt treatments (0 or 125 mM NaCl in full strength Hoagland's nutrient solution; salinity was increased stepwise in aliquots of 50 mM NaCl daily) were begun 19 d after the start of the experiment. The experiment was arranged in a completely randomized design with 4 replicates. Every day, 200 cm^3 of distilled water was added to each pot to compensate evapotranspiration loss.

Just before the onset of the booting stage third leaf from the top was sampled from each plant for the analysis of soluble proteins. Soluble proteins were determined by the modified Bradford method (Macart and Gerbaut 1982). Separation of proteins from the extracted samples was carried out using one dimensional sodium dodecyl sulphate - polyacrylamide gel (SDS-PAGE). After loading the samples the gel was run for 45 min at 200 V and 25 mA.

The cultivars/lines of spring wheat differing in degree of salt tolerance differed significantly in leaf soluble proteins (Table 1). Addition of NaCl in the rooting

medium caused a considerable increase in leaf soluble proteins in all cultivars/lines tested in respect of control. The highest increase in protein concentration was observed in salt-sensitive cv. Potohar and lowest in salt-tolerant S24; the remaining lines being intermediate in protein accumulation. The increase in soluble protein content has already been observed in different plant species in response to NaCl, *e.g.*, in stargrass (Langdale *et al.* 1973, Helal *et al.* 1975), and tobacco (Cusido *et al.* 1987).

Table 1. Total soluble proteins [mg g^{-1}(d.m.)] of leaves of salt-tolerant and salt-sensitive cultivars/lines of spring wheat exposed to 0 or 125 mM NaCl. Means ± SE, $n = 12$. Means with the same letters in each column do not differ significantly at $P = 0.05$.

Cultivars/lines	Control	125 mM NaCl
Kharchia	30.2 ± 4.31a	50.1 ± 4.45a
LU26S	36.6 ± 3.85b	56.9 + 6.94b
S24	31.1 ± 2.94a	39.2 ± 3.11c
S36	38.2 ± 2.71b	44.4 ± 7.89d
Potohar	29.2 ± 1.98a	49.9 ± 4.32a

The high molecular mass (> 52 kD) soluble proteins increased markedly in the leaves of all cultivars/lines in response to salt stress and this increase might resulted in overall increase in total soluble proteins. The similar pattern of increase in high molecular mass proteins has also been observed in wheat in response to cold (Sarhan and Perras 1987). The most prominent protein in all five cultivars was that of 52 kD. This protein was increased considerably in salt-sensitive cv. Potohar (Fig. 1) and to a lesser extent in salt-tolerant S24. But this protein remained almost unchanged in the remaining three salt-tolerant cultivars. The 29 kD and 48 kD proteins were reduced in NaCl-treated cv. Potohar (Fig. 1). But differences in these proteins were not found in all four salt-tolerant lines. The patterns of labelled polypeptides in all cultivars/lines were identical. The only difference in salt-tolerant and salt-sensitive lines was that in the quantity of polypeptides of different mass. These results are quite similar to those of Hurkman *et al.* (1989) showing that salt treatment causes quantitative rather than qualitative changes in polypeptides in barley. However, these quantitative changes in polypeptides may be responsible for adjustment in metabolic pathways in response to salinity (Sarhan and Perras 1987).

Although the possible mechanisms of salt tolerance are not completely uncovered, the increase or decrease in certain polypeptides in response to salt stress could be important in the adaptation of plants to saline substrate. For instance, in tobacco an increase in a 26 kD polypeptide due to salt has been reported and suggested its possible role in osmoregulation (Singh *et al.* 1985, 1987). Similarly polypeptides with molecular mass 26 and 27 kD and pI of 6.3 and 6.5, which are not immunologically related to osmotin, increased in NaCl-treated barley (Hurkman and Tanaka 1987, 1988, Hurkman *et al.* 1988), and a 22 kD polypeptide in *Raphanus*

sativus (Lopez *et al.* 1994). Conversely, in the present study the decrease in the levels of two polypeptides (29 and 48 kD) have been related to salt sensitivity in spring wheat.

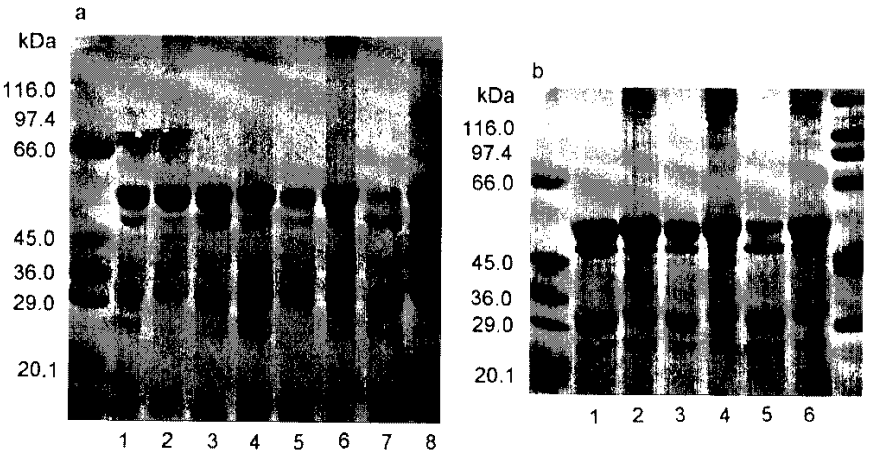

Fig. 1. Electrophoretic banding pattern obtained on polyacrylamide gels of soluble proteins extracted from the leaves of some salt-tolerant and salt sensitive cultivars/lines of spring wheat (Kharchia - bands a1, a2; LU26S - a3, a4; S24 - a5, a6, b3, b4; S36 - b1, b2; Potohar - a7, a8, b5, b6) exposed to 0 (bands a1, a3, a5, a7 and b1, b3, b5) or 125 mM NaCl (bands a2, a4, a6, a8, and b2, b4, b6).

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