INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2002/04–4–462–464
http://www.ijab.org

In Vitro Salt Tolerance in Wheat. II. Organic Solute Accumulation in Callus

FARRUKH JAVED
Department of Botany, University of Agriculture, Faisalabad–38040, Pakistan

ABSTRACT

Two selected genotypes of wheat (*Triticum aestivum* L.) differing in salt tolerance were used in this study. Calli were initiated on Linsmaier and Skoog (LS) basic salt medium supplemented with 5 mg 2,4-D alone. One month old calli were subjected to different concentrations of NaCl in LS-liquid medium. Dry weights of both the calli increased with increase in NaCl concentration in the culture medium. Callus tissues of both genotypes exhibited an increase in total soluble proteins, total free amino acids and total soluble carbohydrates contents as the NaCl concentration increased in the culture medium. The increase was more in LU-265 (salt tolerant) than Potohar (salt sensitive). It is concluded that more the salt tolerant genotype more the accumulation of organic solutes in the callus tissue.

Key Words: Tissue culture; Wheat; NaCl; Salinity; Salt tolerance; Organic solutes

INTRODUCTION

Salinity is a major factor limiting the crop productivity in arid and semi-arid areas of the world. In the last years, *in vitro* selection has seemed to be the methodological solution to cope with this problem, since assessment of salt tolerance by this method requires relatively little space and time, as well as controlled environment (Cano *et al.,* 1996, 1998).

Plants under saline environment accumulated organic solutes such as sugars, amino acids, proteins and/or other compounds against the deleterious effects of Na^+ and Cl^- (Yancey *et al.,* 1982). This process called osmotic adjustment. Accumulation of these organic solutes in response to stress is a metabolic adaptation found in a number of stress-tolerant plants (Yancey *et al.,* 1982; Rhodes & Hanson, 1993).

In vivo studies in wheat reveled that salt tolerance is related to its low uptake of Na^+ and Cl^- (Qureshi *et al.,* 1980; Wyn Jones & Gorham, 1986; Ashraf & O'Leary 1996). The effects of salinity on accumulation of different organic compounds as osmotica are also evident (Cusido *et al.,* 1987; Ashraf *et al.,* 1993; Ashraf & O'Leary, 1999). Hu and Schmidhalter (1998) reported that carbohydrates (sugars) contributed 13% osmotic adjustment in wheat.

The present report describes *in vitro* technique as an efficient method to study the effect of NaCl stress on organic solutes accumulation as osmotica in callus tissue of two wheat genotypes differing in salt tolerance.

MATERIALS AND METHODS

Callus establishment. A salt tolerant genotype LU-26S and salt sensitive Potohar at whole plant level (Ashraf & O'Leary, 1996) were obtained from the Gene Bank, Department of Botany, University of Agriculture, Faisalabad. Seedlings were raised of both the genotypes on agar solidified Linsmaier and Skoog (1965) medium (LS). Callus was initiated from first 3 mm of the leaf base of germinating seeds by culturing on supplemented LS-medium with 5.0 mg 2, 4-Dichlorophenoxy acetic acid (2,4-D) alone, subjected to pH 5.7 before autoclave. The culture were placed under continuous fluorescent light with photosynthetically active radiation (PAR) of 300 µmol m^{-2} s^{-1} at $25^0C \pm 2^0C$.

Salt treatments. Two g of one month old calli were placed in flasks containing 50 mL of liquid LS-medium with 20 g L^{-1} sucrose and 5.0 mg 2, 4-D. The medium was salinized with NaCl to make the final concentrations of 0, 100 and 200 mol m^{-3}. Each treatment per genotype was replicated thrice. The flasks were placed on a gyratory shaker for 15 d of incubation with the same growth conditions described above.

Callus dry weight. Calli were harvested after 2 weeks of incubation on a gyratory shaker and dry weight was determined after 1 week of incubation at 65^o C.

Solute measurement. Total soluble proteins were determined by using the method of Lowry *et al.* (1951). Fresh callus tissues (0.2 g) were chopped in 5ml phosphate buffer (pH 7.0). One ml of copper reagent was added in each test tube containing 1 mL of callus tissues extract. The contents in the test tube were thoroughly mixed and allowed to stand for 10 min at room temperature. Then 0.5 mL of Folin-phenol reagent (1:1 diluted) was added, mixed well and kept for 30 min at room temperature. The optical density (O.D) was read at 620 nm on a spectrophotometer (Hitachi- 2001).

Total free amino acids were determined according to the method of Moore and Stein (1954). One mL of the extract was taken in 20 mL test tube. Then 1 mL of ninhydrin reagent was added. The tubes were covered with an aluminium foil and heated for 20 min in boiling water bath. Then they were cooled in cold water and 5 mL of the

diluent was mixed and again incubated at room temperature for 15 min. The optical density was read at 570 nm on spectrophotometer (Hitachi 2001-Japan).

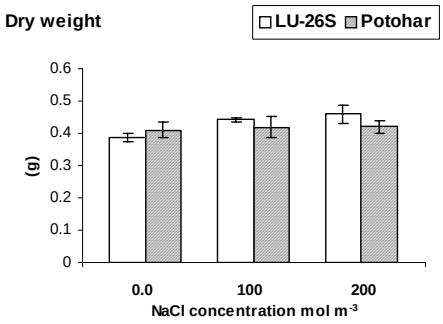
Total soluble sugars were determined according to the method of Yemm and Willis (1954) Calli material (0.1g) was extracted in 80% ethanol solution. Dried callus tissues were ground so as to pass through 1mm sieve of millimicro mill (Model Culatti, DFH-48) and it was shaken for 6 h at 60^0C. This extract was used for the estimation of total soluble sugars.

Statistical analysis. A two-way analysis of variance of data for all the parameters was computed, using the COSTAT computer package (Cohort software Berkeley, California). The least significant differences between means were calculated.

RESULTS AND DISCUSSION

Callus dry weight of both the genotypes increased with the increase in NaCl salt concentration of the culture medium (Fig. 1). The salt sensitive genotype Potohar was the lowest and showed almost the same effect on dry weight at both salt treatments. While, salt tolerant LU-26S was the highest in this attribute at both salt treatments as compared to control.

Fig.1. Dry weight of two wheat genotypes calli after treatment with different concentration of NaCl for 15 d. Standard error are shown.



Presence of NaCl salt in the culture medium significantly ($P<0.01$) increased the total free amino acids, total soluble carbohydrates and total soluble proteins in calli tissues of both the genotypes. Salt sensitive genotype Potohar was the lowest and salt tolerant genotype LU-26S the highest in these attributes as compared to controls (Fig.2-4).

Under osmotic stress, levels of total amino acids and carbohydrates were increased in sweet potato (Heng-Long *et al.*, 1999) and in soybean (EL-Sayed & Kirkwood, 1992) cultured cells. In the present studies, an increase in total amino acids, total soluble carbohydrates and in total soluble

Fig. 2. Callus total soluble proteins of two wheat genotypes after treatment with different conc. of NaCl for 15 d. SE are shown.

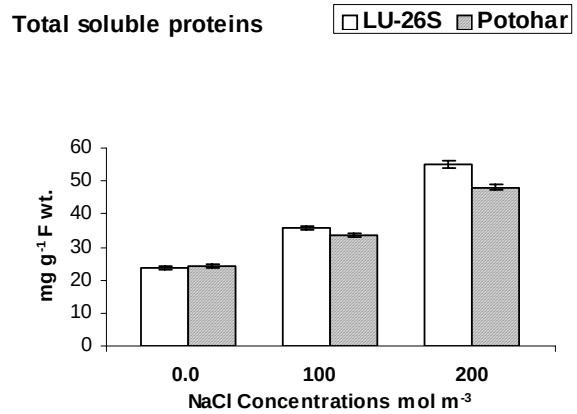


Fig. 3. Callus free amino acids of two wheat genotypes after treatmemt with different conc. of NaCl for 15 d. SE are shown.

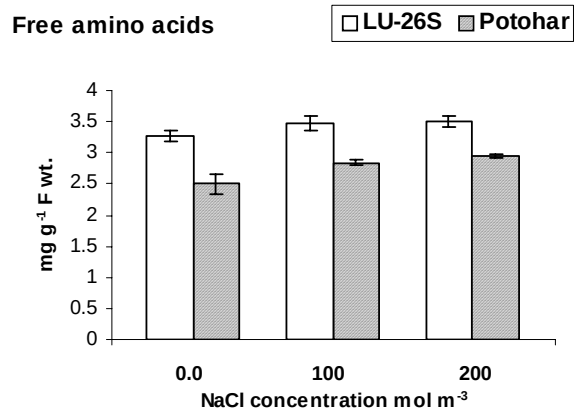
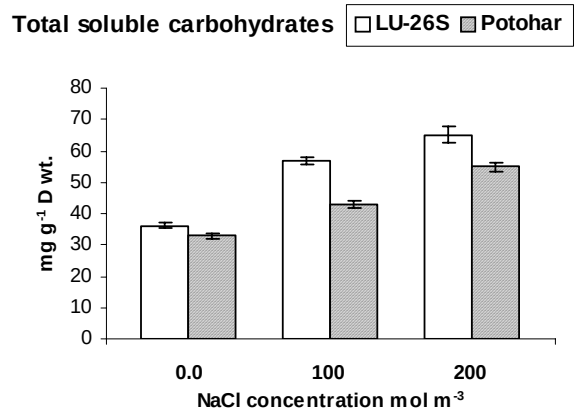


Fig. 4. Callus soluble carbohydrate of two wheat genotypes after treatment with different conc. of NaCl for 15 d. SE are shown.



proteins was observed in both the genotypes at both salt concentrations. The increase was more in salt tolerant LU-26S calli and low in salt sensitive genotype Potohar as compared to their controls. This indicated that LU-26S performed better against NaCl salt stress compared to salt-sensitive Potohar due to the more accumulation of these compounds as osmotica. These results are in accordance with the findings of Watad *et al.* (1983) and Pandey and Ganapathy (1985), in which that salt tolerant calli of *Nicotiana* and *Cicer* had more organic solutes than salt sensitive calli.

REFERENCES

Ashraf, M. and J. W. O'Leary, 1996. Responses of some newly evolved salt-tolerant genotypes of spring wheat to salt stress. I. Yield components and ion distribution. *J. Agron. Crop Sci.,* 176: 91–101.

Ashraf, M. and J. W. O'Leary, 1999. Changes in soluble proteins in spring wheat stressed with sodium chloride. *Biol. Plant.,* 42: 113–7.

Ashraf, M., M.I. Naqui and Z.U. Zafar, 1993. Physiological and biochemical aspects of tolerance of three grass species to varying Na/Ca ratios. *Biol. Plant.,* 35: 425–33.

Cano, E.A., F. Perez-Alfocea, V. Moreno and M.C. Bolarin, 1996. Responses to NaCl stress of cultivated and wild tomato species and their hybrids in callus cultures. *Plant Cell Rep.,* 15: 791–4.

Cano, E. A., F. Perez-Alfocea, V. Moreno, M. Caro and M.C. Bolarin, 1998. Evaluation of salt tolerance in cultivated and wild tomato species through in vitro shoot apex culture. *Plant, Cell Tissue Org. Cult.,* 53: 19–26.

Cusido, R.M., J. Palazon, T. Altabella and C. Morales, 1987. Effect of salinity on soluble protein, free amino acids and nicotine contents in *Nicotiana rustica* L. *Plant Soil,* 102: 55–60.

EL-Sayed, H. and R.C. Kirkwood, 1992. Solutes accumulation in soybean (*Glycine max* L.) cell adapted to NaCl salinity. *Phyton. (Horn),* 31: 233–49.

Heng-Long, W., L. Ping-Du, L. Li-Fei and S. Jong-Ching, 1999. Effect of sorbitol induced osmotic stress on the changes of carbohydrate and free amino acid pools in sweet potato cell suspension cultures. *Bot. Bull. Acad. Sin.,* 40: 219–25.

Hu, Y. and U. Schmidhalter, 1998. Spatial distributions of inorganic ions and sugars contributing to osmotic adjustment in the elongating wheat leaf under saline soil conditions. *Australian J. Plant Physiol.,* 25: 591–7.

Linsmaier, E. M. and F. Skoog, 1965. Organic growth factor requirements of tobacco tissue cultures. *Physiol. Plant.,* 8: 100–127.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randal, 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.,* 193: 265–75.

Moore, S. and W.H. Stein, 1954. *In:* Colowick, S. P. and N. O. Kaplan (ed.), *Methods in Enzymology*, Vol. III. Academic Press Inc. New York.

Pandey, R. and P.S. Ganapathy, 1985. The praline enigma: NaCl-tolerant and NaCl-sensitive callus lines of *Cicer arietinum. Plant Sci.,* 40: 13–7.

Qureshi, R.H., R. Ahmad, M. Ilyas and Z. Aslam, 1980. Screening of wheat (*Triticum aestivum* L.) for salt tolerance. *Pakistan J. Agri. Sci.,* 17: 19–26.

Rhodes, D. and A.D. Hanson, 1993. Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Ann. Rev. Plant Physiol. Plant Mol. Bio.,* 44: 357–84.

Watad, A.A., L. Reinhold and H.R. Lerner, 1983. Comparison between a stable NaCl selected *Nicotiana* cell line and the wild type. *Plant Physiol.,* 73: 624–9.

Wyn Jones, R.G. and J. Gorham, 1986. The potential for enhancing the salt tolerance of wheat and other important crops. *Outlook Agri.,* 15: 33–9.

Yancey, P.H., M.E. Clark, S.C. Hand, R.D. Bowlus and G.N. Somero, 1982. Living with water stress: Evolution of osmolytesystems. *Science,* 217: 1214–22.

Yemm, E.W. and A.J. Willis, 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.,* 57: 769–73.

(Received 15 August 2002; Accepted 13 September 2002)