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In Vitro Salt Tolerance in Wheat. I. Growth and Ions Accumulation

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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. The RGR (fresh) of both wheat calli decreased with increase in salt concentration of the culture medium. The reduction was more in Potohar (salt-sensitive) callus than that of LU-26S (salt-tolerant). Na^+ and Cl^- contents increased, while K^+ and K^+/Na^+ ratio decreased in calli of both the wheat genotypes at both salt concentrations. The accumulation of these ions was more in LU-26S calli than those of Potohar. Moreover, Cl^- content was higher than Na^+ and K^+ contents in both calli at all NaCl concentrations. In conclusion, the mechanism of salt tolerance at callus/cellular level was found to be associated with the better compartmentation of high amount of Na^+ and Cl^-. Since Na^+ exclusion mechanism which is marker of salt tolerance of whole plant was not expressed at cellular level.

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

INTRODUCTION

Salinity is a global problem that largely limits crop production in irrigated areas of the world. Many techniques have so far been adapted to alleviate this problem. Of these, one is the selection of salt tolerant genotypes. This technique has successfully been used by many workers for the last many years. They reported that changes in salinized plants growth appear to be associated with accumulation of toxic elements and/or osmotic adjustment and turgor maintenance against these elements (Munns *et al.*, 1983; Morgan, 1984; Shannan, 1998).

In the last decade, this conventional technique was supplemented with *in vitro* technique. Some workers reported that *in vitro* selection of plants cell lines that exposed to saline environment can be selected for enhancement of tolerance to salinity (Kirti *et al.*, 1991; Barakat & Abdel-Latif 1995, 1996). Moreover, studies at cellular level provide better knowledge to understand the mechanism of salt tolerance, since they require relatively little space and lower time for the selection, as well as controlled environment (Cano *et al.*, 1996, 1998).

This valuable technique is used for the assessment of NaCl tolerance in callus tissue of two wheat (*Triticum aestivum* L.) 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 the first 3 mm of the leaf base of germinating seedlings 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 was 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 (control), 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.

Relative growth rate. After 15 d of incubation on gyratory shaker, the calli were harvested and the relative growth rate (fresh) of callus was calculated as:

In (final fresh weight) - In (Initial fresh weight)

Where In is the natural log

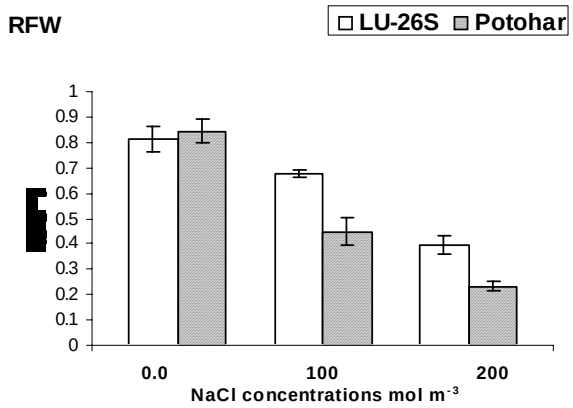
Ions measurement. Sodium and potassium were determined by flame photometric method (Model PFP-7, Jenway, UK). Fifty milligram oven dried (65^oC) samples per replicate per treatment per genotype were digested in 5 mL of nitric acid (100%). Chloride was measured with chloride meter (Model PCLM3, Jenway, UK). For chloride determination 100 mg oven dried samples per replicate per treatment per genotype were extracted in 10 mL of deionized water at 80^oC for 4 h.

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

Increased salt concentration in the culture medium significantly decreased callus growth in both genotypes. The RGR (fresh) of LU-26S callus was greater than that of Potohar at both NaCl concentrations (Fig. 1). Callus RGR was reduced as compared to their respective controls by 51.41 and 72.51% at higher salt concentration in LU-26S and Potohar, respectively.

Fig. 1. Callus relative fresh weight growth of two wheat genotypes after treatment with different NaCl concentrations for 15 d. SE are shown.



Both Na^+ and Cl^- in the callus tissues increased with increase in salt concentration of the culture medium (Fig. 2-3). Callus of LU-26S had a higher Na^+ and Cl^- concentration than that of Potohar at both salt treatments as compared to their respective controls. Chloride content was highest in LU-26S callus compared to Potohar callus. Moreover, Cl^- content was higher than that of any individual cation content

Fig. 2. Callus Na^+ concentration of two wheat genotypes after treatment with different NaCl conc. for 15 d. SE are shown

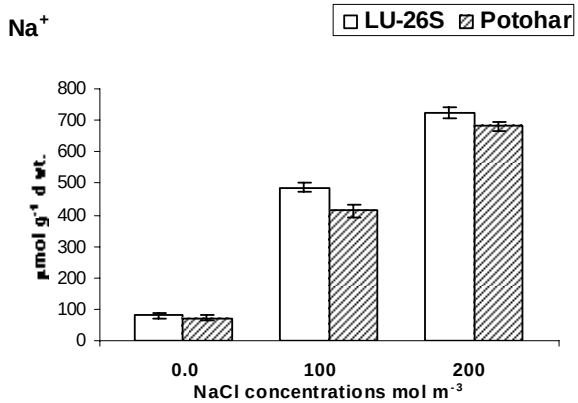
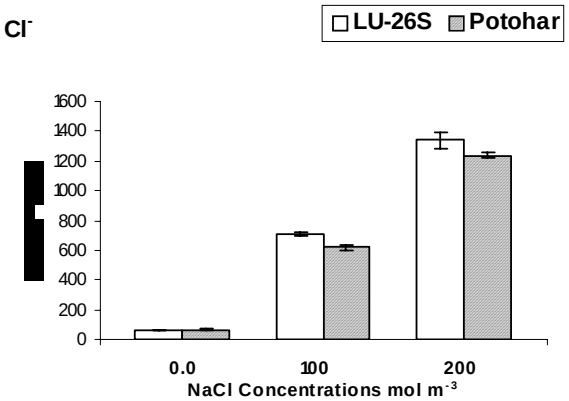


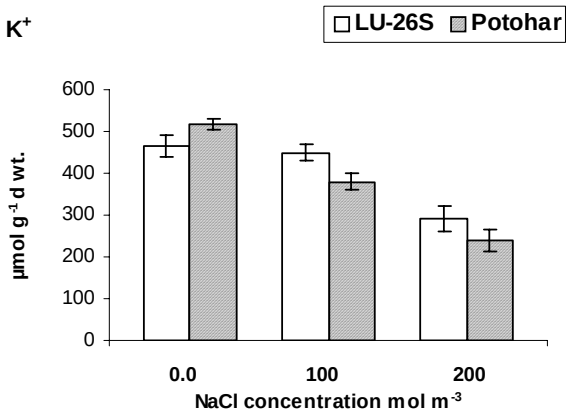
Fig. 3. Callus Cl^- concentration of two wheat genotypes after treatment with different NaCl conc. for 15 d. SE are shown



in both the genotypes calli at all the NaCl concentrations. K^+ content decreased in callus of both the genotypes compared to their respective control. The decrease was more in Potohar callus than that in LU-26S callus at both NaCl treatments (Fig. 4). Moreover, K^+/Na^+ ratio decreased in both wheat calli with increasing NaCl concentrations of the culture medium (Fig. 5). The K^+/Na^+ ratio was lower in Potohar callus than that of LU-26S at both NaCl concentrations.

Growth reductions and salt damage appear to be associated with ions toxicity (Heyser & Nabors, 1981), or disturbance of cellular and tissue water status (Weimberg *et al.*, 1982, 1984), or perhaps increased demand on ATP for osmotic adjustment. EL-Sayed and Kirkwood (1992) reported that salinity reduced callus fresh weight and

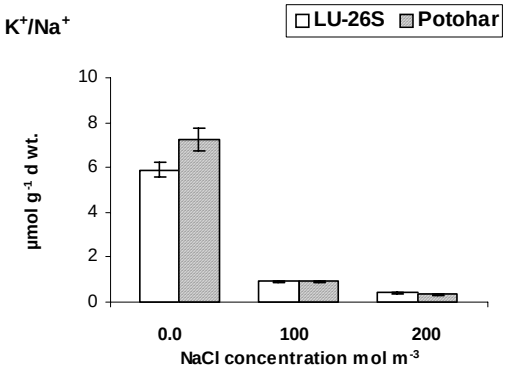
Fig. 4. Callus K^+ concentration of two wheat genotypes after treatment with different NaCl conc. for 15 d. SE are shown.



increased dry matter. In the present studies, the callus relative fresh weight of both the genotypes decreased with increasing salt concentration of the culture medium. The salt tolerant genotype LU-26S gained more callus relative growth rate (fresh) than that of salt-sensitive Potohar at both salt concentrations.

Callus tissue of salt tolerant LU-26S accumulated more Na^+ and Cl^- than that of salt sensitive genotype (Fig. 2-3). The data indicated that Na^+ exclusion operative in wheat whole plants (unpublished data) was not expressed in callus tissue. These results are in accordance with the earlier findings in sorghum (Yang *et al.*, 1990) and tomato (Cano *et al.*, 1996; 1998), in which Na^+ exclusion as a salt tolerant mechanism operated at whole plant level, was not expressed in the cell cultures.

Fig. 5. Callus K^+/Na^+ ratio of two wheat genotypes after treatment with different NaCl conc. for 15 d. SE are shown.



Accumulation of K^+ and K^+/Na^+ ratio in callus tissue, reduced in both the genotypes under varying salt concentration in culture medium. The reduction was lower in salt tolerant LU-26S than that in salt sensitive genotype Potohar (Fig. 4). These results indicated that less reduction in K^+ content and K^+/Na^+ ratio, in salt tolerant genotypes and greater in salt-sensitive may be due to greater accumulation of Cl^- in the calli (Fig. 3), since this anion accumulated more than individual cation (Weimberg *et al.*, 1982; Yang *et al.*, 1990; Barakat & Abdel-Latif, 1996).

In conclusion, the response of both the wheat genotypes examined in this study was different to salt stress. The mechanism of salt tolerance at callus/cellular level is found to be associated with the better compartmentation of high amount of Na^+ and Cl^-, since Na^+ exclusion mechanism which is marker of salt tolerance of whole plant was not expressed in the callus tissue.

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