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In Vitro Salt Tolerance in Wheat. III. Water Relations in Callus

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ABSTRACT

Two selected genotypes of wheat (*Triticum aestivum* L.) LU-26S (salt-tolerance) and Potohar (salt-sensitive) 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 (control, 100 and 200 mol m^{-3}) in LS-liquid medium. Relative water content of callus tissues of both genotypes increased as the salt concentration of the culture medium. Water and osmotic potentials of both the calli decreased and turgor potential increased as the NaCl concentration increased. LU-26S callus tissues exhibited more reduction in water and osmotic potentials and less in turgor as compared to salt-sensitive Potohar. It is concluded that more the salt tolerant genotype more is the reduction in water and osmotic potentials and less in turgor of the callus tissue.

Key Words: Tissue culture; Wheat; NaCl; Salt tolerance; Water relations

INTRODUCTION

In the last years, *in vitro* selection has seemed to be a promising solution to cope with the problem of soil salinity because assessment of salt tolerance by this method requires relatively little space and time, as well as controlled environment (Cano *et al.,* 1996, 1998).

In vivo studies revealed that osmoregulation (osmotic adjustment) is regarded as an important adaptation of plants to salinity, because it helps to maintain turgor and cell volume (Hsiao, 1973; Greenway & Munns, 1980; Morgan, 1984; Ashraf & Waheed, 1993). Osmotic stress due to lack of osmotic adjustment, resulting in inhabitation of water uptake and physiological drought has long been considered a major cause of salt injury to plants (Bernstein & Hayward, 1958; Levitt, 1980). The reduction in osmotic potential of plants subjected to salt stress may be to either water loss or an increase in dissolved solutes or a combination of both (Carvagel *et al.,* 1998).

The present report describes *in vitro* studies as an efficient method to study the effect of NaCl stress on callus tissue water relations of two wheat genotypes differed 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 ± 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.

Water relations. The water content of calli was calculated by the following formula:

(FW-DW)/DW.

The solute potential of both culture media and callus were measured with an osmometer (Wescor-5500). Water potential of callus was assumed to be equal to the solute potential of the culture medium. Callus osmotic potential was determined from frozen calli. The turgor potential was calculated as the difference between water potential (ψ_w) and osmotic potential (ψ_s) values.

$$\Psi_p = \psi_w - \psi_s$$

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

The water content of callus tissues of both the genotypes increased with increase in salt concentration in culture medium (Fig. 1). LU-26S calli tissues exhibited more WC than Potohar. Water and osmotic potentials ($\psi_{w,}$ ψ_s) of both the genotypes calli decreased significantly with increase in salt concentration of the culture medium (Fig.2-3). The salt sensitive genotype Potohar was the highest (less –ive) and salt tolerant genotype LU-26S the lowest as

Fig. 1. Relative water content of two wheat genotypes calli after treatment with different conc. of NaCl for 15 d. SE are shown.

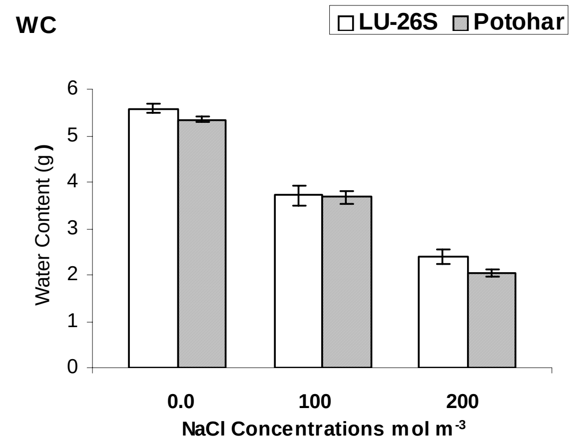


Fig. 2. Water potential of two wheat genotypes after treatment with different conc. of NaCl for 15 d. SE are shown.

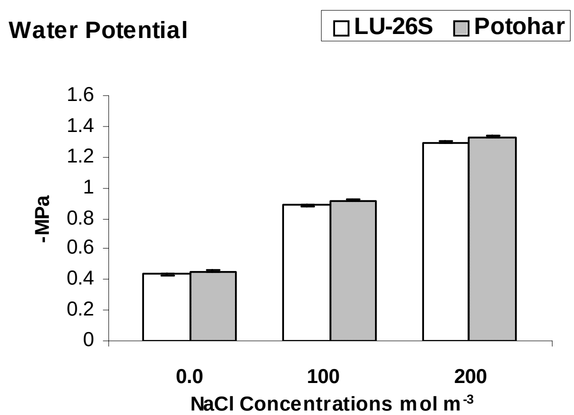
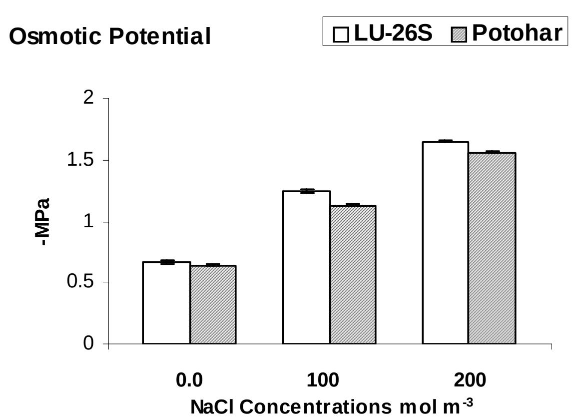


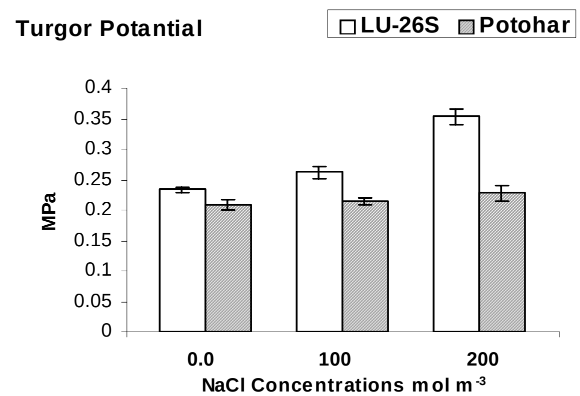
Fig. 3. Callus osmotic potential of two wheat genotypes after treatment with different conc. of NaCl for 15 d. SE are shown



concentration. In contrast, salt-tolerant LU-26S was the highest (more –ive) and salt sensitive Potohar the lowest (less –ive) in osmotic potential.

Callus turgor potential (ψ_p) of both the genotypes increased significantly with increase in salt concentration of the medium (Fig. 4). Salt-tolerant LU-26S was the highest and salt sensitive genotype Potohar the lowest in turgor potential at both salt treatments.

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



Callus water and osmotic potential increased with increasing salt concentration in the culture medium. Yang *et al.* (1990) reported that osmotic adjustment in callus results from both Na^+ and Cl^- accumulation. Similar results were reported for calli of both wheat genotypes (Javed, 2002) that salt-tolerant LU-26S accumulated more Na^+ and Cl^- than salt sensitive Potohar. Concerning the water content and turgor potential of calli, full turgor maintenance was observed with Na^+ and Cl^- concentrations. LU-26S calli maintained more turgor and exhibited more water content than salt sensitive Potohar. Yang *et al.* (1990) also reported that in sorghum callus, turgor maintenance was associated with Na^+ and Cl^-.

In conclusion, the responses of both the wheat genotypes examined in this study were different to salt stress. The mechanism of salt tolerant in studied wheat genotypes at callus/cellular level is found to be associated with high turgor potential and high amount of Na^+ and Cl^-

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