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Morphophysiological Plasticity in a Wheat Variety in Response to NaCl Stress and its Alleviation by Exogenous Abscisic Acid

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Abstract: Nowadays, soil salinity is the most unfavourable abiotic factors for plant growth, causing important yield loss of many crops. A partial solution to this situation is to establish crop varieties in these areas affected which are tolerant to stress. The aim of this study was to evaluate in a wheat variety, the morphophysiological plasticity to sodium chloride (NaCl) stress and the effect of exogenous Abscisic Acid (ABA) on physiological variables. This was carried out by using the BI3000 wheat variety, for regional adaptability experiments. The germination percentage, coleoptile and radicle growth and root anatomic were evaluated, both seedling irrigated with water or saline solution. On the other hand, ABA sprays were applied to wheat plants and their biomass, pigment, stomatal behaviour and cellular membrane injuries were determined after salt treatments. In this study, it was possible to determine that the BI3000 wheat variety can grow in high electrical conductivity, with good germination and seedling growth. This variety showed less radical anatomic variations under salinity, what allows a faster plasticity to adapt. ABA applications suggest a protective role in plants under salinity, due to an increase in chlorophyll and carotene content, stability of cell membranes and stomatal behavior. This study is a contribution to a better understanding of the morphophysiological responses of glycophytic plants to salt stress. This have been pointed out as a useful approach to show more tolerance to salt stress crops in the future and it suggests that ABA could help improve agriculture production in areas affected by this stress.

Key words: *Triticum aestivum* L., abscisic acid, salt stress, morphophysiological responses

INTRODUCTION

Nowadays, soil drought and salinity are the most unfavourable abiotic factors for plant growth, causing important yield loss of many crops (Munns, 2002). A partial solution to this situation is to establish crop varieties in these areas affected which are tolerant to stress. For this, it is necessary to previously evaluate the behaviour of these crops, including the greatest diversity of other possible crops (Nonhebel, 1993).

Wheat (*Triticum aestivum* L.) is able to grow and bear fruit in different environments and is a glycophyte species, as most cultivated plants. However, wheat has shown moderate tolerance towards salinity (Mass and Hoffmann, 1977); this behavior has made wheat a sustainable option to make a better use of the areas affected by salinity. The anatomic and morphologic characteristics of roots can also have great influence on their capacity to adapt to salinity (Reinhardt and Rost, 1995; Maggio *et al*., 2001). Certainly, it would be very beneficial to intensify wheat tolerance, since it is not only profitable for the grain it produces but also for the increase in the balance of soil carbon through stubble.

Plants possess mechanisms that enable them to perceive stress and regulate their metabolism and physiology. The vegetal hormone Abscisic Acid (ABA) plays a key role in physiological answers. ABA participation is widely documented in numerous responses to water deficit (Bray, 1997; Gomez *et al*., 1989; Seo and Koshiba, 2002; Zhu, 2002). Typically, the levels of ABA also increase in response to salinity in a similar way when stress does it in response to drought. ABA high levels are important for the rapid osmotic adjustment in both individual cells and intact plants. It has been informed that ABA treatment is beneficial before exposing the plants or tissues to adverse environmental conditions. ABA application improved salt and osmotic stress (Singh *et al*., 1987; Nayyar and Kaushal, 2002; Nayyar and Walia, 2003; Perales *et al*., 2005). It can be expected that ABA exogenous applications have a similar effect on

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endogenous increase and relieve salt and water stress. Although both types of stress induce the plant dehydration and show closely connected responses and mechanism superposition, other responses can be different. Thus, ABA application can enhance some mechanisms important for the plant rapid adaptation to any type of stress.

With the aim of obtaining better productivity in salt stress, a better understanding of the morpho-physiological basis could be used to select new resistant crop varieties. Therefore, the purpose of this study was to evaluate in a wheat variety, selected from 24 varieties for regional adaptability experiments, the morphophysiological plasticity to salt stress and the effect of exogenous ABA applied at different phenological stages on physiological variables until post-flowering. The results obtained in this study may provide interesting information to increase wheat yields and other cultivars under stress.

MATERIALS AND METHODS

Experiments were conducted in the plant growth cabinets with wide ranges of temperature, humidity and lighting control, during crop season in the experimental field of the Universidad Nacional de Río Cuarto campus, Río Cuarto, Córdoba, Argentina (33°07′ S, 64°14′ W).

This experiment was carried out by using the long growth cycle BI3000 wheat variety, which was chosen out of 24 varieties after preliminary experiments (Travaglia *et al*., 2009b) and sent by the Argentine Farming Cooperative (ACA) for regional adaptability experiments.

Experiment I: Characterization of BI3000 wheat seedlings variety in salinity.

Twenty-five seeds per each Petri dish were taken at random and incubated at 27°C with distilled water (control) and sodium chloride (NaCl) saline solution, at a concentration of 65 mM (value that exceeds the threshold electric conductivity for barley and wheat during germination, Richards *et al*. (1987). Three repetitions were carried out in each treatment. After 48 h, the germination percentage was evaluated and after 96 h the coleoptile and radicle growth was observed according to the ISTA Plant Evaluation Manual (ISTA, 2003).

Adventitious root samples, both seedling irrigated with water or saline solution, were taken for their anatomic analysis. Freehand sections were cut from fresh material and treated with 1% watery safranine and set in glycerine water (v:v). The histological preparations were assessed with a standard Ziess 16 model microscope and photomicrographs were taken with a Zeiss Axiophot microscope, with AxioVision 4.3 screen shot and image digitalization equipment.

Experiment II: Effect of exogenous ABA applied in wheat plants under salinity.

Wheat seeds were sown in 24 L pots filled with a mixture (1:1) of vermiculite and soil during crop season in the experimental field. After one week, wheat plants were thinned out to 2 in each pot. After 10 days of post-sowing, plants were subjected of salt treatment 200 mM sodium chloride (NaCl) or water at field capacity (without stress as a control treatment). Salt treatments were maintained for 60 days, 25 mM NaCl was added over 3 days to final concentration of 200 mM (concentration that inhibits the growth in nonhalophytes plants (Greenway and Munns, 1980). Once a week conductivity was recorded in pots drainage water by using a digital conductivity meter to estimate evaporation and the water consumption of the plants. Conductivity was conserved in about 16-18 dS m^{-1}.

Plants were treated with water foliar sprays or a 300 mg L^{-1} solution of ABA (Lomon Biotech, Beijing, China, 90% purity) at the beginning of shoot enlargement and repeated at anthesis. Both solutions included 0.1% ethanol (a minimum amount to dissolve the ABA) and 0.1% of Triton X and spraying was done at dawn to prevent ABA photo destruction. The dose of ABA was chosen after preliminary experiments (Travaglia *et al*., 2007) and according to the experience with other species (Sansberro *et al*., 2004). The plants were located at random with 6 replicas per treatment, with a perimeter border to offer equal conditions and were subjected to a natural photoperiod. The climatic behavior during the experiment was similar to the historical series, showing low and high temperatures, an average of around 1.9 and 34.9°C, respectively and total rains of 131 mm. After 10 days of post-flowering (75 days post-sowing), different samplings were taken to determine the following variables (n = 15 plants per treatment).

Aerial part and root biomass was determined on a Dry Weight (DW) basis by placing sample aliquots for 7 days at 65°C in a fan-ventilated oven. For pigment measurement, 50 mg fresh weight of flag leaf was homogenized in a mortar with 10 mL of 80% acetone. The homogenate was loaded in Eppendorf tubes and after 1 h at 4°C to allow pigment extraction in darkness conditions; it was centrifuged (twice) for 5 min at 5000 rpm. Aliquots were taken and chlorophyll a and b levels were measured by spectrophotometry at 650 and 665 nm, respectively. Five millimeters of 1 M NaOH and 15 mL of diethyl ether were added to the total volume. Carotene content was assessed from the ethereal fraction by spectrophotometry at 450 nm (modified from Mackinney, 1938). Stomatal conductance, transpiration rate (mmol m^2 sec^{-1}) and leaf temperature (°C) were measured on flag leaf at 01:00 PM by using a portable porometer LI-COR model LI-1600. In order to determine the cellular membrane injury, leaf pieces were quickly washed three times and then immersed in 10 mL of deionized water for 24 h at 10°C. The electrical conductivity was measured and then the leaf tissues were killed by autoclaving for 15 min, cooled to 25°C and the electrical conductivity was measured for the second time. Membrane injury was evaluated as the percentage injury index following the Sullivan´s formula (Sullivan, 1971):

$$J = \frac{1-(1-T_1/T_2)}{(1-C_1/C_2)} \times 100\%$$

where, C_1 and C_2 represent conductivity measurements of control samples before and after autoclaving respectively and T_1 and T_2 represent conductivity measurements of water-stressed samples before and after autoclaving, respectively.

Statistical analysis: The results were analyzed for variance using the InfoStat statistical analysis software (professional version 1.1) and the LSD Fisher a 5% test was used to compare differences among treatments.

RESULTS AND DISCUSSION

Experiment I: Even though the approach used to define plant tolerance to salinity is different from what different authors have said (Kingsbury and Epstein, 1984; Shalaby *et al*., 1993; Mass and Hoffmann, 1977; Richards *et al*., 1987), in general, seedling germination, emergency and growth are the most critical crop stages since seeds and seedling are exposed to higher salt concentrations than in other growth stages (Bernstein and Fireman 1957; Mass and Hoffmann, 1977). In this study, the BI3000 wheat variety could be selected as a variety capable of growing in high electrical conductivity soils as it has 94 and 92% of germination percentage under both water and salinity conditions, respectively. This result confirmed previous studies that determined that this wheat variety showed a good germination percentage compared with other wheat varieties (ACA304, ACA523B and Malevo, ACA901, BI1004 below 90 and 80%, respectively, among others, Travaglia *et al*. (2009b).

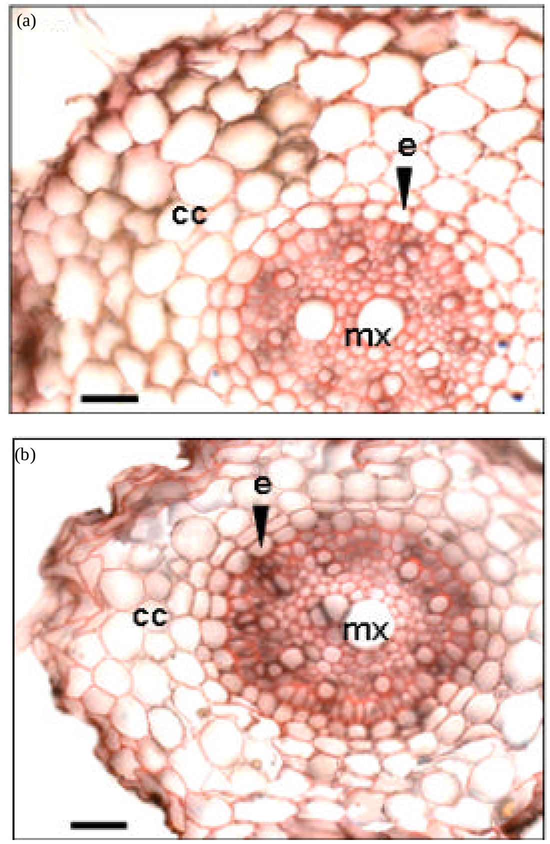

Fig. 1(a-b): Cross sections of wheat plant roots grown under (a) Control conditions and (b) Salinity conditions due to NaCl, The cross-sections were obtained at 20 mm from the adventitious root tip. mx: Methaxylem, e: Endodermis, cc: Cortical cells, scale bar = 50 µm

Table 1: Radicle and coleoptile length 96 h post-germination and dry weight of roots and aerial part of wheat plants, at 10 days after flowering, Treatments: control and salinity due to NaCl

Parameters	Control	NaCl
Radicle length (cm)	3.46[b]	0.80[a]
Coleoptile length (cm)	1.97[b]	0.98[a]
Root biomass (g)	1.02[b]	0.59[a]
Shoot biomass (g)	3.03[a]	3.16[a]

Data represent the means from 15 samples and the different letters mean significant differences from control at p<0.05 for the fisher test

Root long and biomass in salt stress conditions were lower compared to control plants (Table 1). Also in the anatomic study, the BI3000 variety under salinity decreased 23% its radical diameter (Fig. 1b) compared to its control sample (Fig. 1a). This decrease was mainly due to plasmolysis of sub-epidermal parenchyma cells of the cortex. The vascular tissues, pericycles and endodermis of these roots in salinity showed similar development to that observed in control roots. Therefore, both roots showed xylematic tissue with thick lignified wall elements and an endodermis with important developed walls, especially the inner periclinal and radial walls (Fig. 1a, b). This wheat variety would allow these plants to have greater possibilities of efficiently filtering soil solution to prevent the passage of ions excess to the xylem. This similarity between treated and control roots can be assumed as an adaptive characteristic per se, what allows this variety a faster plasticity to adapt to salinity. In addition, without change of xylem area, no resistance is offered to the flow of water or no more energy is needed to transport any amount of water from the roots to the leaves (Munns and Termaat, 1986), unlike that observed in sensitive wheat varieties by salinity stress (Akram *et al*., 2002). Their diameter decrease was one important anatomical change observed in the roots from the BI3000 plants under salinity conditions. This decrease was mainly due to plasmolysis of sub-epidermal parenchyma cells of the cortex. This fact agrees with other results in wheat under salinity in that there was no significant change in the number of cortical cell layers but it was the size of the affected cells (Akram *et al*., 2002). An inhibitory effect of toxic ions on the root tissue mainly appeared due to their toxicity, which prevented the expansion and enlargement of different cells (Curtis and Lauchli, 1987; Javed *et al*., 2001). A marked reduction in the development of the primary root cortex is one feature that characterizes halophytic species. According to Waisel, 1972), the cortex of primary roots of *Suaeda monoica* was only about 2 to 5 cells thick, similar to the tree cells thickness found in *S. maritime*. It is well known that salt tolerance in glycophytes is associated with the ability to limit the uptake and/or transport of salt ions (mainly Na^+ and Cl^-) from root to shoot (Greenway and Munns, 1980; Tester and Davenport (2003). This suggests that the cortical layers of plasmolysis cell in the BI3000 variety may have some physiological and/or adaptive implications that help reinforce protection against salinity more efficiently.

Experiment II: Saline treatments decreased root biomass relative to the control, which is consistent with the ones observed in the anatomical analysis where salt decreased their diameter mainly due to plasmolysis of sub-epidermal parenchyma cells of the cortex. The root and shoot biomass in salt stress conditions was not significantly different from that in ABA treatments (Table 2). Although in this study ABA application did not enhance plant growth salt stress conditions, an increase in ABA content could be beneficial for plants under environmental stress since there is extensive bibliography describing which changes induced by ABA help mitigate the effects of stress at the cell and whole plant level (Hasegawa *et al*., 2000; Bray, 2002; Finkelstein *et al*., 2002). In *Phaseolus vulgaris* the addition of 1 μM ABA to the nutrient solution before the exposure to salt stress reduced the negative effect of NaCl (Khadri *et al*., 2006).

Table 2: Biomass dry of roots and aerial part, chlorophyll a, b, total and carotene content, membrane injury, total conductance, foliar temperature of plants in salinity with ABA at different phenological stages, expressed as percentage relative to control

Parameters	% of control
Biomass root	-7.56
Biomass aerial part	2.91
Chlorophyll a	18.37*
Chlorophyll b	24.30*
Chlorophylls total	18.73*
Carotene	77.16*
Membrane injury	-23.84*
Conductance	30.65*
Foliar temperature	-2.02*

Data represent the means from 15 samples, * - significant differences from control at $p<0.05$ for the fisher test

The chlorophyll and carotene contents increased in plants with exogenous ABA under salt stress respect to control (Table 2). These results coincide with other previous studies carried out with soybean and wheat under water stress (Travaglia *et al*., 2007, 2009a, 2010). Thus, an increase in chlorophyll content as a function of osmotic stress may be related to the ABA signaling pathway. Other studies indicated that drought-tolerant genotypes were able to maintain higher chlorophyll content than susceptible genotypes in wheat (Chandrasekar *et al*., 2000). The increase in the relationship chlorophyll a, b under water stress is another benefit of ABA applications in wheat plants since decrease in this relationship is characteristic of foliar senescence in many vegetal species (Wolf, 1956). By extending the photosynthetic apparatus stability, there will be greater photosynthetic activity as time passes, what would mean a greater dry matter accumulation in crop products (Thomas and Howarth, 2000; Radford, 1967). Carotenes are found accompanying chlorophylls, making up complex photosynthetic which acts as radiant energy capture and as protecting agents of the photosynthetic apparatus on possible injuries from visible light (Gross, 1991). That is why it is essential to stand out in this study the importance of carotene increase in ABA treatment, mainly under drought conditions. Also, the benefit of ABA exogenous application was proved in barley plants against injuries due to light intensity and low temperatures, where the carotene levels were higher than those in the control plants (Ivanov *et al*., 1995). ABA treatment favoured the cellular membrane stability in salt stress (Table 2), coinciding with carotene accumulation as protecting agents. ABA has been implicated to have a role in protecting cellular structures during dehydration (Stewart, 1980; Singh *et al*., 1989; Ristic *et al*., 1992; Morgan, 1983; Frahm *et al*., 2004). Kacperska (1995) claimed that ABA functions, as a regulator in processes, enable the plant to reduce injuries possibly caused by different environmental stress factors. This was also observed in Vigna (Mukherjee and Choudhuri, 1985), jute (Chowdhury and Choudhuri, 1989) and barley plants (Bandurska, 1998), in which ABA treatments decreased the membrane injury under water deficit.

It is known that lack of water in the soil makes plants transpire at a lower rate than the atmosphere evaporation demands, what produces leaf warming when the refrigerating effect of transpiration decreases (Clark and Hiler, 1973). When evaluating the stomatal behaviour, it was determined that conductance under salinity was low under high foliar temperatures. This coincides with the results found in sorghum and barley in which the saline osmotic effect showed an important reduction of the stomatal conductance, suggesting the almost complete stomata closure, while the vegetal cover temperature increased linearly along with salinity (Kluitenberg and Biggar, 1992). ABA participation in stomatal responses to a variety of environmental and endogenous signaling is well-known (Schroeder *et al*., 2001; Dodd, 2003; Roelfsema and Hedrich, 2005; Davies and Zhang, 1991; Sauter *et al*., 2001; Davies, 1995).

In this study ABA treatment in plants grown under salinity kept low foliar temperature and enhanced stomatal conductance compared to control plants (Table 2). These results can be beneficial if we take into consideration that plants close their stomata and foliar temperature could be higher under stress conditions (Rizhsky *et al*., 2002). In non-salinized citrus plants, ABA reduced stomatal conductance and CO_2 assimilation, whereas in salinized plants the treatment slightly increased these two parameters (Gomez-Cadenas *et al*., 2002). The results suggest a protective role for ABA in plants under salinity. This show an effect of ABA opposite to what may be expected on the basis of literature data about ability of ABA to close stomata. This is quite justified, since the long-term effect of ABA may be different or even opposite to its direct fast effect. In previous results, ABA had a long-term effect alleviating the stomata closure partially in wheat and soybean under water stress, which was reflected in a stomatic conductance and transpiration rate increase (Travaglia *et al*., 2009a, 2010). This could be the reason for an effective balance between water loss and CO_2 intake for the photosynthesis during the day. Higher stomatal conductance in salt is related to higher assimilation and there was a positive relationship between stomatal conductance and relative growth rate in salt, suggesting stomatal conductance can be used as a surrogate for growth rate. These relationships showed that stomatal conductance can be a reliable indicator of growth rate and also can be used as a reliable screen in tolerance to osmotic stress caused by salinity for wheat genotypes.

CONCLUSION

In conclusion, in this study it was possible to determine that the BI3000 wheat variety can grow in high electrical conductivity, with a germination and seedling growth response optimum and with less radical anatomic variations under salinity, what allows this variety a faster plasticity to adapt to salinity. In this variety, ABA applications suggest a protective role in plants under salinity, due to an increase in chlorophyll and carotene content, stability of cell membranes and stomatal behavior. This becomes important in this current situation in which precipitations are scarcer and the soil chemical composition suffers from variations accumulating, for example, a great amount of sodium chloride. The capacity of the plant to avoid abiotic stress conditions is reinforced when the plant hormonal sign is enhanced, thus, facilitating its growth and increasing its agriculture potential. This study is a contribution to a better understanding of the morphophysiological responses of glycophytic plants to increase wheat yields and other cultivars in areas affected by this stress.

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