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Effect of Salicylic Acid on the Growth and Mineral nutrition in Salt Stressed Wheat Plants

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Abstract: The effect of 0.1 mM salicylic acid (SA) on the counteracting of the NaCl 100 mM-induced deleterious effects on wheat (*Triticum durum* Desf., var. Razzak) was studied. Effects of SA on salt tolerance of wheat were determined by measuring the growth parameters: shoot and root dry weights. Photosynthetic pigments chlorophylls *a* and *b* content and mineral nutrition were investigated in response to the interactive effects of SA and NaCl treatment. Application of 0.1 mM SA through the rooting medium increased plant growth significantly both in saline and control conditions. The results showed that the growth of shoot, and not that of roots, is reduced by 36 % in the presence of NaCl, and only of 14.5 % when SA is present in the medium. NaCl significantly reduced photosynthetic pigments content. Whereas, the addition of SA induce an increase of these contents. Also, SA inhibited Na^+ accumulation, but stimulated K^+ and Ca^{2+} contents of salt stressed plants. These results suggest that SA could be used as a potential growth regulator to improve plant salinity stress resistance.

Keywords: Growth, Nutrition, Salicylic acid, Salt stress, Wheat

1. Introduction

The role of salicylic acid (SA) in defence mechanisms against pathogen attack has been known for several years (Yalpani *et al.*, 1993). In recent years its role has been widely investigated in both biotic and a biotic stress (salinity, drought, water deficit and so on) (Gunes *et al.*, 2007). Excessive soil salinity is an important constraint limiting the distribution of plants in natural habitats, and is an increasingly severe agricultural problem in arid and semi-arid regions (Lauchli and Epstein, 1990). About 15 million hectares are affected by salinity in the Maghreb and the Middle East. In Tunisia, the (semi) arid Mediterraneanan bio-climatic regions are frequently irrigated with water reached in salt. Consequently, about 10% of the whole territory area and 20% of the cultivated lands are salinized (Hachicha *et al.*, 1994). Convincing data have been obtained on the SA-induced increase in the resistance of wheat seedlings to salinity (Afran *et al.*, 2007), and water deficit (Bezrukova *et al.*, 2001), of maize to low temperature (Janda *et al.*, 1999) of tomato and bean plants to low and high temperature (Senaratna *et al.*, 2000) as well as on the detrimental action of heavy metals on rice plants (Mishra and Choudhuri, 1999). However, the beneficial effect of SA application depends on type of species or cultivar and with the concentration of SA applied. Of the varing SA level used, Arfan *et al*. (2007) and Stevens *et al*. (2006) reported that the most effective levels for promoting growth were the lower concentration of SA applied (0.1- 0.75 mM). The aim of this work was to study the responses of growth and mineral nutrition in wheat (*Triticum durum* Desf., cv Razzak) to the interactive effects of salicylic acid (SA) 0.1 mM and NaCl treatment 100 mM.

2. Materials and Methods

Sterilized seeds of wheat (*Triticum durum* Desf., var. Razzak), obtained from the wheat section, Agricultural Research Institute, Tunis, Tunisia, were germinated for 4 days at room temperature, then grown in hydroponics nutrient solution. The composition of this one was made of K^+, 3.0 mM; Ca^{2+}, 3.5 mM; Mg^{2+}, 1.0 mM; NO_3^-, 8.0 mM; SO_4^{2-}, 1.0 mM; P, 1.5 mM, Fe, 1.4 ppm; Mn, 0.25 ppm; B, 0.16 ppm; Cu, 0.03 ppm; Zn, 0.03 ppm and Mo, 0.01 ppm. The experiment was carried in a growth chamber under artificial light (150 µmol m^{-2} s^{-1}; 16h photoperiod), at 25 ˚C day/20 ˚C night and air humidity 60-80%. Three days after, four treatments were applied: (1) control (nutrient solution), (2) SA (0.1 mM), (3) NaCl (100 mM) and (4) SA (0.1 mM) with NaCl (100 mM). pH was adjusted with KOH 1.0 N. After 13 days of treatments, plants were harvested and divided into roots and shoots. Estimation of pigment contents was achieved by application of the method of Torrecillas *et al.* (1984): 80% acetone extract was colorimetric all assayed at 649 and 665 nm. Extraction of ions was achieved in HNO_3 (0.1 N). Na^+, K^+

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and Ca^{2+} were assayed by flame emission photometry. Analysis of variance was used for the statistical analysis of data. Mean separation procedures were carried out using the multiple range tests with Fisher's least significant difference procedure ($p<0.05$).

3. Results and Discussion

Salinity decreased significantly wheat plant growth significantly (**Fig. 1A**). The depressive effect of NaCl appears mainly in shoots than in roots. The shoot biomass was about 64 % of the control. Whereas, the growth of root system was stimulated by salinity. Which results in reduction of shoot/root dry weight. But this reduction was suppressed when adding SA 0.1 mM to the culture medium. Salicylic acid-treated wheat plants exhibited an increase in tolerance to salt treatment. This increase in salt tolerance was reflected in the measured growth criteria: dry weight of shoots were increased comparing with plants received NaCl only. In the presence of SA 0.1 mM, the sensitivity to NaCl of the whole plant was decreased about only 7% compared to the control treatment (Fig. 1A).

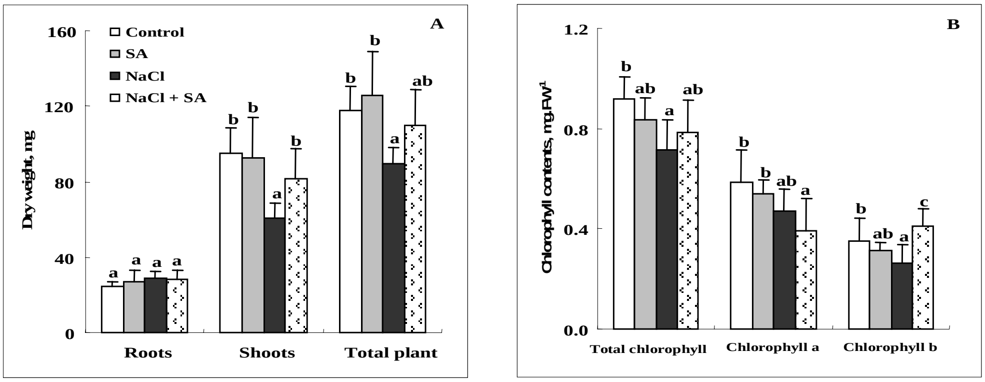

Fig. 1. Production of whole plant, roots and shoots dry weight (A) and Chlorophyll contents in leaves (B) of wheat plants grown for 13 days in nutrient solution with 100 mM NaCl ± 0.1 mM salicylic acid. Different letter on top of bars indicate significant difference according to LSD test ($p< 0.05$) at each treatment. ($n= 10 \pm SE$).

The improvement of the tolerance to salt by SA can be related to two factors: the stimulation of the root growth activity and the attenuation of the depressive effect of NaCl on the shoots. Salinity and SA treatment affect photosynthetic pigments such as chlorophyll a, chlorophyll b and total chlorophyll contents (**Fig. 1B**). The figure showed that the pigments (chlorophyll a, b) content of NaCl-treated wheat plants was significantly decreased below that of the control. Whereas, the addition of SA in the culture medium induces an increase in these parameters. In the presence of NaCl, the amounts of K^+ and Ca^{2+} absorbed by roots and transported to the shoots were restricted. Potassium and calcium content of plants decreased significantly in saline conditions (**Fig. 2**), which suggests a deficit of provisioning of the plants in essential ion to the growth. The addition of salicylic acid in the culture medium improves accumulation of K^+ and Ca^{2+} on the shoots. The absorption and the transport to shoots of high contents of sodium in saline conditions were attenuated by the addition of SA 0.1 mM. Indeed application of SA induced a significantly decreases in Na^+ content as compared to the plants treated only by NaCl 100 mM (Fig. 2). Positive effect of SA on the K^+ and Ca^{2+} uptake, and inhibitory effect on Na^+ uptake should also be responsible for managing salinity of wheat plants.

Salinity decreased K/Na selectivity in the two organs. Whereas SA addition ameliorates selectivity in favour of K^+. In fact, at salinity level 100 mM NaCl, the K/(K+Na) ratio in shoots was equal to 0.368, these ratio was enhanced (about 0.417) when adding SA 0.1 mM to the culture medium (**Table 1**).

Thus SA ameliorate the aptitude of whole plant to ensure a sufficient supply K^+ by maintaining a high selectivity for this essential nutriment, in spite of excess of Na^+ in the medium. Dry weights of roots and shoots decreased by salt (Fig. 1A). These results are in agreement with those of Munns (2002) who showed that salinity caused a marked reduction in growth parameters. Exogenous application of SA

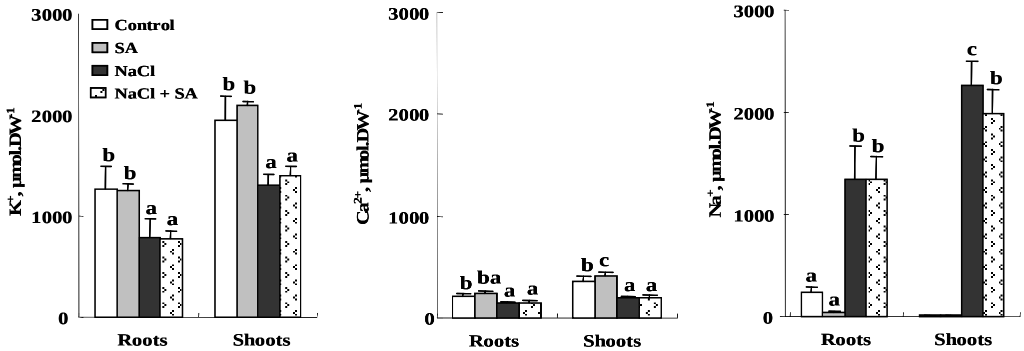

Fig. 2. K^+, Ca^{2+}, and Na^+ content in wheat plants grown for 13 days in nutrient solution with 100 mM NaCl ± 0.1 mM salicylic acid. Different letter on top of bars indicate significant difference according to LSD test ($p< 0.05$) at each treatment. (n= 10 ± SE).

Table 1. K/(K+Na) ratio in roots and shoots of wheat plants grown for 13 days in nutrient solution with 100 mM NaCl ± 0.1 mM salicylic acid. Different letter on top of bars indicate significant difference according to LSD test ($p< 0.05$) at each treatment. (n= 10 ± SE).

Treatment	K/(K+Na) Roots	K/(K+Na) Shoots
Control	0,993 ± 0.001 b	0.838 ± 0.031 c
SA 0.1 mM	0.968 ± 0.005 c	0.994 ± 0.001 c
NaCl 100 mM	0.373 ± 0.050 a	0.368 ± 0.032 a
NaCl 100 mM + SA 0.1 mM	0.374 ± 0.055 a	0.417 ± 0.039 b

through the rooting medium had an ameliorative as well as growth promoting effect under both non-saline and saline conditions (Fig. 1A). It was shown in a previous work that 0.5 mM of SA added in the hydroponics solution of maize increased its tolerance to salt stress (Németh *et al*., 2002). Application of 0.1 mM SA also increased fresh and dry weights of both shoots and roots under non-saline conditions (Gunes *et al*., 2007). Dhaliwal *et al*. (1997) attributed this amelioration in growth production to an increase in photosynthesizing tissue of leaves. Which was in agreement with our results, in fact application of 0.1 mM SA through the rooting medium increased pigments chlorophyll b in salt stressed plants (Fig. 1B). This effect of SA application on photosynthetic pigments was expected in many studies that showed increased or decreased photosynthetic pigments following SA application, depending on species (Arfan *et al*., 2007). The presence of salt in root medium causes disturbances in the mineral nutrition of the plant. NaCl reduces K^+ and Ca^{2+} uptake and translocation from roots to shoots more severely (Fig. 2). Greenway and Munns (1980) suggested that the susceptibility of many glycophytes to salt is due to an insufficient ion supply. Indeed, in wheat, salt reduces K^+ uptake and induces Ca^{2+} deficiency (Ouerghi, 2001). Application of SA through the rooting medium inhibited Na^+ accumulation, but stimulated K^+ and Ca^{2+} concentrations of salt stressed wheat plants (Fig 2). Al-Hakimi and Hamada (2001) studied the effect of SA on ion uptake in wheat. They found that SA decreased Na content of wheat shoot and root tissues under salinity. These results suggest that SA could be used as a potential growth regulator to improve the resistance of plant to salinity stress.

4. Conclusions

It might be concluded that SA treatment of salt stressed wheat could stimulate their salt tolerance via increasing their performance to absorb K^+ and reducing the transport of Na^+ to shoots. This study suggests that SA could be used as a potential growth regulator to improve plant growth and nutrient utilization under salt stress.

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