

Effect of salicylic acid on Na⁺ accumulation in shoot and roots of tomato in different K⁺ status

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ABSTRACT

Tomato plants grown in nutrient solutions with different K⁺ levels were used to study the effect of salicylic acid and Potassium status on Na⁺ uptake and Na⁺ accumulation in the shoot and root. Changes in the nutrient K⁺ levels induced evident differences in internal K⁺ content. When low and normal – K⁺ plants treated with SA were exposed in saline condition (50 mM NaCl) during experiment, Na⁺ uptake in low- K⁺ plants was greater than in normal – K⁺ plants. At the presence of SA, Na⁺ uptake in low- K⁺ plants was lower than low- K⁺ plants alone. Normal – K⁺ plants plus SA showed lowest amount of Na uptake and accumulation. In addition, K⁺ starvation favored the Na⁺ uptake and the Na⁺ accumulation both in the root and in the shoot. When the plants were exposed to heat stress by a sharp increase of the temperature to 32^o C during the same period of time, the stimulating effect of K⁺ starvation on the water uptake was even greater. The high temperature increased Na⁺ uptake in both types of plants, but the Na⁺ accumulation in the shoot was only favored in low K⁺ plants.

Key Words: K starvation, *Lycopersicum esculentum*, potassium status, sodium accumulation, tomato, water uptake

INTRODUCTION

Excess salt in soil is an important environmental factor limiting plant growth and the yield of crops. About 6% of total global land area and one third of the world's irrigated land are significantly affected by soil salinity, and one of the major components limiting plant growth in such soils is high Na⁺ (Bergmann, 1992). Plants exposed to high salt concentrations must withstand both the water deficit and the ion imbalance imposed by the salt excess. Although water deficit always has a negative effect, many crop plants are primarily sensitive to the Na excess (Greenway and Munns, 1980) due to its adverse effects on K⁺ nutrition, cytosolic enzyme activities, photosynthesis and metabolism (Niu et al., 1995). Tolerance to salinity involves processes in many different parts of the plant, and more than one of these processes can operate concurrently within a particular plant. These mechanisms can occur at a wide range of organizational levels, from the cellular (e.g. compartmentation of Na⁺ within cells) to the whole plant (e.g. exclusion of Na⁺ from the plant, and intraplant allocation of Na⁺). They can occur in all cells within the plant, or in specific cell types. This reflects adaptations at two major levels of organization: those that confer tolerance to cells, and those that contribute to tolerance not of cells per se, but of the whole plant. Ions transports cross the plasma membrane or the tonoplast operate as factors of Na⁺ tolerance in plants. The function of these processes is to restore the ionic homeostasis and to keep the cytoplasm of actively growing or photosynthesizing cells of free of Na⁺ as possible (Schachtman et al., 1991). In glycophytes, which include most crop plants, the primary strategy is to exclude Na⁺ from the shoots by retaining it in root and lower stem. Potassium is an essential nutrient and the most abundant cation in plants, whereas the closely related ion sodium (Na⁺) is toxic to most plants at high millimolar concentrations. K⁺ deficiency and Na⁺ toxicity are both major constraints to crop production worldwide. K⁺ counteracts Na⁺ stress, while Na⁺, in turn, can to a certain degree alleviate K⁺ deficiency. Several earlier studies reported that K⁺ starvation induces increased low-affinity Na⁺ uptake (Pitman, 1967) and low-affinity K⁺ uptake (Maathuis and Sanders 1995). Phytohormones play an essential role in regulating plant growth and development. Application of growth regulators to restrict the harshly effect of salinity reported by some researcher. Also, adding the soil amendent like zeolite have beneficial effect (eshghi et al., 2010). One of the plant growth regulators that showed benefit effect in salinity condition is salicylic acid. SA has been shown as an important signal molecule for modulating plant responses to environmental stress (Breusegem et al., 2001; Rowshan et al., 2010). The ameliorative effects of SA have been well documented in inducing salt tolerance in many crops (El-Tayeb, 2005). The soaking of wheat seed in 0.05 mM SA also reduced damaging of salinity on seedling growth accelerated the growth processes (Shakirova et al., 2003). Exogenous application of salicylic acid can induce salt tolerance and water stress tolerance in wheat

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(Arfan *et al.*, 2007). In the present study we addressed the question of whether Na⁺ accumulation in tomato are affected by different k⁺ status, Temperature and exogenous SA application via seed soaking.

MATERIALS AND METHODS

Plant material, growth conditions and salicylic acid treatment

Tomato seeds (*Lycopersicon esculentum cv khorma*) were surface-sterilized in 5% v/v sodium hypochlorite for 1 min, then seeds were two group: (i) seeds soaked with SA (levels for 12 h in the dark at 22 °C) (10⁻⁶ mM), (ii) without SA Soaking. Then seeds germinated in the dark at 25^{0c} on wetted filter paper for six or seven days. Then the seedling were transferred individually to box containing 1000 ml of a K Free standard nutrient solution with the following composition: 2.5Mm Ca⁺ (No)₂, 1.0mM MgSo₂, 2.5 mM Ca₂(HPO₄), 0.25 μM CuSo₄, 0.1 ZnSo₄, 0.1 μM MnSo₄, 0.2 μM₇O₂₄ and 12.5 μM Fe-ethylenediamine-di-o-hydroxy-phenylacetic acid. Then the boxes were maintained in the greenhouse under natural light (>800 μmol m⁻² s⁻¹) at a day temperature of 22±1 °C and night temperature of 18±2 and a RH of 75±5%. In order to obtain plants whit different K status, the plants were grown hydroponically for 15 d in standard nutrient solution supplemented with different concentration of KCL (i) 0.5 Mm KCL over 14 d, and 0.1 mM on the last day in order to favor the k starvation (low-k plants), (ii) or 2.5 mM Kcl over all 15 d (normal K-plant). In all cases, Ca (OH)₂ was used to adjust the pH of the nutrient solution to 5.5. The final concentration of calcium in the standard nutrient solution was never higher than 3mM. The nutrient solutions were renewed at day 5.

Water uptake and Na determination

Water uptake was determination in 30-d- old plants. In the some flasks in which the plants had grown , the nutrient solution was exchanged for a new solution that had the same basic composition as the K -free standard nutrient solution supplemented with 0.1 mM KCl (Low- k plants)or 2.5 mM KCL (normal – K plants) , plus 50 and 75 mM NaCl. Immediately after transferring the plants to the new solution, the plants were exposed to tow different growth chamber temperatures. A group of plants were kept at the same temperature of the growth period (22), and other groups were kept in other chamber with similar conditions but at 32. The water uptake was determined by measuring consumption of nutrient solution after 10 h, and the values were referred to the fresh weight of the root and shoot. All water uptake experiments were performed at the same time of the day, 1 h after switching on the lights of the growth chamber. During the experiment growth chamber conditions were the same as for the growth period, except the temperature was 32 in same experiment. Na and K were determined by flame photometer (model Jenway PFP7), after extraction from the plant materials (roots or shoots) with 10 % acetic acid solution (Benlloch *et al.*, 1989). Na uptake was calculated from the total Na accumulated in the plants (Root plus shoot) during the uptake period, and the values were referred to the fresh weight of the root.

Experimental design and statistical analyses

This experiment was conducted as a complete randomized design in a factorial arrangement with four replicates. Duncan's multiple range test (DNMRT) was used for comparison among treatment means.

RESULTS

Low- K+ plants showed a reduction in plant weight, but neither reduction in water content or visible symptoms of K+ deficiency were observed (data not shown) .Both shoot and root of plants grown at 0 .5/0.1 Mm KCL had a lower k+ content than those of plants grown at 2.5 Mm KCL. For investigate the effect of the temperature and SA on Na⁺ distribution in different K status, the plants were exposed to two different temperature regimes: 22 and 32 °C. In the first temperature condition in (22^{0c}), the water uptake was greater in low- K⁺ than in normal- K⁺ plants (Table 1). In the same experiment period (9 h), the rise in temperature in the growth chamber (32^{0c}) favored and the Na⁺ uptake both in low – K⁺ plant and in normal – K⁺ plants, and this effect was in the same direction that the one observed on the water uptake. When the Na⁺ distribution between the shoot and root was determined at 22^{0c}, the Na⁺ accumulated in the root was greater than the Na⁺ accumulated in the shoot in both types of plants. The same result observed at the presence of SA. But, accumulation on Na in both shoot and root

were lower than only low-K plants. In addition, Na in the root and in the shoot was higher in low – K plants than in normal- K plants (Table 1). When the temperature was increased at 32^{0C}, Na accumulation in the roots and shoots in low- K plants was favored. However, in normal – K plants the rise in temperature only increased the Na accumulation in root and shoot was dependent (P<.05) on the water uptake, while in normal K plants, this dependence (P<.05) was only absorbed in the roots but not in the shoot (Table 1). The same effect described above was also observed in both types of plants when 75 mM NaCl was added to the external medium (Data not shown). The only difference was that the Na accumulation was higher in this case. Adding SA in different K status showed significant effects. Present of SA in low-k plant decreased Na accumulation in both root and shoot and the rate of decreasing were higher in shoot.

Table 1. Effect of different temperature and SA on fresh weight, Na uptake, Water uptake and K⁺ content in tomato plants with different K⁺ nutrition plus 50 mM Na

Temp. (C ⁰)	Plant Type	Fresh Waight (g)		K ⁺ (μ mol g ⁻¹ FW)		Na uptake (μ mol g ⁻¹ FW _{root})	Water uptake (mL g ⁻¹ FW _{root})	Na accumulation (μ mol g ⁻¹ FW _{shoot})	
		Shoot	Root	Shoot	Root	Root	Shoot	Root	Shoot
22 ^{0C}	Low – K ⁺	12.7 c	9.2 c	64.9 c	135.2 a	83 b	4.5 bc	63 ab	18.4 b
	Low–K ⁺ + SA	14.7 c	11.1 c	68.9 c	137.2 a	25 c	4.8 bc	34 c	13.4 c
	Normal–K ⁺	19.9 b	18.2 b	145.7 b	129.4 b	10 e	4.2 bc	7 e	3.2 d
32 ^{0C}	Normal–K ⁺ + SA	25.9 a	23.9 a	155.7 a	135.4 a	16 d	4.3 bc	5 e	1.3 d
	Low –K ⁺	14.5 c	10.2 c	64.9 c	135.2 a	117 a	8.2 a	69 a	29.4 a
	Low–K ⁺ + SA	16.3 c	12.1 c	68.9 c	37.2 c	21.4 c	8.5 a	48 b	22 b
	Normal –K ⁺	21.8 b	19.2 b	145.7 b	129.4 b	17.1 d	5.1 b	18 d	3.3 d
	Normal–K ⁺ + SA	27.2 a	25.1 a	155.7 a	135.4 a	15.2 d	5.8 b	7.3 e	2.9 d

Means within a column followed by the same letters are not significantly different by new Duncan's multiple range test (P > 0.05).

DISCUSSION

Salinity dominated by Na and Cl ions has been shown to decrease the concentration of essential macro and micro elements in several crops. Both shoot and roots of tomato plants decrease dramatically at the presence of Na in medium. Application of SA increased the amount of K uptake in different k status than other treatment without SA application. Sivritepe et al. (2003) found that NaCl salinity increased Na content in plant tissue of some crops. Essa (2002) reported that NaCl salinity may produce extreme ratios of Na/Ca and Na/K in the plants, causing them to be susceptible to osmotic and specific-ion injury, as well as to nutritional disorders. Salt stress increased the Na content in both organs of tomato plants (Table 1). However, a large body of literature indicates that exogenous application of salicylic acid to the stressed plants can potentially alleviate the toxic effects, generated by salinity. An enhanced tolerance against salinity stress was observed in wheat seedlings raised from the grains soaked in salicylic acid (Hamada and Al-Hakimi, 2001). Similar observations were also made in tomato plants raised from the seeds soaked in salicylic acid and was presumed to be due to the enhanced activation of some enzymes viz. aldose reductase and ascorbate peroxidase and to the accumulation of certain osmolytes such as proline (Szepesi et al., 2005). Exogenous application of salicylic acid enhanced the photosynthetic rate and also maintained the stability of membranes, thereby improved the growth of salinity stressed barley plants (El Tayeb, 2005). The damaging effects of salinity were also alleviated by exogenous application of SA in Arabidopsis seedlings (Borsani et al., 2001). Kaydan et al. (2007) observed that pre-sowing soaking treatment of seeds with SA positively affected the osmotic potential, shoot and root dry mass, K⁺/Na⁺ ratio and contents of photosynthetic pigments (chlorophyll a, b and carotenoids) in wheat seedlings, under both saline and non-saline conditions. SA reduced the Na uptake of plants and increased the uptake of K as compared to non SA treatment (Table 1). These results are consistent with those of El-Tayeb (2005) for barley and Szepesi et al., (2005) for tomato who found out that exogenous SA application inhibited Na accumulation, but stimulated K uptake. An increase in concentration of K in plants (normal- K) under salt stress the deleterious effects of salinity on growth were cumulative. Alteration of mineral uptake from SA applications may be one mechanism for the alleviation of salt stress. K starvation increases the plants susceptibility to the water stress (Hsiao and

Läuchli, 1986) and salinity. It is likely that the K nutritional stress provokes in the plant and loss of its regulation capacity on the water flux and Na transport. In sunflower plants, support this assumption: the water use efficiency and ABA sensitivity were lower in K^+ -starved plants than in plants with normal-K status. In relation to Na transport, Benlloch et al. (1994) have reported that K starvation can increase the salt stress susceptibility in sunflower and bean plants, since the root showed a lower capacity to discriminate between K/Na, and this contributed to a higher Na accumulation in the shoot. It has also been previously reported that transpiration is positively correlated with salinity susceptibility (An et al., 2001) this effect has been ascribed to the greater Na accumulation in shoot, most likely Na and water apoplastic flows by transpiration (Yeo et al., 1987). In the present investigation, the water uptake increased when the plants were exposed to sharp heat stress by rising the temperature in the growth chamber ($32^{\circ}C$) (Table 1). This effect was higher in low-K plants than in plants with normal K status. The raise of temperature increased Na uptake in both types of plants (Table 1). However, differential effect was observed on the distribution of Na between root and shoot: while in low-K plants the heat stress, and so the higher evaporative demand, favored the Na accumulation in the shoot is related to the transpiration stream (the higher water uptake, the higher the Na accumulation to the shoot) in K starved plants but not in plants with normal K status (Table 1). Therefore, our result partially agrees with the effect of the transpiration on Na accumulation in the shoot noted above (Yeo et al., 1987). In fact, this effect is dependent on K status, at least in tomato plants. Interestingly, An et al. (2001) have shown that the Na translocation to the shoot was independent of the transpiration rate in a salt-tolerant cultivated soybean treated with 40 and 80 mM NaCl, whereas the Na^+ translocation was dependent on the transpiration in a salt-sensitive cultivar. To conclude, the results reported here show that in tomato plants exposed to salinity conditions and additional heat stress, characteristic in dry climates increases both the water uptake and the Na accumulation in shoot of K starved plants. However, the greater water uptake induced by heat stress does not provoke Na over accumulation in shoot of plants with normal K status. Therefore, these plants maintain their Na-excluding nature under these stressing climatic conditions.

ACKNOWLEDGEMENTS

We thank from Dr. S. Eshghi for the critical reading manuscript. This work was supported by the Persian Gulf Research Institute Center, Agricultural College, Department of Horticultural Science.

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