Effect of salicylic acid and salt stress on Na and K content in *Ocimum basilicum* L.

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Abstract

In this research the role of Salicylic acid (0.1, 0.01 mM) in amelioration of salt stress in species of sweet basil (*Ocimum basilicum L.*) has been investigated. We studied the effects of salicylic acid on the Na^+, K^+ content of those plants which were under salt stress for 24, 48 and 72 hours. In stress conditions, while Na^+ content of leaf increased compared with those plants pretreated with salicylic acid, there was a reduction in K^+ content. Salicylic acid pre-treatment alleviated the adverse effects of salt stress based on Na^+, K^+ measurement.

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Introduction

Soils with high salt contents already existed before the appearance of man, but problems have only arisen since the spread of agriculture and particularly irrigation. The ionic equilibrium is disturbed by a high salt concentration, leading to hyperosmotic stress in plants. The regulation of ion homeostasis is a fundamental criterion for physiological activities in plants (Janda et al., 1999). It has been reported that salinity reduces plant growth and photosynthetic pigments altering ionic relation by ionic and osmotic effects (Molassiotis et al., 2006; Parida and Das 2005; Silvia et al., 2008). Crop

performance may be adversely affected by salinity as a result of nutritional disorders, competitive uptake and transport or partitioning within plant (Silvia et al., 2008). The osmotic effect involves limited water absorption due to salinity in the rhizosphere and the ionic effect consists of intracellular toxicity or imbalance due to excess ions (Silvia et al., 2008). The term salt resistance has been described as the ability of plants to tolerance excess salt in their habitat without any significant impairment of their vital functions (Walter et al., 1985). It is believed that a complex combination of various mechanisms, and not a single process or adaptation exists; therefore, salt resistance is certainly not controlled by a single gene (Munns et al., 1993).

Following the terminology of Levitt (1980), plants can achieve resistance to salt stress either by tolerating the stress or by avoiding it. Several mechanisms have been reported to

counteract salt stress, the most important of which are: the transportation of sodium and chloride ions from the cytoplasm to the vacuoles with the aid of Na^+/K^+ ATPase, leading to higher concentration of K^+ ions in the cytoplasm, the synthesis of the compatible osmolytes and the conversion of certain ions into less soluble forms. Also modifications in photosynthesis, respiration and the hormone metabolism cannot be ignored (Janda et al., 1999).

Many attempts have been made to approach the mechanisms for salt tolerance using physiological traits to select germplasm. For example in wheat, it has been reported that the salt tolerance is associated with low rates of transport of Na^+ to shoots, with high selectivity for K^+ over Na^+ (Gorham et al., 1987). Ashraf and O'Leary (1997) and El-Hendawy et al., (2005) have shown that Na^+ exclusion are not the only mechanism of salt tolerance but in many plants the resistance to salt stress involves a more or less efficient restriction of salt uptake. Such a restriction can concern either the whole plant such as mangroves, or sensitive organs, tissues, or cells as is found in various species of Fabaceae (Lauchli ,1984) where this process is effective only at low levels of salinity. Under conditions of prolonged salt stress the retention mechanism must be supported by retranslocation of accumulated salt ions within the plants. In various crop plants, like bean, maize, and pepper, Lessani and Marschner (1978) observed a transport of Na^+ and Cl^- ions from leaves to roots. Part of the ions was recreated back into the medium. This shows how long-distance transport processes can be involved in plant adaptations to salt stress. The efficiency of such avoidance mechanisms is always limited. Consequently, additional mechanisms have evolved for the regulation and stabilization of internal salt levels (Breckle et al., 1990; Flowers et al., 1986; Waisel, 1972). As salt tolerance in plants depends on both the accumulation of Na^+ ion in leaves and the time in which the content of Na ion reduces in the leaves, in this study salicylic acid (SA) was used to see whether SA can reduce the accumulation of Na^+ ion in leaves and the time needed for Na^+ ion to reach the leaves.

Materials and Methods

Plant materials and culture conditions

A pot experiment was conducted at the growth chambers in International Center for Science, High Technology and Environmental Science, Kerman, Iran. Seeds of *Ocimum basilicum* (provided by Yasateb Research Center, Isfahan, Iran) were preliminarily screened for germination in 100 and 200 mM NaCl and various concentrations (0.01, 0.1, 0, 0.5, 1, 1.5, 2, 3 mM) of salicylic acid to obtain the optimum response of germination. Eventually, concentrations of 0.01, 0.1 mM of SA were selected. Salicylic acid was dissolved in deionized water and the pH was adjusted to 5.5 ± 0.2 with KOH (1N). The seeds were divided into 3 parts and soaked for 6 h at dark at 25 $^\circ$C, in 0.01 and 0.1mM SA, and in deionized water as control .Twenty-five seeds were then germinated in each petri dish (9 cm diameter) provided with two filter papers saturated with deionized water, 0, 100 and 200 mM NaCl. The seeds were kept under aseptic conditions for 1 day in the dark and 5 days in 16 h / 8 h light /dark cycle, with a light intensity of 350 µmole m^{-2} s^{-1} and a relative humidity of 65% at 25 $^\circ$C.

Three replicates were prepared for each treatment. The seeds were transferred into vases including perlite. These plants were divided into two parts and then when the third leaf emerged, SA was sprayed for 5 consequent days on treated plants. NaCl dissolved in nutrient solution and applied once. The leaves were then harvested in 24, 48 and 72 hours. Shoot and root samples were wet digested with a nitric acid and hydrochloric acid mixture and $Na^+$and K^+ contents measured by atomic absorption (Varian Spectra AA 220, Australia) (Kaydan et al., 2007).

Statistical analyses

All analysis was done on a completely randomized design. Obtained data were then subjected to one -way analysis of variance (ANOVA) and the mean differences were compared by the least significant differences (L.S.D test). The experiments were repeated twice with three replicates for each (n=3) and
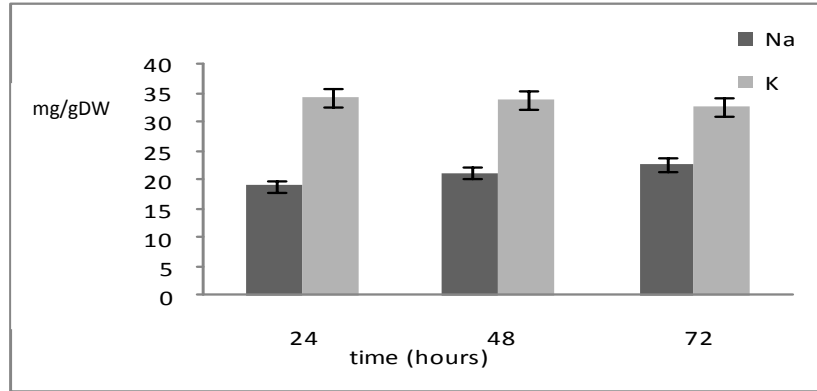
Fig. I. The effect of 100 mM NaCl on Na$^+$ and K$^+$ content of leaf of *Ocimum basilicum*. The third leaves were harvested 24, 48, and 72 hours after the salt treatment had started.

comparisons with p≤0.05 were considered significantly different .

Results

In those plants treated with 100 mM salinity, Na$^+$ ion increased, and K$^+$ decreased slightly. Fig. I shows that the maximum concentration of Na$^+$ was increased in 72 hours, and minimum K$^+$ concentration was also 72 hours after salinity treatment.

Fig. II shows that Na$^+$ increased significantly while K$^+$ decreased slowly with 200 mM saline solution. The maximum Na$^+$ was measured 72 hours after salinity conditions and maximum K$^+$ concentration was observed 72 hours after salinity. When plants were pretreated with 0.01mM of SA and then treated with salt (100mM NaCl), the concentration of Na$^+$ decreased and that of K$^+$ increased slightly during time course of experiment (Fig. III). All the above

changes happened 72 hours after treatment.

Fig. IV shows when plants were pretreated with 0.01 mM of SA and then treated with salt (200 mM NaCl), the concentration of Na$^+$ increased and that of K$^+$ decreased slightly during time course of experiment (Fig. IV). All the above changes happened 72 hours after treatment.

Fig. V. shows that Na+ and K+ decreased slowly during experiment where plants were pretreated with 0.1 mM SA and then treated with salt (100 mM NaCl). The maximum Na+ and K+ were measured 24 hours after pretreatment with 0.1 mM SA and salinity conditions.

Fig. VI. shows SA treatment (0.1 mM) had only an effect on Na+ ion of leaves when salinity conditions were 200 mM NaCl. When plants were pretreated with 0.1 mM of SA and then treated with salt (200 mM NaCl), the concentration of Na+ increased and that of K+ decreased slowly during time course of
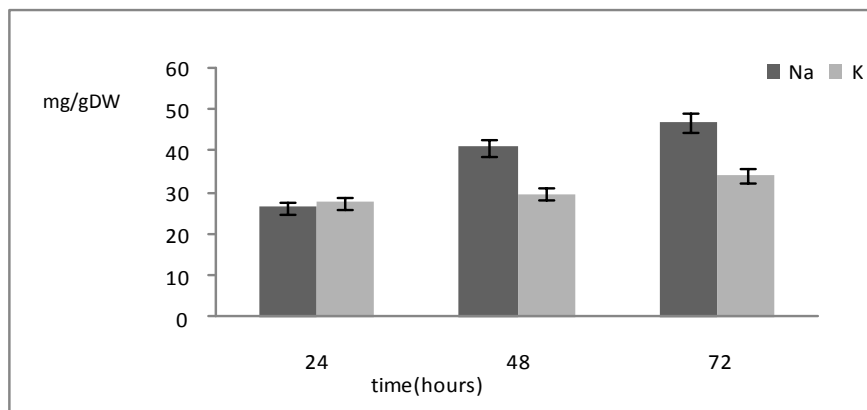

Fig. II. The effect of 100 mM NaCl on Na$^+$ and K$^+$ concentrations in leaf of *Ocimum basilicum* . The third leaves were harvested 24, 48 and 72 hours after the salt treatment had started.
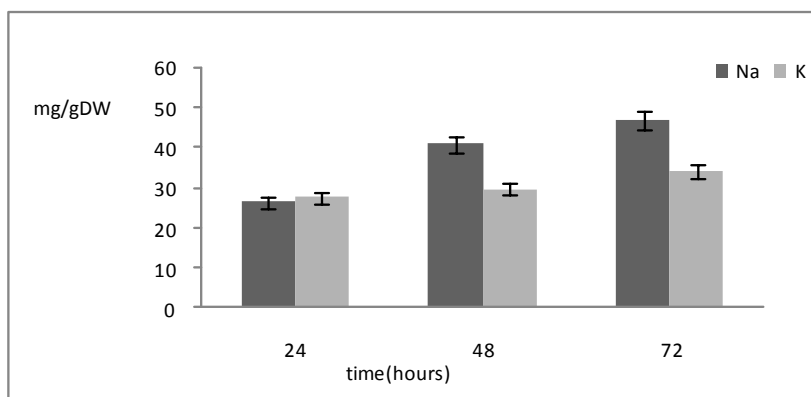
experiment (Fig. VI) .

Fig.III. Effect of 100 mM NaCl Plus 0.01 mM Salicylic acid on Na^+ and K^+ concentrations in leaf of *Ocimum basilicum*. Third leaves were harvested 24, 48 and 72 hours after spraying SA (0.01mM) and being treated by salt (100 mM Na Cl).

Fig. IV. Effect of 200 mM NaCl plus 0.01 mM Salicylic acid on Na^+ and K^+ concentrations in leaf of *Ocimum basilicum*. Third leaves were harvested 24, 48 and 72 hours after spraying SA (0.01 mM) and being treated by salt (200 mM NaCl).

Fig. V. Effect of 100 mM NaCl plus 0.1 mM Salicylic acid on Na^+ and K^+ concentrations in leaf of *Ocimum basilicum*. Third leaves were harvested 24, 48 and 72 hours after spraying SA (0.1 mM) and being treated by salt (100 mM NaCl).
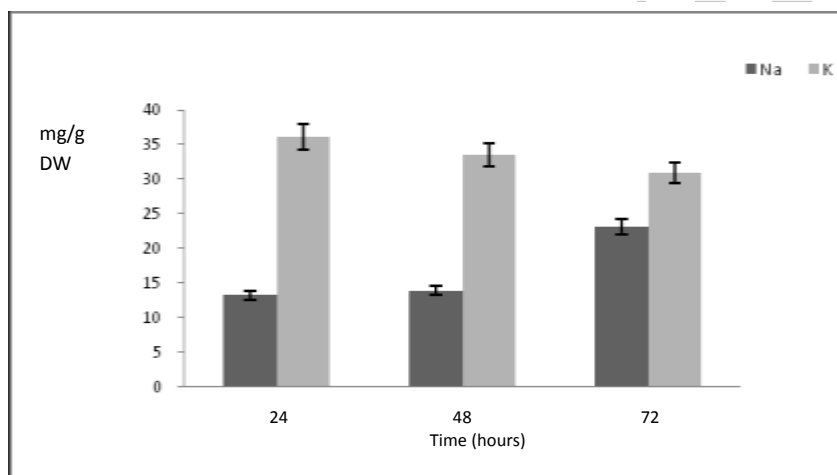
Fig. VI. Effect of 200 mM NaCl plus 0.1 mM Salicylic acid on Na^{+} and K^{+} concentrations in leaf of *Ocimum basilicum*. Third leaves were harvested 24, 48 and 72 hours after spraying SA (0.1 mM) and being treated by salt (200 mM NaCl).
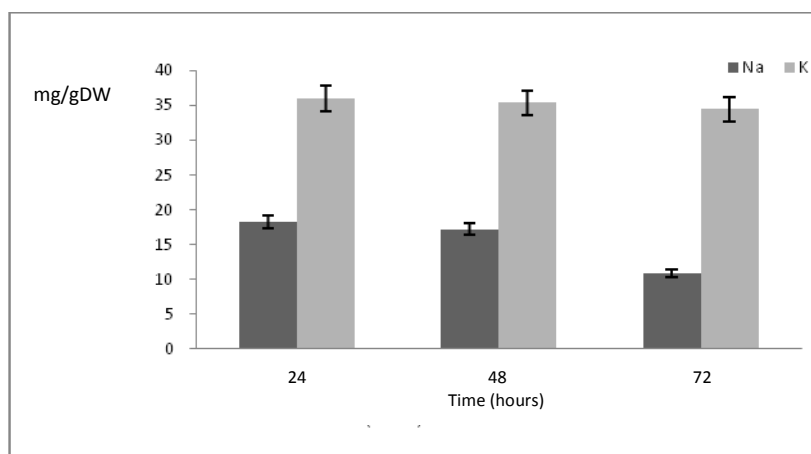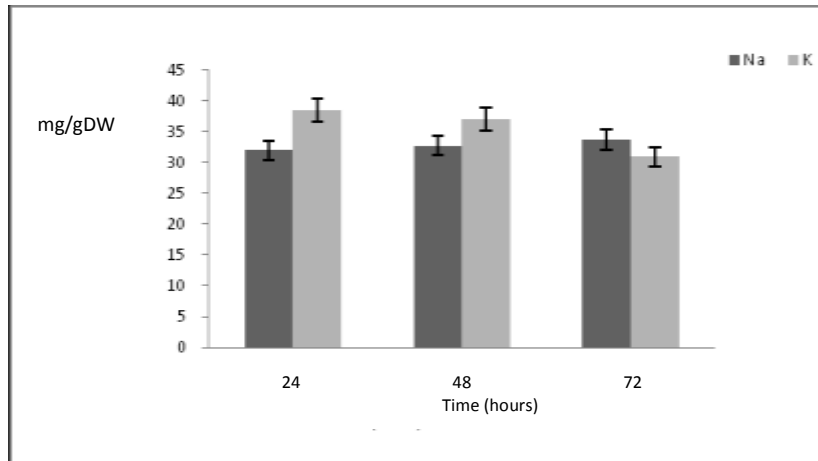
Discussion

Roots have a remarkable ability to control their Na^{+}, Cl^{-} concentrations, which do not increase in proportion to the external concentrations. The present study also shows the same changes in sweet basil, as illustrated in Fig. I. In sweet basil, once the external NaCl exceeds 100 mM, organic solutes must make a significant contribution to turgor maintenance, because the internal Na^{+} and Cl^{-} concentrations did not increase in proportion to the external solution and there is also enough salt inside to balance the osmotic pressure of the salt outside. K^{+} is usually only 100 mM or less in leaves and declines with salinity but this was increased after two and three days of treatment with NaCl. Results in this research indicate that there must be increasing concentrations of K^{+}. Figs. I and II may indicate cell-specific localization of Na^{+} in the root which can provide insight into the major control points that limit Na^{+} loading of the xylem Na^{+} transport speed to leaf (Lauchli et al. 2005). In this survey, the highest concentrations of Na^{+} were 72 hours after the treatment by 100 and 200 mM NaCl indicating that it may provide a major control point in limiting Na^{+} uptake 24 hours after treatment of Na^{+} especially in 100 mM NaCl. The K^{+} accumulation distribution pattern was the reverse of the Na^{+} distribution pattern. It seems that the shoot ion uptake rate is not determined by the transpiration rate.

However it has been reported that the flux of ions to the shoot is largely independent of the flux of water; because water moves across root membranes through aquaporin and ions move across root membranes through ion channels or transporters (Tyerman et al., 2002). So, when transpiration rates fall, ion concentrations in the xylem sap increase (Amtmann and Sanders, 1999). It is well established that K^{+} uptake is not influenced by transpiration rates in non-saline soils (Smith et al., 1991). Na^{+} fluxes are also independent of transpiration rates in saline soils (Munns, 1985; Ball, 1988). The independence of K^{+} flux into leaves over Na^{+} flux rates can be seen in Fig. II. The low Na^{+} trait did not restrict turgor maintenance as K^{+} uptake was enhanced. It has been reported that species which cannot exclude 98% of the salt from the transpiration stream must have ways to handle the salt arriving in leaves as the water evaporates, and salt gradually builds up with time in the other leaves, the salt concentration will soon become high enough to kill the cells, unless they can compartmentalize the salt in vacuoles, thereby protecting the cytoplasm from ion toxicity (Rivelli et al., 2002). This compartmentation is exemplified by halophytes, which hold concentrations of over 500 mM on leaf basis, but which show no sign of injury (Greenway, 1962). Barley leaves like other numerous species have been reported to tolerate concentrations close to this without showing injury (Greenway, 1962; Rawson et al., 1988). In these species, salt must be sequestered in

vacuoles. Although this is difficult to measure directly, it must happen. In this research when plants were pretreated with SA and then exposed to 100-200 mM NaCl, the sensitivity of sweet basil to salinity stress was ameliorated. Under salt stress, basil seedlings accumulated more inorganic Na^+ ions similar to the results were reported in sugar beet cultivars (Ghoulam et al., 2002) in rice (Lutts et al., 1996) and in sorghum bicolor (Colmer et al., 1996). Decrease in K^+ uptake reportedly depressed growth at higher Na^+ concentrations (Sairam and Srivastava., 2002). It has been reported that The control of Na^+ accumulation and high shoot $K^+ : Na^+$ ratios may enhance salt tolerance in tomato crops (Perez-Alfocea et al., 1996; Cuartero and Fernandez-Munoz, 1999; Al-Karaki, 2000). In our experiment, the results for K^+ and Na^+ ratio were similar to those indicated by other researhers (Aqueel Ahmad et al., 2007; Molassiotis et al., 2006). SA application sharply decreased Na^+ content in stressed basil seedlings. This may indicate that pretreatment with SA induced a reduction of Na^+ absorption and toxicity, high water content and dry matter production. K^+ accumulation increased in shoots of the seedlings grown from SA spraying compared to those of untreated ones. On the contrary, salinized plants displayed shoot Na^+ accumulation as well as decrease in K^+. This imbalance has been reported widely and described as a mechanism of competition between cations (Agarwal and Pandey, 2004).

Conclusion

The differences observed in Na^+ and K^+ concentrations under salt stress showed that *Ocimum basilicum* is more tolerant under low salt stress than high salt stress. This species probably maintains the osmotic adjustment with accumulation of Na^+ and other compounds induced by Na^+ stresses and by Na^+ compartmentation. Meanwhile, under salt stress conditions, SA significantly improved the performance of this species that can be of potential importance in *Ocimum basilicum* production.

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