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Ameliorative Effects of Exogenous SA on Germination of Sorghum Under Salinity Stress

[1]Jahanbakhsh Behzadnejad, [2]Enayatolah Tohidinejad

[1] *Shahid Bahonar University, Department of Agronomy and Plant Breeding, Faculty of Agriculture, Box.76169. Kerman. Iran*
[2] *Shahid Bahonar University, Department of Agronomy and Plant Breeding, Faculty of Agriculture, Box.76169. Kerman. Iran*

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ABSTRACT

Background: Salicylic acid (SA), an endogenous plant growth regulator has been found to generate a wide range of metabolic and physiological responses in plants thereby affecting their growth and development**. Objective:** Experiment was conducted to investigate the effects of SA on sorghum plants under saline conditions at germination stage. Germination experiment was performed with three levels of SA and three levels of salinity as a factorial plan based on a completely randomized design. The treatments were included three levels of SA (0, 0.5 and 1 mM) and salinity (0, 75 and 150 mM). **Results:** Results revealed that salinity caused a significant decrease in germination characteristics and seedling growth of sorghum. Increasing in salinity concentration led to a significant decrease in the germination percentage, germination rate, mean germination time, seed stamina index and seedling dry weight while relative water content were reduced. Application of SA under salinity stress improved germination traits and increased dry weight. These results suggest that SA might induce salt tolerance in sorghum by preventing oxidative damage**. Conclusion:** Based on the results SA as a phytohormone play critical roles in plant responses to salinity and it can be concluded that hormonal priming with salicylic acid increase the ability of sorghum to grow successfully under saline conditions. Finally, in future, this hormonal priming treatment may be used for improving plant growth and yield in saline areas.

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INTRODUCTION

Salinity is one of the most important limiting factors for agriculture development around the world (Abdel Latef, 2010). Over 800 million hectares of land are salt-affected throughout the world (Munns, 2005).

Several physiological processes have been affected by salinity stress which results to a decreased growth and productivity (Yurtseven *et al*., 2005). Salinity disrupts cellular processes through several mechanisms such as inducing osmotic stress by limiting water absorption, and ionic stress as a result of high concentrations of toxic salt ions (Kohler *et al*., 2009). Salinity also causes nutritional disproportion through an increased uptake of Na+ or a decreased uptake of Ca2+ and K+ (Neel *et al*., 2002). The mechanism of salinity tolerance varies at cellular, molecular and the whole-plant levels (Munns and Tester, 2008).

Successful establishment of plants largely depends on successful germination. Germination is a crucial stage in the life cycle of plants and tends to be highly unpredictable over space and time. Several environmental factors such as temperature, salinity, light, and soil moisture simultaneously influence germination (El-Keblawy and Al-Rawai, 2006; Huang *et al*., 2003; Zia and Khan, 2004).

Salicylic acid (SA), an endogenous plant growth regulator has been found to generate a wide range of metabolic and physiological responses in plants thereby affecting their growth and development. In the present review, we have focused on various intrinsic biosynthetic pathways, interplay of SA and MeSA, its long distance transport and signaling. The effect of exogenous application of SA on bio-productivity, growth, photosynthesis, plant water relations, various enzyme activities and its effect on the plants exposed to various biotic and abiotic stresses has also been discussed (Hayat *et al*., 2010).

A high salinity induces serious metabolic perturbations in plants, as it generates ROS which disturb the cellular redox system in favour of oxidized forms thereby creating an oxidative stress that may damage DNA, inactivate enzymes and cause lipid peroxidation (Smirnoff, 1993). 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

Corresponding Author: Jahanbakhsh Behzadnejad, Shahid Bahonar University, Department of Agronomy and Plant Breeding, Faculty of Agriculture, Box.76169. Kerman. Iran.

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 (Tari *et al*., 02004; Szepesi *et al*., 2005). Accumulation of large amounts of osmolytes (proline) is an adaptive response in plants exposed to stressful environments (Rai, 2002). Wheat seedlings accumulated large amounts of proline under salinity stress which was further increased when salicylic acid was applied exogenously, thereby alleviating the deleterious effects of salinity (Shakirova *et al*., 2003). The exogenous application of salicylic acid prevented the lowering of IAA and cytokinin levels in salinity stressed wheat plants resulting in the betterment of cell division in root apical meristem, thereby increasing growth and productivity of plants (Shakirova *et al*., 2003). These authors also reported that the pre-treatment with SA resulted in the accumulation of ABA which might have contributed to the pre-adaptation of seedlings to salinity stress as ABA induces the synthesis of a wide range of anti-stress proteins, thereby providing protection to the plants. Further, the treatment also lowered the level of active oxygen species and therefore the activities of SOD and POX were also lowered in the roots of young wheat seedlings (Shakirova *et al*., 2003). These findings indicate that the activities of these antioxidant enzymes are directly or indirectly regulated by salicylic acid, thereby providing protection against salinity stress (Sakhabutdinova *et al*., 2004).

Regards to the importance of investigating the effects of SA on germination properties under salinity stress, the present study was conducted in order to evaluate germination responses of sorghum plants to exogenous application of SA under saline conditions.

1. Methodology:

Experiment was conducted to investigate the effects of SA on sorghum plants under saline conditions at germination stage. Germination experiment was performed with three levels of SA and three levels of salinity as a factorial plan based on a completely randomized design. The treatments were included three levels of SA (0, 0.5 and 1 mM) and salinity (0, 75 and 150 mM). Seeds were surface sterilized with %1 sodium hypochlorite (NaClO) for 5 minutes and washed with distilled water extensively. Then they were primed with different levels of SA and placed in petri dishes. Distilled water was used for control treatments. Salinity was applied in petri dishes and germination properties were recorded daily. After 10 days, seedlings were used to determine seedling dry weight and relative water content. All data were analyzed using Analysis of Variance (ANOVA) and the LSD was calculated at P = 0.05.

Germination, germination rate, mean germination time and seed stamina index calculated for sorghum by fallowing equations that were previously reported by others.

$G = (n / N) \times 100$ (Jefferson and Penachchio, 2003)

$RG = \Sigma (Ni / Di)$ (Jefferson and Penachchio, 2003)

$MGT = \Sigma (Ni \times Di) / (\Sigma Ni)$ (Khaled *et al*, 2007)

$SSI = [G \times (HL + RL)] / 100$ (Abdul-Baki and Anderson, 1970)

G: germination percentage, n: number of seeds germinated, N: number of seed planted, RG: rate of germination (seed day^{-1}), Ni: germinated seeds in each numeration, Di: day of each numeration, MGT: mean germination time (day), SSI: seed stamina index, HL: average of hypocotyls length (mm), RL: average of Radicles length (mm).

Relative water content (RWC) was determined using fresh leaf discs with 2 cm2 diameter. After weighting, the leaf discs were immersed on deionized water until 24 hours. Saturated leaf weight was recorded and the dry mass was noted after drying at 70 ◦C for 48 h. the following formula was used to calculate RWC.

$$RWC = \frac{\text{(Fresh weight-dry weight)}}{\text{(Turgor weight-dry weight)}} \times 100$$ (Hayat *et al*., 2005)

2. Results:

Germination percentage was significantly reduced due to the applied salinity level, but exogenous application of SA could improve germination ability of sorghum seeds (Fig. 1). Although the germination percentage was increased with application SA, but the differences between 0 and 1 mM SA were not significant. The highest and lowest of germination percentage belonged 0 mM salinity+0.5 mM SA and 150 mM salinity+1 mM SA respectively.

Deleterious effect of salinity treatments on germination rate was obvious (Fig.2). But exogenous application of SA improved germination rate of sorghum seeds under salinity levels. The trend of reducing germination rate of seeds under salinity stress was slower when plants exposed to levels of 0.5 and 1 mM SA. The germination rate of sorghum at the level of control (0 mM salinity+0 mM SA) was 10.7 seed day^{-1}. Application of 0.5 mM SA affected the germination rate of sorghum significantly but the higher concentration of SA (1 mM) had not significant effect on germination rate of sorghum in compared to control (0 mM salinity+0 mM SA). In general, the lowest germination rate was recorded at the highest extract concentration for these three crops (Figure 2).

The lowest mean germination time was recorded for level of 1 mM SA in all salinity levels. Rising levels of salinity concentration resulted in a significant decrease in this trait in sorghum so that the highest and lowest of

MGT were belonged to 0 and 150 mM of salinity. The effect of the first levels of SA (0.5 mM) on MGT was more effective in increase MGT in compared to control (0 mM SA).

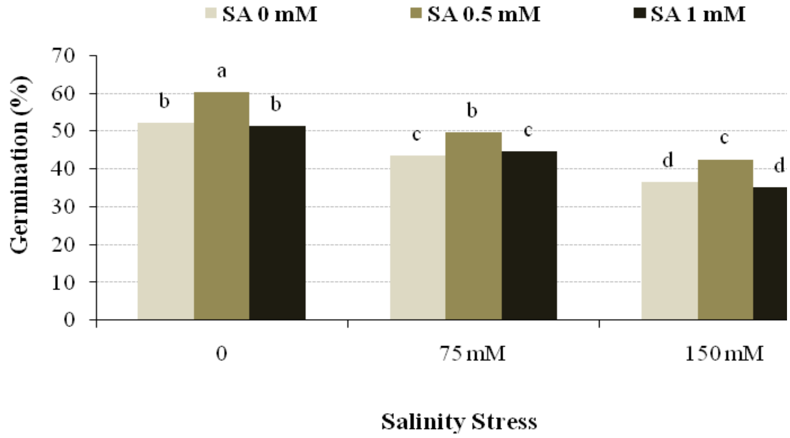

Fig. 1: Interaction of salinity and SA on germination percentage of sorghum.

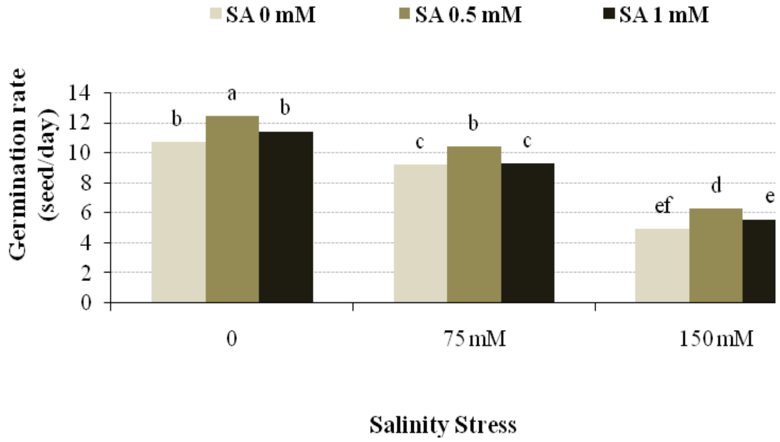

Fig. 2: Interaction of salinity and SA on germination rate of sorghum.

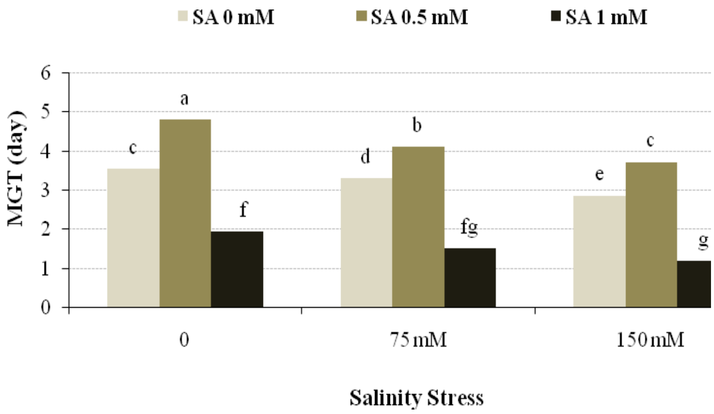

Fig. 3: Interaction of salinity and SA on mean germination time of sorghum.

In all three salinity levels, the lowest and highest seed stamina index was belonged to levels of 0 and 0.5 mM SA, respectively. Application of 1 mM SA in levels of 0 and 75 mM salinity had not significant effect on this trait in compared to 0 mM SA. While the effect of 0.5 and 1 mM SA in highest level of salinity stress was the same statistically (Figure 4).

Seedling dry weight as an important index of plant growth was affected by the interactions of salinity and SA. Salinity-reduced seedling dry weight was improved by applied SA treatments. Enhancing effect of SA on seedling dry weight was not elevated with increasing SA level and it was even slightly decreased. As it was shown in figure 5, priming with the first level of SA (0.5 mM) in all salinity levels resulted to a significant

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increase in seedling dry weight compared to control plants. The highest and lowest of seedling dry weight in all SA concentrations belonged to 150 mM and 0 mM salinity respectively (Figure 5).

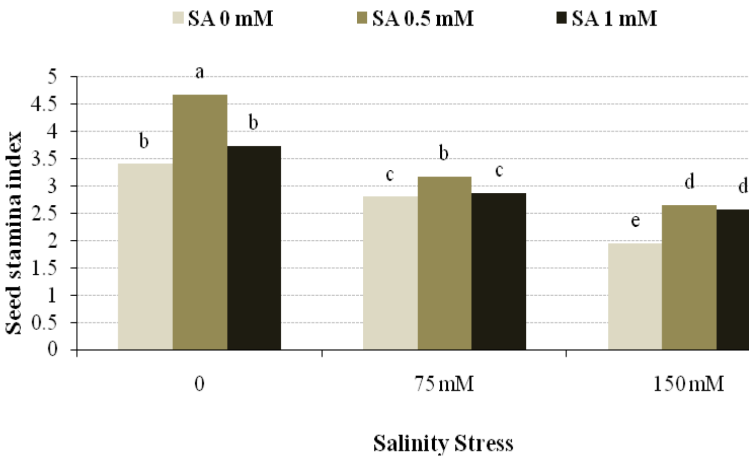

Fig. 4: Interaction of salinity and SA on seed stamina index of sorghum.

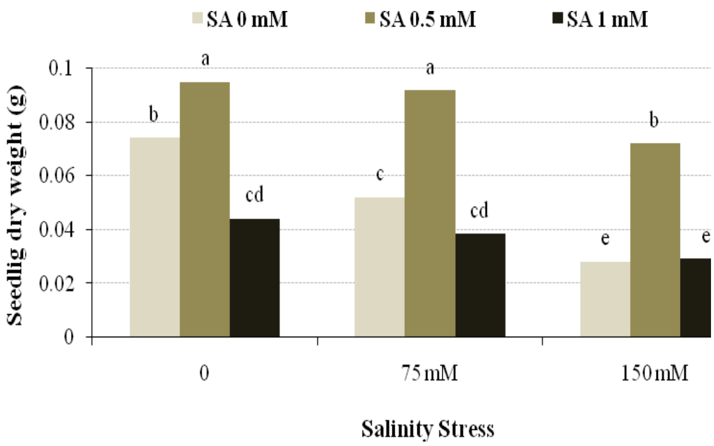

Fig. 5: Interaction of salinity and SA on seedling dry weight of sorghum.

The interaction between SA and salinity for RWC was significant (Fig. 6). RWC in sorghum leaves decreased with increasing in salinity concentration. As shown in Fig 6, in all salinity levels applying the first level of SA (0.5 mM) resulted to a significant increase in RWC compared to control plant. Percentage reduction in RWC under salinity stress for level of 0.5 mM SA was less than 1 mM. Results of membrane damage investigation based on RWC showed that membrane damage in leaves of sorghum increased along with increasing stress severity.

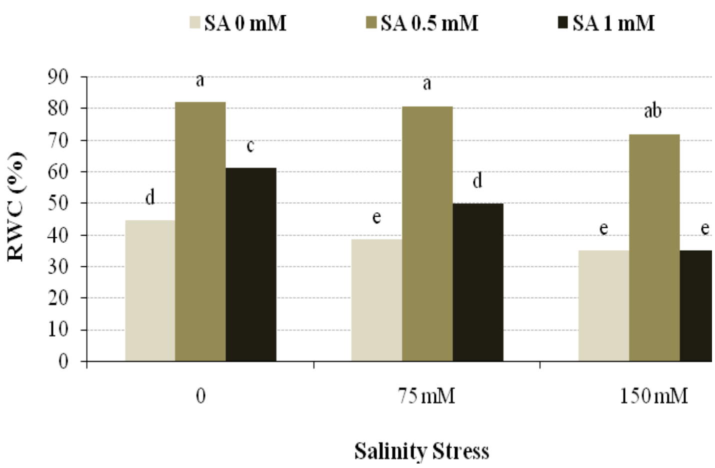

Fig. 1: Interaction of salinity and SA on relative water content of sorghum.

3. Discussion:

The germination characteristics and growth of sorghum seeds pre treated with different levels of SA were studied at Salinity stress induced by NaCl. SA could be a promising compound for the reduction of abiotic stress sensitivity in plants and under certain condition, it has been found to mitigate the damaging effects of various stress factors (Horvath *et al*., 2007). Soaking seeds in SA solution before sowing probably caused a concentration dependent increase in both the free and bound SA contents of the seeds. Previous studies demonstrated that SA or related compounds could be used as effective preventive compounds against oxidative damage in plants.

The reduced rate of germination by NaCl may be due to the reduced water potential and the resulting slower rate of imbibitions'. From present investigations, it is quite clear that various concentrations of SA proved to be effective in inducing salt tolerance at the germination stage of sorghum. These results are in agreement with those obtained by other researchers such as Pasandi Pour *et al*. (2012); Farahbakhsh and Pasandi Pour (2012), showing wheat germination significantly decreased by salinity. The results of germination percentage can be related to findings in which El-Tayeb (2005) found an improvement in seeds pretreated with SA solution than those of un-treated (control) seeds.

Dry weights of seedlings decreased progressively due to salinity compared to control. These results are in agreement with those of Pasandi Pour *et al*. (2012), who showed that salinity caused a marked reduction in growth parameters of shoots and roots of fenugreek plants but shoot and root dry weight were increased in seedlings raised from seeds primed with 10 µM SA, which confirms our results. Singh and Ushu (2003) also found that SA application increased the dry mass of wheat seedlings under water stress. It supposed that the protective and growth promoting effect of SA are due to increased level of cell division within the apical meristem of seedling root, which caused an increase in plant growth. These observations are in consistent with those of Khodary (2004), who reported that SA increases the fresh and dry weight of shoot and roots of stress maize plants. Several reports have published in the last decade demonstrating the role of SA in various physiological processes, especially weights (Korkmaz, 2005).

RWC in leaves is considered as an alternative measure of plant water status, reflecting the metabolic activity in plant tissues (Flower and Ludlow, 1986). Results show that salt stress significantly declined RWC compared to the control treatment. The decrease in RWC under salinity stress has already been reported (Srivastava *et al*., 1998). This decrease could be attributed to root systems which are not able to compensate for water lost by transpiration through a reduction of the absorbing surface (Gadallah, 2000; Yildirim *et al*., 2008). SA treatments elevated RWC to a level higher than the non-treated salt stressed plants. Increased RWC by SA application under salinity stress were reported (Pasandi pour *et al*., 2012; Farahbakhsh and Pasandi pour, 2012).

4. Conclusion:

In general, based on the results SA as a phytohormone play critical roles in plant responses to salinity and it can be concluded that hormonal priming with salicylic acid increase the ability of sorghum to grow successfully under saline conditions. Finally, in future, this hormonal priming treatment may be used for improving plant growth and yield in saline areas.

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