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Salinity Tolerance of Kentucky Bluegrass as Affected by Salicylic Acid

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Abstract

Salinity is one of the greatest environmental challenges facing plant growth and development in the 21st century. Salicylic acid (SA) is a phenolic compound and signal molecule involved in the regulation of plants responses to biotic and abiotic stresses. This greenhouse experiment was conducted to determine effects of SA application on Kentucky bluegrass (*Poa pratensis* L.) responses to salinity stress. The three salinity levels (0, 40 and 80 mM NaCl) were applied in nutrient solutions, and foliar SA treatments (0, 1 and 2 mM) were applied at 2-weeks intervals. The study was carried out as a factorial experiment based on completely randomized experimental design with four replications. Salinity reduced root and shoot dry weight, visual turf quality, leaf chlorophyll and potassium content, whereas electrolyte leakage, proline and sodium content were increased with salt concentration in nutrient solution. Salicylic acid application ameliorates adverse effects of salinity in all factors and this effect was more pronounced in 80 mM NaCl. In terms of root dry weight, leaf sodium and proline content as well as electrolyte leakage, SA application at 2 mM had better results than 1 mM. These results suggest that further studies are required to find proper SA application rate in different salinity levels.

Keywords: Electrolyte leakage, Growth, Salt stress, Signal molecule, Turfgrass.

INTRODUCTION

Salinity is one of the greatest environmental challenges facing plant growth and development in the 21st century (Handmer *et al*., 2012). Rapidly expanding population growth is occurring in many arid regions, where soil and water salinity are problems. Fresh water shortage has resulted in restrictions on the use of potable water for turfgrass irrigation, and highly saline secondary water sources are increasingly being used to irrigate landscape and large turf facilities (Leinauer *et al.,* 2010).

The detrimental effects of salinity on turfgrass growth include ionic toxicity, osmotic stress (osmotic inhibition of plant water absorption), and secondary stresses such as nutritional disorders and oxidative stress. Salt tolerance in plants is a complex phenomenon involving morphological, physiological and biochemical processes (Pessarakli, 2010). Kentucky bluegrass (*Poa pratensis* L.) is a cool season grass widely used for home lawns, sport fields, and commercial landscapes in temperate climates and considered to be salt sensitive with an average threshold EC of 3 dS m-1 (Koch *et al*., 2011).

Salicylic acid (SA) (2-hydroxybenzoic acid, $C_7H_6O_3$) is a phenolic compound and signal molecule involved in the regulation of growth and development of plants and responses to biotic and abiotic stresses (Bartoli *et al.,* 2013). Recent studies have investigated the influence of SA on enhancing environmental stress tolerance such as drought (Yazdanpanah *et al*., 2011), heat (Khan *et al*., 2013), salinity (Li *et al.*, 2013) and heavy metal stress (Zhang *et al.*, 2015).

Effects of SA on turfgrass responses to environmental stresses are less understood and are still unsettled. He *et al.* (2005) reported that SA application enhanced heat tolerance in Kentucky bluegrass and SA could be involved in the scavenging of active oxygen species by increasing superoxide dismutase and catalase activities under heat stress. Chen *et al*. (2014) showed that suitable exogenous SA (0.5 mM) helps zoysia grass to perform better under drought stress. They suggested that SA has this effect by enhancing the net photosynthetic rate and antioxidant enzyme activities while decreasing lipid peroxidation as compared to the controls. Also, Hosseini *et al.* (2015) showed that under drought stress condition, SA foliar application increased the content of chlorophyll a, b and reduced electrolyte leakage, proline accumulation and antioxidant enzyme activity in perennial ryegrass. However, Azimi *et al.* (2014) showed that SA application decreased annual bluegrass (*Poa annua* L.) growth.

The present study aimed at investigating the effect of SA application on salinity tolerance of Kentucky bluegrass and comparing morphological and physiological effects of different rates of SA application on Kentucky bluegrass under salinity levels.

MATERIALS AND METHODS

Turfgrass culture and growth condition

'Barimpala' Kentucky bluegrass (*Poa pratensis* L.) was seeded in plastic pots filled with washed sand. Plants were grown in a greenhouse at the University of Zanjan with average day/night temperatures of 25/15 °C under natural light. Pots were fertigated daily with half strength Hoagland's solution as long as drainage occurred from the bottom of the containers for 4 months prior to initiation of treatments. Turf was hand-clipped weekly at a 5-cm height.

Treatments and experimental design

Three salinity treatments (0, 40 and 80 mM NaCl) were obtained by adding NaCl gradually (to avoid salinity shock) to nutrient solutions during a 5-day period. Salicylic acid was applied at 0, 1 and 2 mM, on three occasions, at the start of the treatments, 2 and 4 weeks after salt treatments were initiated. Grasses were exposed to salinity and SA treatments for a period of 6 weeks. The study was conducted as a factorial experiment based on completely randomized design with four replications.

Measurements

During treatments period, grass clippings (leaves and some stems) that have been cut off by mowing, were harvested on a weekly basis and were dried at 70 °C for 48 h for dry weight determination. Following the final clipping harvest after 8 weeks of salinity treatments, grass swards were harvested and divided into verdure and roots. Each fraction was, then, dried at 70°C for 48 h to determine dry mass. Shoot dry weight was calculated based on cumulative clipping and verdure dry weight (Qian *et al.,* 2000).

Data on leaf chlorophyll, proline, electrolyte leakage, K+ and Na+ content, and turf quality were determined at the end of experiment.

Turf quality was visually rated on a scale of 1 to 9 based on color, density, and uniformity (Turgeon, 2002). Plants rated 1 were completely desiccated with a completely necrotic turf canopy. A rating of 9 represented healthy plants with dark green, turgid leaf blades, and a full turf canopy.

Chlorophyll was extracted by homogenizing 0.1 g fresh leaves in 8 mL of 80 % acetone. Absorbance of the extract at 663 and 645 nm was measured with a spectrophotometer and total chlorophyll concentration was calculated using the formulas described by Arnon (1949).

Proline content was measured according to the method of Bates *et al.* (1973). A 0.1 g sample of fresh leaves was homogenized in 1.5 mL of 3 % aqueous sulfosalicylic acid and the residue was removed by centrifugation at 15000 × g for 20 min. Then, 1 mL of extract was mixed with 2 mL of ninhydrin (1.25 g ninhydrin warmed in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid until dissolved) and 2 mL of glacial acetic acid and heated at 100 °C for 1 h. The reaction was terminated in an ice bath. Then 4 mL of toluene was added to the mixture and content of tubes were stirred for 15 to 20 s. The chromophore was aspirated from the aqueous phase, and the absorbance was read at 520 nm. The amount of proline was determined from a standard curve.

Cell membrane stability was estimated by measuring electrolyte leakage (EL) from leaf tissues by the modified method of Wang and Huang (2004). Samples of fresh leaves (0.1 g) were rinsed and immersed in 20 mL of deionized water. The conductivity of the solution was measured after the leaves were shaken for 24 h. Leaves were, then, heated in boiling water bath for 20 min. The conductivity of the killed tissues was measured after samples were cooled down to room temperature. Relative EL was calculated as the percentage of initial conductivity over conductivity of the killed tissues.

To determine K+ and Na+ content, leaves were rinsed thoroughly and dried at 70 °C for 2 d. Ground samples were dry-ashed at 550 °C for 4 h, mixed with hot 2 M HCl, filtered, and then brought to a final volume of 50 ml with distilled water. K+ and Na+ content were determined in these digests using an Eppendorf flame photometer (Chapman and Pratt, 1982).

The data were statistically analyzed using the analysis of variance procedure (SAS Institute, 2001). Differences between treatment means were compared by Duncan's multiple range test at 0.05 probability level.

RESULTS AND DISCUSSION

Shoot and root dry weight

Salinity reduced shoot dry weight regardless of SA levels while decline in root dry weight was observed only in 80 mM NaCl. SA-treated plants showed higher shoot dry weight than untreated plants in both 40 and 80 mM NaCl. No significant difference existed between 1 and 2 mM SA treatments. Under 40 mM NaCl, root dry weight was not affected by SA treatments whereas, in 80 mM NaCl, root dry weight was increased with SA application rate (Fig. 1, A and B).

Fig. 1. The salinity and salicylic acid interaction effect on shoot dry weight (A), root dry weight (B), turf quality (C), leaves total chlorophyll (D), sodium (E), potassium (F), proline (G) and electrolyte leakage (H) of Kentucky bluegrass. In each figure, values followed by the same letter(s) are not significantly different at 5% level (DMRT).

Table 1. Analysis of variance of the effects of salinity and salicylic acid on measured characteristics in Kentucky bluegrass.

S.o.V	df	Mean square							
		Shoot dry weight	Root dry weight	Turf quality	Total chlorophyll	Sodium	Potassium	Proline	Electrolyte leakage
Salinity	2	57647.1**	5417.0**	19.28**	0.0967**	455.61**	356.82**	437.56**	1017.7**
Salicylic acid (SA)	2	692.0**	203.8**	0.80**	0.1630**	21.08**	11.654**	21.56**	59.2**
Salinity × SA	4	847.5*	171.6**	0.08*	0.0786**	5.80**	2.733*	60.83**	44.1**
Error	27	197.1	21.89	0.029	0.0142	0.1617	1.0114	0.7115	3.260
CV (%)	-	3.96	5.85	2.65	5.06	5.74	3.31	7.29	6.51

* and **: Significant at the 1% and 5% level of probability, respectively.

It is well understood that salinity reduces turfgrass shoot and root growth due to decline in leaf area, photosynthesis, stomatal conductance, and relative water content (Uddin and Juraimi, 2013). There are some other reasons for growth loss by salinity such as the production of compatible osmolytes like proline and glycine betaine. Production of these high carbon compounds reduces plant growth indirectly. Additionally, under salt stress condition plants consume considerable energy for active ion transport that makes plants weak (Taiz and Zeiger, 2002). Root development is one the salinity tolerance mechanisms in plants that enhances plant ability for the uptake of water and nutrient, and in some turfgrass species root growth enhancement under salinity stress has reported (Alshammary *et al.*, 2004). Similarly, in present study, a little increase in root dry weight was detected in 40 mM NaCl. However, severe shoot growth decline in 80 Mm NaCl affected root growth, and plants showed significantly lower root dry weight than control.

Increased shoot and root growth by SA have also been observed in different turfgrasss species (Beiraghdar *et al.*, 2014; Nasri and Ghaderi, 2014). Positive effect of SA on growth could be related to the increase in net photosynthetic rate and carboxylation efficiency by salicylic acid (Fariduddin *et al.*, 2003; Babar *et al.,* 2014).

It is reported that treatment of SA (0.5 mM) alleviated the negative effects of salt stress and improved photosynthesis and growth through the increase in enzymes of ascorbate-glutathione pathway, which suggests that SA may participate in the redox balance under salt stress (Nazar *et al.,* 2015).

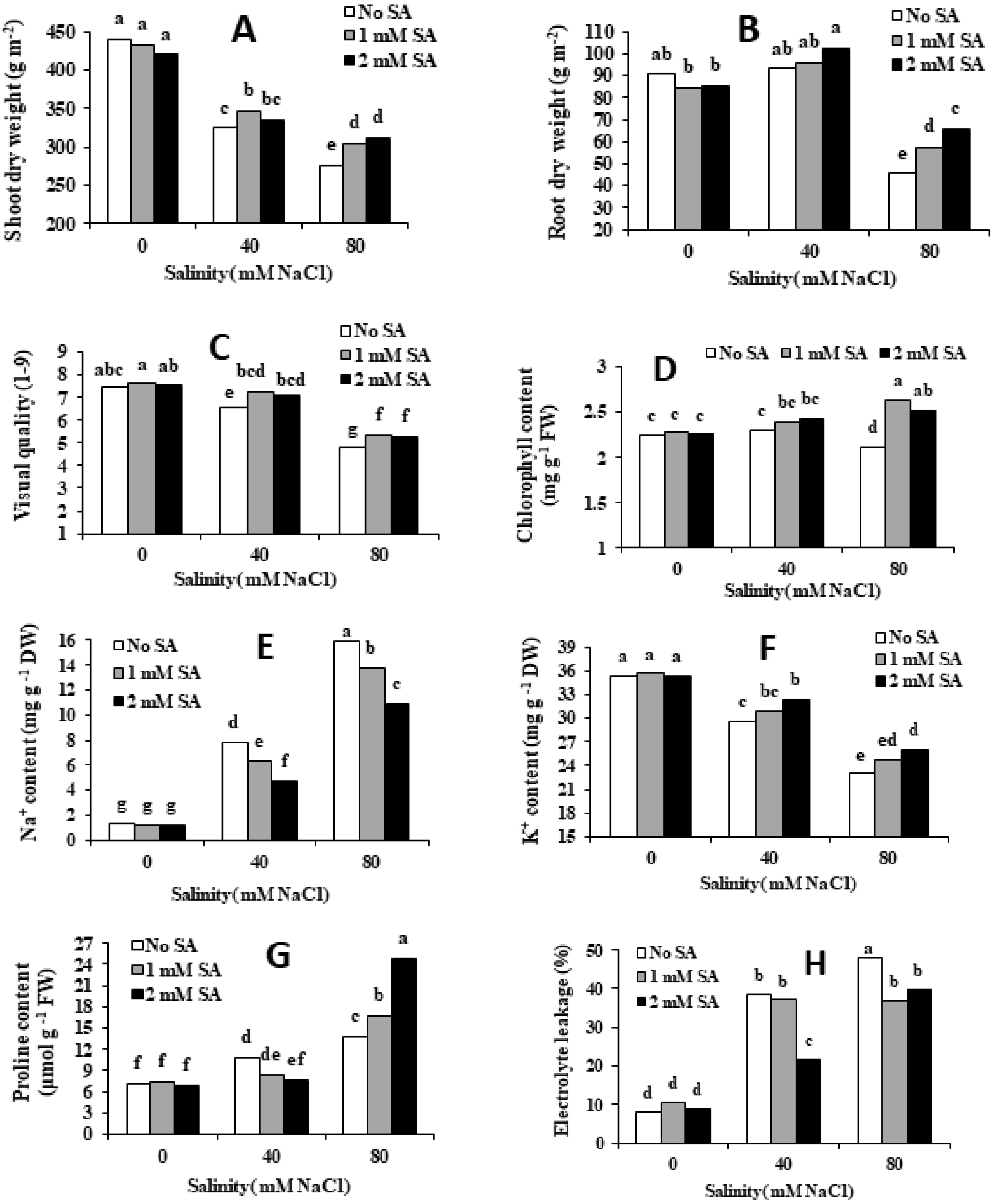
Turf quality

Turf quality declined with the increase in salinity, while plants treated with SA maintained a higher quality compared with untreated plants. No significant difference was observed between 1 and 2 mM SA treatments (Fig. 1, C).

In this research, turf quality was visually rated based on color, density, and uniformity. Decline in turf quality by salinity may be correlated with the effect of salt stress on shoot growth that reduces turf density and uniformity. Also, salinity had a negative effect on turf color by reducing leaf chlorophyll content, as shown in other studies (Arghavani *et al.*, 2012; Uddin and Juraimi, 2013). Enhanced turf quality by SA could be associated with its positive impact on Kentucky bluegrass shoot growth and chlorophyll content under salt stress conditions. Previous work on ryegrass (*Lolium perenne* L.) has suggested that higher quality of turf induced by SA is due to enhancement of turf density (Beiraghdar *et al.*, 2014). Also, our results were consistent with Shahgholi et al. (2013) who reported that SA improved turfgrass quality in ryegrass.

Total chlorophyll content

Leaf chlorophyll content in unstressed plants and 40 mM NaCl treatment did not change



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significantly, and there was not a significance difference among SA treatments, while in 80 mM NaCl, SA-treated turfs at 1 and 2 mM showed higher chlorophyll content than untreated plants (Fig. 1, D).

In high salinity level, chlorophyll content of untreated plants was decreased maybe due to an increase in chlorophyll degradation or to a decrease in chlorophyll synthesis (Santos, 2004). Salt stress, like other abiotic stresses induces oxidative stress, resulting from the increase in reactive oxygen species (ROS) production such as superoxide, hydrogen peroxide and hydroxyl radicals. Accumulation of ROS causes extensive damage such as chlorophyll breakdown, loss of membrane integrity, and photosynthesis. Our results showed that SA treatment helped to maintain higher chlorophyll content under salt stress that is in agreement with the findings of some recent researchers (Agamy *et al.*, 2013; Li *et al.,* 2014; Salachna *et al.*, 2015). This enhanced chlorphyll content could be related to enhanced activities of ROS scavenging enzymes by SA that has been observed in several studies (Li *et al.*, 2013; Hasanuzzaman *et al*., 2014; Abdul Qados, 2015; Dong *et al.*, 2015). Additionally, SA has been reported to increase leaf iron (stimulator of synthesis of chlorophyll) concentration (Kong *et al*., 2014).

Sodium and potassium content

Leaf Na+ content was increased and K+ content was decreased with salinity. Salicylic acid application reduced Na+ content and increased K+ content in both salinity levels even though leaf K+ content was not significantly different between untreated plants and turf treated with 1 mM SA. Also, there was no significant difference between 1 and 2 mM SA treatments with respect to K+ content (Fig. 1, E and F).

Plant cells need to maintain a favorable K+/Na+ ratio in the cytosol. Under salt stress, high sodium uptake competes with the uptake of K+, which is necessary for the maintenance of cell turgor and normal enzymatic activities (Demidchik and Maathuis, 2007). It has found that SA application may enhance the activity of H+-ATPase, decrease NaCl-induced membrane depolarization, and minimize NaCl-induced K+ leakage from the cell (Jayakannan *et al*., 2013). Our results were confirmed with those obtained by Dong et al. (2015) and Salachna *et al.* (2015) who reported that SA increased shoot K+ and decreased Na+ accumulation under salt stress.

Proline content

Leaf proline content was increased with salinity. However, in 0 and 40 mM NaCl, no significant difference existed in proline level among SA treatments. In 80 mM NaCl, proline content was increased with the increase in SA application rate (Fig. 1, G).

Proline is one of the most common compatible solutes or osmoprotectant whose accumulation has been correlated with salinity and tissue Na+ concentration for several turfgrass species (Manuchehri and Salehi, 2015; Alshammary *et al*., 2004). Our results showed that under high salinity level, SA treatment increased leaf proline content. This result agrees with some previous reports (Agamy *et al.,* 2013; Abdul Qados, 2015). Effect of SA on proline accumulation might have been due to the enhancement in proline metabolism. It has been found that under salt stress, proline biosynthesis enzymes, pyrroline-5-carboxylate reductase and gamma glutamyl kinase activities are increased (Misra *et al.*, 2010).

Electrolyte leakage

A sharp increase in leaf electrolyte leakage was observed with the increase in salinity. In 40 mM NaCl, untreated turfs and plants treated with 1 mM SA had higher proline content than 2 mM SA treated plants while in 80 mM NaCl, the highest electrolyte leakage was observed in untreated turfs turf that was lower than that detected in both SA treatments (Fig. 1, H).

Cell membranes are one of the targets of reactive oxygen species that are produced under stress conditions. Therefore, electrolyte leakage from the plasma membrane has been used as a measure for plant tissue injuries. In several studies on grasses, electrolyte leakage has been increased by salinity (Liu *et al*., 2011; Wang *et al.*, 2011; Manuchehri and Salehi, 2015). Our results were confirmed with the reports of other studies where salt-induced increase in the electrolyte leakage is alleviated by SA application. (Agamy *et al.*, 2013; Dong *et al.,* 2015). Less electrolyte leakage of leaves by SA could be associated with the increase in antioxidant enzymes in treated plants (Hasanuzzaman *et al*., 2014; Li *et al*., 2014; Abdul Qados, 2015; Dong *et al*., 2015). Moreover, SA has been reported to stimulate calcium uptake, and calcium is known to help cell membrane integrity (Noreen *et al.*, 2011).

CONCLUSION

In summary, based on results SA application was beneficial for Kentucky bluegrass survival under salt stress, as manifested by improved turf dry weight and quality under stress conditions. Increased salt tolerance due to SA application could be related to the effects of SA on the increased proline, K+ and chlorophyll content and decreased Na+ uptake. Our data indicate that, in some characteristics, SA application at 2 mM had better results than 1 mM, suggesting that additional studies are required to find proper SA application rates in different salinity levels.

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