Cercetări Agronomice în Moldova
Vol. XLVII , No. 3 (159) / 2014

COMPARISON OF DIFFERENT TREATMENT METHODS OF SALICYLIC ACID ON SOME PHYSIOLOGICAL TRAITS OF WHITE BEAN UNDER SALINITY STRESS

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Received February 3, 2014

ABSTRACT. It has been shown that salicylic acid (SA) acts as an endogenous signal molecule responsible for inducing biotic and abiotic stress tolerance in plants. The effect of three application methods (Soil, Foliar and Priming) and four salicylic acid concentrations (0, 0.1, 0.5 and 1.0 mM) on chlorophyll a, b and total chlorophyll, carotenoids, proline, protein and soluble sugars of NaCl (4 ds/m) stressed white bean (*Phaseulus vulgaris* L.) was investigated. The results showed that the effect of applied concentrations and application methods on chlorophyll a and total chlorophyll, proline, protein and soluble sugars were significant. The interaction of concentrations and application methods used was significant on protein, proline and soluble sugars. According to the results, the greatest impact was belonged to the soil treatment which was not significantly different from priming. Among applied concentrations, the concentration of 0.1 and 0.5 mM were the most effective and the concentration of 1 mM was not significantly different from the control.

Key words: Application method; Salinity; Salicylic acid; White bean.

INTRODUCTION

Salinity is one of the important environmental problems limiting plant production. Under salt stress, plants cope with water deficit induced by low external water potential, and with ion toxicity due to accumulation of harmful ions inside the plant (Munns, 2005). Salt specific effects on plants develop with time and degree of ions accumulation, which eventually rise to toxic level and impose an additional stress on physiological and bio-chemical processes (Munns, 2002). Survival under these stressful conditions depends on the plant's capability to perceive the stimulus, generate and transport signals and provoke biochemical changes that adjust the metabolism accordingly (Hasegawa *et al.,* 2000). Salt stress has toxic effects on plants and lead to

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metabolic changes, like loss of chloroplast activity, decreased photosynthetic rate and increased photorespiration rate which then leads to an increased reactive oxygen species production (Parida and Das, 2005). Poor germination and seedling establishment are the results of soil salinity. It is an enormous problem adversely affecting growth and development of crop plants and results in low agricultural production (Garg and Gupta, 1997).

Strategies for alleviation of salt stress involve developing salt resistant cultivars, leaching excess soluble salts from soil layers, flushing soils that contain salt crusts at the surface, reducing salt by harvesting salt accumulating aerial plant parts in areas with negligible irrigation water or rainfall for leaching, and amelioration of saline soils under cropping and leaching (Qadir *et al.,* 2000).

Breeding for tolerance to salinity in crops has usually been limited by a lack of reliable traits for selection. Multiple genes seem to act in concern to increase salinity tolerance, and certain proteins involved in salinity stress protection have also been recognized (Murillo-Amador *et al.,* 2006). Other strategies may also be costly. Therefore, the development of methods and strategies to ameliorate deleterious effects of salt stress on plants has received considerable attention (Senaratna *et al.,* 2000).

Salicylic acid is a common plant-produced phenolic compound that can act as growth regulator and has been reported to induce significant effects on various biological aspects in plants (Horvath *et al.,* 2007). This compound influences in a variable manner: inhibiting certain processes and enhancing others (Raskin, 1992). SA influence a range of diverse processes in plants, including seed germination (Cutt and Klessig, 1992), ion uptake and transport (Harper and Balke, 1981) photosynthesis and growth rate (Khan *et al.,* 2003). This substance naturally is produced in plants in very low amounts. SA appeared as a signal molecule or chemical messenger and its role in defense mechanism has been well established in plants (Gunes *et al.*, 2007). SA plays an important role in abiotic stress tolerance, and more interests have been focused on SA due to its ability to induce a protective effect on plants under certain adverse environmental conditions. It is well known the fact that SA controls tolerance to salinity (Sakhabutdinova *et al.,* 2003; Shakirova and Bezrukova, 1997), osmotic stress (Singh and Usha, 2003), mineral deficiency and oxidative stress (Gunes *et al.*, 2007). However, the physiological and biochemical basis for its mechanism of action in abiotic stress tolerance is still not clear (Gautam and Singh, 2009).

Bean is considered as a sensitive plant to salinity and is adversely affected by salt stress in terms of growth and yield. Therefore, this experiment focused on the effect of different methods and concentrations of SA on bean under salt stress.

MATERIALS AND METHODS

A pot experiment was conducted at the research farm of agricultural college of Urmia University, Urmia, Iran, in 2011. The experiment was a factorial based on complete randomized block design with two factors and four replicates. Factor A was three methods of application (Soil application, Foliar application and Seed Priming) and factor B was four concentrations of SA (0, 0.1, 0.5 and 1.0 mM). The pots were filled with soil having salinity of 5 dS/m. Before priming, the seeds were surface sterilized with 1% sodium hypochlorite solution for 5 min, then washed with sterilized water and air-dried. Then seeds were soaked in specified amount of each solution for 12 h and redried in room temperature. Seeds were sown with a density of six plants per pot. Other seeds were planted without any treatment. Soil and foliar treatment was carried out at the beginning of vegetative phase and beginning of reproductive stage. Soil treatment was done by adding of 0.5 liter of each solution of salicylic acid in each pot. Foliar treatment was carried out on plants with the spray of 60 ml of all concentrations of the solution mentioned above. Pots irrigations in all stages of plant growth were conducted up to field capacity, so that no water will be removed from the pots. Sampling was performed to determine traits such as chlorophyll a and b, total chlorophyll, carotenoid, proline, protein and soluble sugars at the beginning of pod formation stage. The data collected was analyzed using the SAS and MSTAT-C software's and the treatment means were compared by using Duncan's multiple range test at 0.05 probability level.

RESULTS AND DISCUSSION

Analysis of variance results showed that application methods and concentrations had significant effect on chlorophyll a, total chlorophyll, protein, proline and soluble sugars at 1% level of probability (*Table 1*). Interaction of application methods and concentrations was significant on proline, protein and soluble sugars at the 1% level of probability but there isn't any significant effect on chlorophyll b and carotenoids (*Table 1*).

Chlorophyll a

Comparison of results showed that among application methods, soil application had the greatest impact on chlorophyll a which had not significant difference with priming. Foliar application had the least effect on chlorophyll a (*Table 2*). The most effective concentration was 0.1 mM which its difference with treatment of 0.5 mM was not significant and the least effect was related to the control (*Table 3*).

Salt stress decreases the chlorophyll content in leaves of many crops (Parida and Das, 2005). The decrease is because of the adverse effect of ions of different salts in chlorophyll biosynthesis. Salinity affects the amount of chlorophyll by stopping certain enzymes responsible for synthesis of green pigments in plants (Ashraf, 1989). One of the most important reasons that reduce chlorophyll content in salt stressed plants, is destructive effect of reactive oxygen species (ROS). SA decreases the damaging influences of reactive oxygen species on chlorophyll by activating antioxidant systems (Kaya *et al.,* 2001). It has been shown that the use of salicylic acid, increased the content of chlorophyll a in bean plants in salinity stress (Türkyilmaz *et al.,* 2005).

Table 1 - Effect of application methods and concentrations of salicylic acid on some bean traits under salinity stress

Source of Variation	Degree of freedom	Chlorophyll a	Chlorophyll b	Chlorophyll total	Carotenoids	Protein	Proline	Soluble sugars
Replicate	3	0.003^{ns}	0.003^{ns}	0.009**	0.00^{ns}	0.887^{ns}	8.72*	16*
Application method (A)	2	0.011**	0.004^{ns}	0.116**	0.00^{ns}	22.62**	27.02**	55.79**
Concentration (C)	3	0.034**	0.009^{ns}	0.072**	0.0000^{ns}	24.97**	302**	330.2**
A× C	6	0.003^{ns}	0.003^{ns}	0.02^{ns}	0.000^{ns}	4.27**	21.35**	34.72**
Experimental error	33	0.002	0.003	0.014	0.0001	0.874	3.10	4.99
Coefficient of variation	-	5.85	9.66	8.89	4.49	3.77	4.97	4.97

*, **, ns: significant at p ≤0.05, p ≤0.01and not significant, respectively.

Table 2 - Comparison of salicylic acid application methods on bean chlorophyll content

Treatment	Chlorophyll a	Total chlorophyll
Soil application	0.803a	1.385a
Foliar application	0.751b	1.260b
Priming	0.788a	1.293ab

Different letter in each column indicate significant differences at 5% level of probability.

Table 3 - Comparison of different concentration of salicylic acid application on bean chlorophyll content

Treatment	Chlorophyll a	Total chlorophyll
Control	0.715c	1.268b
0.1 mMl	0.836a	1.386a
0.5 mM	0.809a	1.333ab
1.0 mMl Priming	0.762b	1.263c

Different letter in each column indicate significant differences at 5% level of probability.

Total chlorophyll

Mean comparison showed that soil application had the greatest impact on total chlorophyll which was not significantly different from priming treatment. Foliar application was the least effective (*Table 2*). Among applied concentrations the most effective was the concentration of 0.1 mM which its difference with concentration of 0.5 mM was not significant (*Table 3*).

Chlorophyll reduction in salinity is probably due to the loosening of chlorophyll binding with chloroplast proteins, and the toxic effect of sodium and chloride ion concentration under salinity. Also reduced chlorophyll content under salinity is due to degradation of pigments and instability of pigment protein compounds (Levitt, 1980). SA is supposed to increase the functional state of the photosynthetic machinery in plants either by the mobilization of internal tissue nitrate or chlorophyll biosynthesis (Shi *et al.,* 2006). SA has also been reported to have stimulatory effects on photosynthetic capacity in maize plants through the induction of Rubisco activity (Khodary, 2004). SA decreases the ACC synthase activity which causes the production of ethylene in the plant (Li *et al.,* 1992).

Ethylene will cause the loss of chlorophyll in plants (Arfan, 2007). Azooz (2009) in bean and Arfan (2007) in spring wheat showed that pretreatment with salicylic acid, significantly increased the total chlorophyll content in salinity condition.

Seed protein content

Comparison of means indicated that the interaction of soil application with concentrations of 0.1 mM of SA was the most effective treatment in term of seed protein content and its difference with soil treatment of 0.5 and priming of 0.1 mM was not significant. The least effective treatment was foliar application of 1 mM which it's difference with soil and priming of 1 Mm was not significant (*Table 4*). Decrease in protein content in plants under salt stress may be due to the reducing availability of amino acids and deformation of enzymes that are necessary in the synthesis of amino acids and proteins (Levitt, 1980). Salicylic acid increases the uptake of potassium in salinity conditions, potassium being essential for protein synthesis. Moreover, salinity causes production of reactive oxygen species in plant. This ROS with oxidative damage can cause the loss of protein and nucleic acids (Kim *et al.,* 2005).

Salicylic acid enhances antioxidant system and reduces the destructive effects of ROS on proteins (Ghorbani *et al.,* 2004). Arfan (2007) showed that salinity significantly reduced the mean protein in wheat under salinity stress, but application of salicylic acid significantly increased protein content in plants.

Proline

Results showed that soil application with concentrations of 0.1 mM of salicylic acid had the greatest impact which was not significantly different from soil treatment of 0.5 and priming of 0.1 and 0.5 mM. Foliar application of 0 mM had the least impact which hadn't any significant difference with soil and priming of 0 mM and foliar application of 1 mM (*Table 4*).

Table 4 - Comparison of interaction between methods and concentrations of salicylic on physiological traits of bean

Treatment		Protein	Proline	Soluble sugars
Soil application	0 mM	22.91d	28.75c	39.63 f
	0.1mM	28.72a	42.75a	55.30 a
	0.5 mM	27.62a	40.25ab	52.94 b
	1 mM	24.97bc	30c	39.86 f
Foliar application	0 mM	22.73d	28.5 c	39.85 f
	0.1mM	24.58bc	37 b	45.87bcd
	0.5 mM	24.07bc	37.25b	44.86cde
	1 mM	23.35cd	31c	40.72 ef
Priming	0 mM	23.48cd	30c	43.94de
	0.1mM	26.61ab	39 ab	50.85 bc
	0.5 mM	25.16bc	38.25 ab	47.06 bcd
	1 mM	24.66bc	36.25b	43.96 de

Different letter in each column indicate significant differences at 5% level of probability.

Salinity induces accumulation of proline in plants. Researchers suggest that proline is an important part in the spectra of SA-induced ABA-mediated protective reactions of wheat plants in response to salinity, which contribute to a reduction of deleterious effects of stress factors and acceleration of recovery processes during the period after stress, which might be appearance of the protective action of SA on wheat plants (Kuznetsov and Shevyakova, 1999). Since proline is one of the important compounds of defense reactions of plants to salinity, it might be expected that pretreatment with SA contributes to accumulation of this amino acid under stress through maintaining an enhanced level of ABA in seedlings (Kuznetsov and Shevyakova, 1999). Sakhabodinova (2003) and Arfan (2007) reported proline increase in wheat under saline condition.

Soluble sugars

Soil application with concentration of 0.1 mM of SA had the greatest impact on soluble sugar and soil treatment of 0 mM showed the least effect which its difference with foliar of 0 and 1 mM was not significant (*Table 4*).

It is well known that organic solutes play a major role in mitigation of salt stress (Azooz, 2009). Accumulation of these compatible solutes reduced osmotic potential in the cytoplasm and contributes to maintaining water homeostasis among several cellular compartments (Sairan and Tyagi, 2004). Among all organic solutes, soluble sugars demonstrate the most osmotically active organic solute (Ashraf and Harris, 2004). The stimulation effect of SA on the biosynthesis of soluble sugars and proteins was associated to an increase in photosynthetic pigments and consequently the photosynthetic system (Yildirim *et al.,* 2008).

Increase of soluble sugars like monosaccharide's and disaccharides as osmotic compounds in water deficit due to saline conditions (Sanchez *et al.,* 1998) and protection of proteins against oxidative damage of free radicals in this circumstance is important (Muchow, 1989). Increase of soluble sugars as a mechanism for adjustment the osmotic potential between the cytoplasm and vacuole of cell may act under salinity stress. Accumulation of soluble sugar in plant leaves in terms of salinity is due to reduction of the activity of glucokinase. Reduction of glucokinase activity by accumulation of soluble sugars considered as one of the important aspect of salt tolerance in SA treatment condition (Poór *et al.,* 2010). Hamid (2008) showed that pretreatment of wheat with salicylic acid increase total soluble sugar content in salinity conditions.

CONCLUSIONS

According to the results, SA had a positive effect in improving plants performance in salinity condition. Among three application methods, soil treatment was the most effective method, but its difference with priming was not significant, so priming can be recommended instead of soil application that is easier to use and less costly. Among concentrations used, the effect of 0.1 and 0.5 mM concentration of SA were similar, so application of 0.1 mM can be offered to reduce the adverse effects of salinity stress in bean crop.

The better effect of soil method compared to the others may be possibly due to the positive effect of SA on the soil micro-organisms. Also it possibly seems that by reducing soil acidity, SA increases the solubility of nutrients required for plant like manganese, boron, copper, zinc and iron. It seems that salicylic acid in high concentration (1 mM) inhibit activity of ascorbate peroxidase which can disturb the H_2O_2 pathway and cause excessive accumulation of this substance in cell (Durner and Klessig, 1995).

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