EFFECT OF SALICYLIC ACID ON SALINITY STRESS IN COWPEA (*Vigna unguiculata* L. Walp)

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Abstract: This study was conducted to determine the effects of seed soaking in salicylic acid (0.25 mM, 0.50 mM, 0.75 mM, 1.0 mM and control) on the growth and some seedling properties in cowpea (Vigna unguiculata L. Walp) under different NaCl doses (2.5 ds m-1, 5.0 ds m-1, 7.5 ds m-1, 10.0 ds m-1 and control). In this work; seedling length, cotyledon width, cotyledon length, fresh-dry weight, chlorophyll a, chlorophyll b and total chlorophyll were investigated. In result, according to NaCl x SA interaction, seedling length, dry weight, total chlorophyll values changed between 5.05-13.58 cm, 0.055-0.138 g, 1.705-3.690 mg/g fresh weight, respectively.

Keywords: Cowpea (Vigna unguiculata L. Walp), salinity, salicylic acid, seedling properties

Introduction

Cowpea, Vigna unguiculata belongs to the group of crops known as legumes. It is a important source of human food. Its vegetative part can be buried into the soil to improve its fertility, and also, cowpea is a well known leguminous cover crop (Onyesom et al. 2005). Cowpea has a good tolerance to salinity, heat, drought, and it has a high yield potential under irrigation (Murillo-Amador & Troyo-Diéguez 2000).

Soil salinity has become an environmental problem and it adversely affects the growth and productivity of many crops. Salinity affects the crop physiology, and causes changes within cellular processes and plant levels (Yusuf et al. 2008). High salt concentrations have toxic effects on plant growth. Soil salinity level decreases availability of nutrients to plants and create high osmotic pressure which leads to physiological drought (Endris & Mohammed 2007). Soil salinity also affects seed germination and limit the agricultural production (Dantas et al. 2005). Salinity creates abiotic stress conditions for plants such as ionic and osmotic stress and these stress conditions cause production of reactive oxygen species and oxidative damage, metabolic toxicity and membrane deterioration. As a result plants developed complex responses to overcome harmful effects of salinity (Szalai & Janda 2009).

Salicylic acid (SA) is a plant phenolic compound and now considered as a hormone-like endogenous regulator. It play important roles to defend plants against both biotic and abiotic stress conditions. Earlier reports show that SA plays important regulatory roles in plants against to a wide range of oxidative stresses (Choudhury & Panda 2004; Deef 2007).

The aim of present work was to reveal whether cowpea plants pre-treated with different concentrations of SA could tolerant salt stress.

Materials and Methods

Cowpea seeds (Vigna unguiculata L. Karnıkara cv.) were used in this study. The experimental design consisted of 25 treatments replicated 3 times in a split plot design. Seeds of cowpea were surface-sterilized for 5 minute in sodium hypochlorite solution. They were then washed 3 times with sterilized distilled water. After sterilization, seeds were soaked in 0, 0.25, 0.5, 0.75 and 1 mM SA for 12 h in dark. The seeds were sown in plastic pots containing vermiculite and torf (1:2), maintained the same environmental condition and watered with different NaCl doses (0, 2.5, 5, 7.5 and 10 ds/mol) in total 50 ml during 10 days. After 10 days, the following were recorded: seedling length (cm), cotyledon width and length (cm), fresh and dry weight (g), chlorophyll a (mg/g fresh weight), chlorophyll b (mg/g fresh weight) and total chlorophyll (mg/g fresh weight).

Chlorophyll concentration was determined from cotyledons. A leaf sample of 0.2 g was ground and extracted with 15 ml of 80% (v/v) acetone in the dark (Haklı 2008). The mixture was filtered and absorbancies (T80 UV/VIS, Spectrophotometer) were determined at 645 and 663 nm. Concentration of chlorophyll a, chlorophyll b and total chlorophyll were estimated by the equations of (Witham et al. 1971).

All of the data were analyzed statistically using CoStat computer program. Duncan's test was used to compare averages at the 0.05 significance level.

Results

NaCl ($P<0.01$) and SA ($P<0.05$) applications were found statistically important on seedling length. According to the salt concentration, seedling length was changed between 6.21-10.75 cm. The highest seedling length was obtained from 0 ds/mol (untreated control) of salt concentration and the lowest was obtained from 10.0 ds/mol NaCl dose. Under SA applications, seedling length was changed between 6.88-9.43 cm and the highest seedling length was determined in 0.50 mM SA application. NaCl and SA interaction showed that seedling length changed between 5.05-13.58 cm (Table 1).

When Table 2 was investigated, NaCl and SA applications on cotyledon width were statistically important at the level 1%. According to NaCl and SA applications, cotyledon width varied between 2.34-3.82 cm and 2.51-3.49 cm, respectively.

While cotyledon length values which were found statistically significant ($P<0.01$) changed between 3.20-5.77 cm in NaCl treatments, these values changed between 3.79-5.18 cm in SA treatments (Table 3).

When the values of fresh and dry weight of seedlings were investigated, NaCl, SA applications and their interactions were found statistically significant. The 10 ds/mol dose of NaCl gave the lowest value of dry and fresh weight of seedlings. The highest values of fresh and dry weight were determined in 0.50 mM SA treatment (Table 4, 5).

The effects of SA, NaCl x SA applications interactions on chlorophyll a were statistically significant at level 1%. Chlorophyll b values were statistically significant at level 5% under NaCl x SA applications interactions. According to NaCl, SA applications and their interactions, chlorophyll a values were changed between 2.075-2.297 mg/g fresh weight, 1.930-2.271 mg/g fresh weight and 1.006-2.500 mg/g fresh weight, respectively. Chlorophyll b values changed; between 0.836-0.995 mg/g fresh weight under NaCl treatment, between 0.817-0.981 mg/g fresh weight in SA treatments and between 0.641-1.903 mg/g fresh weight in NaCl x SA treatments interactions (Table 6,7).

SA applications ($P<0.05$) and NaCl x SA applications interactions ($P<0.01$) were found statistically significant on total chlorophyll. It was determined that total chlorophyll values changed between 2.990-3.213 mg/g fresh weight in NaCl treatments and between 2.747-3.240 in SA treatments. According to NaCl x SA interactions total chlorophyll values varied between 1.705-3.690 mg/g fresh weight (Table 8).

Discussion

As NaCl concentrations were increased, a decrease in cowpea seedling length, cotyledon width, cotyledon length, fresh weight and dry weight (except for 2.5 ds/mol) was obtained. This situation can be explained by that salt stress unfavorably affected plant growth and productivity during all developmental stages (Tavili & Biniaz 2009). For example Hussein et al. (2007) reported that in general salinity decreases plant development. The same results were obtained by Okçu et al. (2005) who used pea, Kaya & Day (2008) who used sunflower.

The highest seedling length, cotyledon width, cotyledon length, fresh weight and dry weight were obtained from 0.5 mM SA concentration. A correlation was observed between increases in the concentration of these SA (0.75 and 1.0 mM) and inhibition of these parameters. Salicylic acid may influence a range of different processes in plants, including seed germination, ion uptake and transport, and membrane permeability (Dolatabadian et al. 2009). These results are consistent with those of Çanakçı & Munzuroğlu (2007) who showed that SA application induced fresh weight and chlorophyll content.

Chlorophyll a, chlorophyll b and total chlorophyll contents increased in SA treatments according to untreated control. The greatest chlorophyll (a, b, a+b) values were found from 7.5 ds/mol dose of NaCl x 1 mM dose of SA interaction. This case shows that SA treatments decreases the negative effects of salt.

Chlorophyll content of cotyledons increased slightly in general under higher NaCl concentration (7.5-10.0 ds/mol) although their increases were not statistically significant. According to Tohma (2007), Çanakçı & Munzuroğlu (2007), Kuşvuran et al. (2008) the effect of NaCl concentrations on chlorophyll content was found statistically significant. This situation can be explained by differences in species, varieties, medium and concentration of NaCl.

NaCl treatments have a negative effect on seedling length, cotyledon width, cotyledon length, fresh weight and dry weight and data were found statistically significant (P<0.01). In terms of all parameters were examined, 0.5 mM SA treatment reduce saline medium negative effects. This case show the necessity of studies about the application of SA in saline soil.

Table 1. Effect of SA on NaCl stress in cowpea seedling length (cm)

NaCl Doses (ds/mol)	Salicylic Acid Doses (mM) 0	0.25	0.50	0.75	1.0	NaCl Means
0	13.58	9.97	11.03	10.93	8.25	10.75 a**
2.5	9.02	9.95	10.32	9.93	9.70	9.78 ab
5.0	8.82	8.49	10.68	8.80	6.25	8.61 bc
7.5	9.02	9.05	8.82	7.37	5.05	7.86 c
10.0	5.97	6.75	6.32	6.83	5.17	6.21 d
SA Means	9.28 a*	8.84 a	9.43 a	8.77 a	6.88 b	

Significance Levels : *P<0.05, **P<0.01

Table 2. Effect of SA on NaCl stress in cowpea cotyledon width (cm)

NaCl Doses (ds/mol)	Salicylic Acid Doses (mM) 0	0.25	0.50	0.75	1.0	NaCl Means
0	3.73	3.70	3.83	4.00	3.33	3.72 a**
2.5	4.00	4.02	4.00	3.62	3.48	3.82 a
5.0	3.22	2.65	3.93	3.47	1.90	3.03 b
7.5	3.18	3.37	3.20	2.75	1.63	2.83 b
10.0	2.25	2.62	2.47	2.20	2.18	2.34 c
SA Means	3.28 a**	3.27 a	3.49 a	3.21 a	2.51 b	

Significance Levels : **P<0.01

Table 3. Effect of SA on NaCl stress in cowpea cotyledon length (cm)

NaCl Doses (ds/mol)	Salicylic Acid Doses (mM) 0	0.25	0.50	0.75	1.0	NaCl Means
0	5.37	5.63	5.73	6.25	5.05	5.61 a**
2.5	5.85	6.12	5.98	5.57	5.32	5.77 a
5.0	4.83	4.47	5.98	5.23	3.05	4.71 b
7.5	4.95	4.93	4.82	4.23	2.55	4.30 b
10.0	3.08	3.47	3.37	3.08	3.00	3.20 c
SA Means	4.82 a**	4.92 a	5.18 a	4.87 a	3.79 b	

Significance Levels : **P<0.01

Table 4. Effect of SA on NaCl stress in cowpea fresh weight (g)

NaCl Doses (ds/mol)	Salicylic Acid Doses (mM) 0	0.25	0.50	0.75	1.0	NaCl Means
0	1.614 abc	1.555 abc	1.789 ab	1.972 a	1.614 abc	1.709 a**
2.5	1.563 abc	1.747 abc	1.781 ab	1.626 abc	1.821 ab	1.708 a
5.0	1.390 bcd	1.384 bcd	1.841 ab	1.471 bcd	0.849 fg	1.387 b
7.5	1.437 bcd	1.472 bcd	1.306 cde	1.102 def	0.613 g	1.186 c
10.0	0.800 fg	0.906 efg	0.804 fg	0.770 fg	0.619 g	0.780 d
SA Means	1.361 a**	1.413 a	1.504 a	1.388 a	1.103 b	

Significance Levels : **P<0.01

Table 5. Effect of SA on NaCl stress in cowpea dry weight (g)

NaCl Doses (ds/mol)	Salicylic Acid Doses (mM) 0	0.25	0.50	0.75	1.0	NaCl Means
0	0.109 a-d*	0.109 a-d	0.122 abc	0.137 a	0.115 abc	0.118 ab**
2.5	0.117 abc	0.122 abc	0.129 ab	0.123 abc	0.133 a	0.125 a
5.0	0.107 a-e	0.105 a-e	0.138 a	0.113 abc	0.065 fg	0.106 bc
7.5	0.112 abc	0.121 abc	0.109 a-d	0.092 b-f	0.055 g	0.098 c
10.0	0.074 d-g	0.088 c-g	0.072 efg	0.067 fg	0.059 fg	0.072 d
SA Means	0.104 a**	0.109 a	0.114 a	0.106 a	0.085 b	

Significance Levels : *P<0.05, **P<0.01

Table 6. Effect of SA on NaCl stress in cowpea chlorophyll a (mg/g fresh weight)

NaCl Doses (ds/mol)	Salicylic Acid Doses (mM) 0	0.25	0.50	0.75	1.0	NaCl Means
0	2.063 a-d**	2.289 abc	2.449 a	2.345 abc	1.854 cd	2.200
2.5	2.122 a-d	1.996 a-d	2.395 ab	2.122 a-d	1.881 bcd	2.103
5.0	2.229 abc	2.102 a-d	1.634 d	2.244 abc	2.440 a	2.130
7.5	1.006 e	2.100 a-d	2.479 a	2.291 abc	2.500 a	2.075
10.0	2.232 abc	2.382 ab	2.334 abc	2.353 abc	2.193 abc	2.297
SA Means	1.930 b**	2.174 a	2.258 a	2.271 a	2.173 a	

Significance Levels : **P<0.01

Table 7. Effect of SA on NaCl stress in cowpea chlorophyll b (mg/g fresh weight)

NaCl Doses (ds/mol)	Salicylic Acid Doses (mM) 0	0.25	0.50	0.75	1.0	NaCl Means
0	0.789 b-f*	0.898 b-f	1.035 b-e	0.963 b-f	0.641 f	0.865
2.5	0.893 b-f	0.729 def	1.101 bc	0.732 c-f	0.724 def	0.836
5.0	0.922 b-f	0.769 c-f	0.670 ef	0.895 b-f	1.045 bcd	0.860
7.5	0.699 def	0.907 b-f	1.152 b	1.028 b-e	1.903 a	0.995
10.0	0.783 b-f	0.997 b-f	0.948 b-f	0.991 b-f	0.851 b-f	0.914
SA Means	0.817	0.860	0.981	0.922	0.890	

Significance Levels : *P<0.05

Table 8. Effect of SA on NaCl stress in cowpea total chlorophyll (mg/g fresh weight)

NaCl Doses (ds/mol)	Salicylic Acid Doses (mM) 0	0.25	0.50	0.75	1.0	NaCl Means
0	2.852 a-e**	3.187 a-d	3.485 ab	3.308 a-d	2.495 de	3.065
2.5	3.014 a-e	2.725 b-e	3.495 ab	2.854 a-e	2.605 cde	2.939
5.0	3.150 a-d	2.872 a-e	2.304 ef	3.138 a-d	3.485 ab	2.990
7.5	1.705 f	3.007 a-e	3.632 a	3.319 a-d	3.690 a	3.070
10.0	3.016 a-e	3.378 abc	3.283 a-d	3.343 abc	3.043 a-e	3.213
SA Means	2.747 b*	3.034 ab	3.240 a	3.193 a	3.064 ab	

Significance Levels : *P<0.05, **P<0.01

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