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Hydropriming, Ascorbic and Salicylic Acid Influence on Germination of *Agropyron elongatum* Host. Seeds Under Salt Stress*

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Abstract: The objective of this study was to evaluate the effects of ascorbic acid and salicylic acid pretreatment in addition to hydropriming on enhancement of seed germination of *Agropyron elongatum* under salt stress. The experimental design was two factors factorial based on a completely randomized design. Treatments were the combination of four levels of salt stress (0, -0.3, -0.6 and -0.9 MPa) in three concentrations of ascorbic acid and salicylic acid separately (100, 200 and 300 mg L^{-1}) and three levels of hydropriming (0 as a control, 12 and 18 h) with four replications. Results indicated that an increase in salt stress decreased germination components such as germination percentage and rate, coleoptile, radicle and seedling length and vigor index. Salicylic acid pretreatment and hydropriming did not significantly affect germination, but ascorbic acid spatially it's 300 mg L^{-1} concentration alleviated the adverse effects of salinity in all germination characteristics. Totally, it is concluded that ascorbic acid pretreatment results in improvement germination properties of *A. elongatum* under salt stress condition which in turn increases the resistance of *A. elongatum* against salt stress in germination phase.

Key words: Salicylic acid, ascorbic acid, hydropriming, seed enhancement, salt stress, *Agropyron elongatum*

INTRODUCTION

Desertification and salinization are rapidly increasing on a global scale and currently affect more than 10% of arable land, which results in a decline of the average yields of major crops greater than 50% (Wang *et al*., 2009). Therefore, understanding the mechanisms of plant tolerance to drought stress and high salinity is a crucial environmental research topic (Bartels and Sanker, 2005; Wang *et al*., 2009). Salinity causes to oxidative stress production and accumulation of Reactive Oxygen Species (ROSs) that may be cellular damage. An increase in cellular level of an antioxidant such as salicylic acid and ascorbic acid may cause enhancing stress tolerance (Khan *et al*., 2006).

Germination is a critical phase in plant life cycle and salinity tolerance in germination phase may be important for successful establishment for plants growing in this environment

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(Azarnivand *et al*., 2006). Application of pretreatments such as salicylic acid and ascorbic acid in addition to hydropriming of seeds may enhance germination under salt stress by neutralizing the excessive super oxide radical or singlet oxygen.

Salicylic acid is an endogenous plant growth regulator. It is involved in various physiological processes of plant growth and development such as induction of flowering (Cleland, 1974) and root growth stimulation (Coronado *et al*., 1998). It also plays a major role during the early stages of *Rhizobium*-legume symbiosis (Rasmussen *et al*., 1991).

Salicylic acid and ascorbic acid and their related components have been reported to induce significant adverse effects in environmental stress including drought and salinity (Khan *et al*., 2006; Wang and Li, 2006; Hamid *et al*., 2008; Wang *et al*., 2009). Several studies show that salicylic acid, ascorbic acid and hyropriming pretreatment increase tolerance to salinity in wheat (Hamada and Al-Hakimi, 2001), soybean (Coronado *et al*., 1998), maize (Khodary, 2004), *Atriplex stocksii* Boiss. and *Sueda fruticosa* L. (Khan *et al*., 2006), sunflower (Hamad and Monsaly, 1998; Kaya *et al*., 2006) and pigeon pea (Verma and Srivastava, 1998).

The aims of this research were to investigate the effects of salinity on germination of *A. elongatum* and to test the hypothesis that salicylic acid, ascorbic acid and hydropriming pretreatments can mitigate the adverse effects of salinity on seed germination in *A. elongatum*.

MATERIALS AND METHODS

Agropyron elongatum seeds were taken from Gorgan Natural Resource Office. This study was carried out during December 2008 at the Department of Arid and Mountainous Regions Reclamation, College of Agriculture and Natural Resources, University of Tehran, Iran. Seeds were sterilized by sodium hypochlorite solution 5% for two minutes and then washed two times with sterilized water before use.

Germination and seedling growth were studied during 14 days under osmotic potentials of 0 (control), -0.3, -0.6 and -0.9 MPa for NaCl (Coons *et al*., 1990). Distilled water was used as control.

The seeds of *A. elongatum* were soaked for 12 h in 100, 200 and 300 mg L^{-1} salicylic acid and ascorbic acid separately. To hydro prime, seeds were immersed in distilled water for 0 (as control treatment), 12 and 18 h at 25°C in darkness. After soaking, seeds were washed with tap water for three times and once with distilled water (Karaki, 1998). The treated seeds were dried in 25°C±1 for 24 h.

Germination test was conducted by four replications of 25 seeds from every treatment in 10 centimeters Petri dishes. Top of Whatman® paper No. 1 was moistened with 6 mL salt solution. Seeds were kept in germination at 25°C in the darkness for 14 days. Radicle length of 2 mm was scored as germination (Kaya *et al*., 2006). Germination percentage was recorded every day for during the study period. Mean Germination Time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1981). At the end of this period, final germination percentage, coleoptile, radicle and seedling length (cm) were recorded.

A two factors factorial (9×4) based on a completely randomized design was made for the experiment (Kaya *et al*., 2006). The first factor was seed treatment (salicylic acid, ascorbic acid (each 3 levels), hydropriming (2 levels) and control and the second factor was osmotic potential of salt solution (4 levels).

Arc sin transformation was used for germination percentage before analysis (Khan *et al*., 2006). Experimental data was analyzed by MSTAT-C program (MSTATC, 1990).

The differences between the means were compared using Duncan's multiple range test at 5% level of probability.

RESULTS

Results indicated that an increase in salt stress resulted in a decrease in germination components such as germination percentage and rate, coleoptile, radicle and seedling length and vigor index, but pretreatment with ascorbic acid caused a lower decrease. Final germination decreased with an increase in salt concentration. Ascorbic acid increased germination in both stress and non stress conditions. 300 mg L^{-1} concentration of ascorbic acid had higher effect compared to other treatments and increased germination percentage 26.8, 87.5, 38.8 and 42.5% in salt levels 0, -0.3, -0.6 and -0.9 MPa, respectively (Fig. 1).

Salicylic acid caused germination increase in some levels of salinity while no germination increase was observed under hydropriming treatment (Table 1).

Table 1: Effect of seed treatment on enhancement of germination characteristics of *A. elongatum* under NaCl stress

	Characteristics						
Treatment	NaCl (Mpa)	Germination (%)	Germination rate	radicle (cm)	Coleoptile (cm)	Seedling (cm)	Vigor index
Control							
	0	40.9H-K	0.1697N-Q	6.6BC	9.3H-O	15.9C-G	634D-H
	-0.3	34.6J-N	0.1670OPQ	5.1D-H	8.9J-O	14.1G-L	506I-L
	-0.6	33.2K-N	0.1560QR	4.5F-I	8.1O-S	12.6K-N	441KLM
	-0.9	28.0N	0.1364S	4.0G-J	6.0T	10.0P	320NO
Ascorbic acid							
100 ppm	0	39.8IJK	0.2201B-G	8.3A	13.0A	20.0A	833BC
	-0.3	41.7G-K	0.2172B-G	6.5BC	11.3B-E	16.7CDE	718D
	-0.6	41.3G-K	0.2158B-H	5.4C-F	11.0B-F	15.3D-I	656D-G
	-0.9	43.1E-J	0.1600Q	4.6E-I	9.1I-O	12.9J-M	564F-J
200 ppm	0	60.7AB	0.2252BCD	9.1A	12.2AB	21.3A	1087A
	-0.3	30.6LMN	0.2128C-I	6.0B-D	11.8A-C	17.9BC	599E-I
	-0.6	40.1IJK	0.2051F-J	5.3D-G	10.0D-L	15.3D-I	602E-I
	-0.9	50.5C-G	0.1995H-K	5.0D-I	7.3Q-T	12.3L-O	558F-J
300 ppm	0	51.9CDE	0.2225B-E	8.2A	11.8A-C	21.3A	921AB
	-0.3	64.9A	0.2162B-H	6.0B-D	10.7C-H	17.8BC	870B
	-0.6	46.1D-I	0.2147B-H	5.0D-I	10.3D-J	16.4C-F	656D-G
	-0.9	39.9IJK	0.2069E-J	4.8D-I	8.1O-S	13.8H-M	520H-K
Salicylic acid							
100 ppm	0	38.7I-L	0.2470A	5.4C-F	9.9E-M	15.3D-I	589E-I
	-0.3	56.3BC	0.2289BC	5.0D-I	8.5M-Q	13.5I-M	659D-G
	-0.6	37.0I-M	0.1828LMN	4.0G-J	8.2O-S	12.2L-O	458J-M
	-0.9	30.5LMN	0.1778M-P	3.8HIJ	6.9ST	10.7NOP	356MNO
200 ppm	0	51.4C-F	0.2080E-J	4.9J	9.7F-N	14.7E-K	672DEF
	-0.3	42.6F-K	0.1817L-O	4.4F-I	8.7L-Q	13.1J-M	531H-K
	-0.6	40.8H-K	0.1603Q	4.0HIJ	8.4N-R	12.3L-O	490I-L
	-0.9	34.6J-N	0.1428RS	2.9J	7.5P-S	10.4OP	375MNO
300 ppm	0	49.6C-H	0.2040G-J	8.2A	11.4BCD	19.7AB	878B
	-0.3	54.5BCD	0.1969I-L	5.9B-E	9.7F-N	15.6D-I	741CD
	-0.6	37.3I-L	0.1874KLM	4.3F-I	8.8K-P	13.1J-M	492I-L
	-0.9	20.4O	0.1657PQ	3.8IJ	7.0RST	10.9NOP	295O
Hydropriming							
12 h	0	42.6F-K	0.2112D-J	6.7B	10.4D-I	17.1CD	697DE
	-0.3	35.5J-N	0.2079E-J	4.7E-I	10.1D-K	14.9E-J	546G-K
	-0.6	34.7J-N	0.1973I-L	4.4F-I	9.7F-N	14.1G-L	508I-L
	-0.9	28.3MN	0.1700N-Q	3.7IJ	8.0O-S	11.8M-P	377MNO
18 h	0	39.9IJK	0.2301B	6.7B	11.1B-F	17.7BC	698DE
	-0.3	36.0J-N	0.2211B-F	5.0D-I	10.9B-G	15.8C-H	584E-I
	-0.6	33.9J-N	0.1951JKL	4.9D-I	9.5G-O	14.3F-L	512I-L
	-0.9	28.3MN	0.1872KLM	4.1G-J	8.9J-O	13.1J-M	415I-L

Means that have a different letter are significantly different from each other

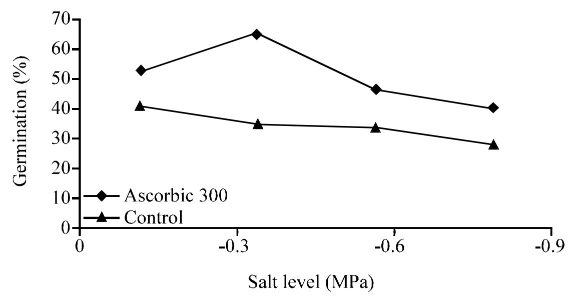

Fig. 1: Comparing germination percentage between control and 300 mg L^{-1} concentration of ascorbic acid pretreatment in *A. elongatum*

Germination rate was adversely affected by salinity, but all treated seeds showed results better than control. The 300 mg L^{-1} concentration of ascorbic acid increased germination rate up to 37% under salt stress.

The growth of coleoptile, radicle and seedling was significantly decreased by increasing salinity level. The ascorbic acid treatment increased coleoptile, radicle and seedling length at all levels. Not only did hydropriming and salicylic acid significantly increase root length, but also 100 and 200 mg L^{-1} salicylic acid decreased root length compared with non treated seeds. All treatments other than 100 and 200 mg L^{-1} salicylic acid increased coleoptile length in both stress and non stress conditions. Any of three concentrations of ascorbic acid had positive effects on seedling length in both conditions, showing that 300 mg L^{-1} was better than other concentrations. The 300 mg L^{-1} salicylic acid increased seedling length, but 100 and 200 mg L^{-1} of mentioned hormone decreased seedling length except in -0.9 MPa of salinity. Hydropriming showed an increase in seedling length in comparison with non treated seeds, while only 18 h of hydropriming in -0.9 MPa salinity was significant.

Seed vigor index obtained best reaction of *A. elongatum* with 300 mg L^{-1} concentration of ascorbic acid in salinity stress. Salicylic acid increased vigor, but only 300 mg L^{-1} in non stress condition was significant. At both times, hydropriming increased vigor index, but there was no significant difference comparing with control (Table 1).

DISCUSSION

Seed germination is commonly inhibited by rising salinity level. Salinity-induced oxidative stress resulted in germination inhibition (Amor *et al.*, 2005). Khan and Ashraf (1988) recommended that nutritional imbalance, specifically ion toxicity and decrease in water potential increased by higher concentration of Na^+ and Cl^-. In addition, salinity led to inhibition of uptake of essential nutrients such as K^+, Ca^{2+} and NO_3^- (Ashraf, 2004; Hamid *et al.*, 2008).

Present study showed that some pretreatments of seeds alleviated adverse salt effects on germination. Ascorbic acid pretreatment, especially it's 300 mg L^{-1} concentration, was better than the other treatments. This result agrees with Hakimi and Hamada (2001), Barh *et al.* (2008) and Burguieres *et al.* (2007) that reported that ascorbic acid advantages mitigate adverse effects of NaCl *via* enhancement of protective antioxidant system. This result is also in agreement with reports of increased protective antioxidant system activity under salt stress in halophytes (Khan *et al.*, 2006).

Ishibashi and Inoue (2006) state that exogenously applied hydrogen peroxide (H_2O_2) ameliorates seed germination in many plants. H_2O_2 is produced during the early imbibition period in several seeds. The plant tissues contain ascorbic acid which acts as an antioxidant that scavenges oxygen species such as hydrogen peroxide. A high level of endogenous Ascorbate is essential to maintain the antioxidant capacity that protects plant from oxidative stresses (Zhou *et al*., 2009).

In low concentration, salicylic acid had no effect on germination. Although in higher concentrations (200 and 300 mg L^{-1}) germination increased but the increases were not significant. The results of this part of present study difference with Hamid *et al*. (2008) and Wang and Li (2006). This may indicates that complete set of antioxidant defense system, rather than a single oxidant, is responsible for protection in stressed plant (Foyer *et al*., 1994). Usually it may not be successful to increase stress tolerance by simply increasing the concentration of single antioxidant in plant (Khan *et al*., 2006).

In addition, available scattered information suggests that the reason for difference in germination responses may be related to seed structure and chemical composition (Khan *et al*., 2006). Seed hydropriming at 12 and 18 h was not significantly effective in improving germination under salt stress. Probably this refers to unfavorable applied duration of hydration. Penàloza and Eira (1993) reported that unfavorable duration of exposure to seed hydration has adverse effect. The main objective of this study was finding that whether pretreatments can mitigate the adverse effects of salinity on seed germination in *A. elongatum.*

CONCLUSION

The evidence in present study suggests an important role of pretreatment with 300 mg L^{-1} ascorbic acid in *A. elongatum* seed enhancement germination and seedling establishment under salinity condition, that this result demonstrated the our hypothesis. We expect that enhancing stress tolerance with ascorbic acid pretreatment might be useful for successful pasture planting in marginal soils, including desertified areas and alkalinized soils.

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