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The effect of seed priming by ascorbic acid on bioactive compounds of Canola (*Brassica napus* L.) under salinity stress

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ABSTRACT: Seed priming is one of the ways to reduce negative effects of salt which is used for increasing germination percentage and seed resistance increase in salty zones. Ascorbic acid is of the most important vegetative antioxidants playing an important role in cell metabolism reactions at the time of germination, cellular disinfection, cell protection against oxidative reactions. So, to examine the effect of ascorbic acid on germination percentage, rootlet length and the extent of activity of peroxidace and catalase existing in canola seed under salt conditions, an experiment was conducted in triplet in factorial form and based on fully randomized plan in vitro. Three levels of ascorbic acid (0, 500 and 1000 μM) and three osmosis potentials produced using chlorine and sodium (0, 60 and 100 μM) were composed the second factor levels. Regarding mean data resulted from the experiment, as salt level increased germination indices decreased, but seed priming with ascorbic acid led to the increase of trait performance and the reduction of the respective enzymes' activity which is probably caused by the neutralization of free radicals resulted from salt stress.

***Keywords*:** Ascorbic acid, salt stress, germination, Canola

INTRODUCTION

Germination is one of the most critical and sensitive stages of canola growth in facing salt stress affecting these settlement of germ and plant survival in saltiness soils. Hence, numerous attempts are made to develop policies for reducing the adverse effects of salt stress. Today, priming technique or seed preparation is one of the physiological methods to increase the germination percentage, seed efficiency, and preparation of speed and consistency of germination and deployment under saltiness conditions. Accordingly, canola seed pre parathion to detect its effects on traits including germination and salt stress tolerance increase in germination stage is of particular significance. Mansouri et al (1999) concluded that salt resistance is a complicated trait resulted from mutual effect of several physiological and morphological processes and leads to the production of strong cellular line while maintaining renewal trait finally transforming to reformed plant. Exterior use of ascorbic acid in *Nicotian atabacum* in saltiness condition will significantly affect poisoning resulted from H_2O_2 by enhancing the activity of catalase and peroxide (Khan &Gul, 2006) .

Studies by Moradshahi et al (2004) regarding the effect of salt stress on nine floral plants demonstrated that as salt stress increases, growth of the roots of all cultivars increases but aerial member of almost all cultivars decreases. Ascorbic acid is a small water soluble antioxidant playing a role in intoxication of oxygen reactive species and in particular H_2O_2 as well as directly neutralizing superoxide radicals, odd oxygen or superoxide and as a secondary antioxidant in reproducing alpha-tocophrol and other volatile oils. Ascorbic acid acts in form of a cofactor for cycle and ulagzantin in chloroplast. Exterior use of ascorbic acid can increase salt stress tolerance and decrease the effect of resulting oxidative stress.Together with glutathione and several antioxidant enzymes, ascorbic acid plays a role in neutralizing oxygen reactive radicals including superoxide ion. It plays a key role in glutathione ascorbatpath which has an active role in removing oxygen reactive species in chloroplastand cytozol. It is shown that ascorbic acid can directly neutralize superoxide and hydroxyl ions. Using extraneousascorbic acid at the time of stress has shown that the adverse effects of stress are partially reduced as a result of interaction with acid. Dowlatabadian et al (2009) demonstrated that feeding flowering plants' leaf by ascorbic acid resulted in the reduction of antioxidant enzymes activity so that ascorbic acid used leads to an alleviation of cell fat oxidation as well as halon di-aldehid content in leaf and root.

By neutralizing oxygen radicals through consuming a variety of active oxygens and producing mono di-hydroascorbat, ascorbic acid prevents from any damage to cell and membrane lipids and consequently lipids peroxidation will decrease. Mono di-hydroascorbat is turned into ascorbat via redoctase. Different studies have shown that ascorbic acid leads to the modification of merozinaz activity in some plant species including mustard. Hata and Tesuro (1986) presented a model for ascorbic acid performance. It does not take part in the reaction catalyzed by mustard merozinaz rather takes part in coupling of subunits.

MATERIALS AND METHODS

To examine the effect on ascorbic acid on germination indices of canola seed in salt stress conditions, it was studied in terms of full randomized blocks factorial plan in Arable Plants Physiology Laboratory, faculty of Agriculture of Saveh Azad University.Accordingly, canola's cultivar Okapy was used, namely, first, 50 seeds were separated and disinfected for each petri dish. For disinfection, the seeds were dipped in %5 sodium hypochlorite for 5min and then cleansed by ample water. Given seeds were placed in three ascorbic acid concentrations of 0, 500 and 1000 μM at 20°C for 24h. Then, they were completely washed using distilled water and transmitted to a sterilized petri dish at the bottom of which a Wattman No.1 filter paper was placed. All petri dishes had a diameter of 9cm. then, 10ml distilled water or chlorine and sodium solutions in concentrations of 0, 60 and 100mM (depending on the type of treatment) was added to each petri dish and all petri dishes were transmitted into a germinator with temperature conditions25$\pm$0.5, light intensity 1500Lux and daylight duration of 16h and darkness of 8h. Counting of the germinated seeds was carried out daily at a certain hour. In counting, seeds were considered as germinated if length of their rootlets were $\geq$2mm.Based on data gained from the counting, germination speed, final germination percentage (percentage of germinated seeds during the experiments out of all seeds) and consistency of germination (the duration required for germination percentage to reach %90 from %10 in hour) were calculated.

Germination speed was calculated in terms of time reverse up to %50 of germination (i.e. 1(D50)) and germination consistency was calculated in terms of duration (in hour) from %10 to %90 of final germination (i.e. $D_{10}$to D_{90}).

RESULTS AND DISCUSSION

Results of the experiment showed that sole effect of ascorbic acid, salt and also the mutual effect of ascorbic acid with salt at %1 level on germination percentage, length of rootlet and the extent of peroxidase and catalase activity of seeds were significant. Comparing mean data from the experiment regarding germination percentage and length of rootlet demonstrated that maximum germination percentage without ascorbic acid(test group) was %88.17 and minimum at third level was %88.17. The comparison for germination at different levels of NaCl showed that maximum germination percentage at the first level (test) was %92.77 and minimum at third level (1000 μM) was %79.26. Comparing mean data from the experiment of using ascorbic acid with NaCl showed that maximum germination percentage at the first acid level with the first salt leveland the minimum was at the first acid level with the third salt level (Table 1). Comparing the mean data regarding the amount of peroxidase at different levels of acid with salt showed that maximum activity of catalase and peroxidase was with acid at the first and salt at the third levels and minimum at the second level of acid with the first level of salt.

Studying the effect of NaCl showed on germination percentage shows that as salt level increases, germination percentage decreases resulted from the disruption in ionic effect, osmosis effect and also nutritional imbalance emanated from the presence of chlorine and sodium ions; namely, the plant consumes more energy to gain a certain amount of water. As a result, a part of energy the plant requires to grow is consumed to gain water.

Another possibility for proving the matter can be mentioned so that the increase of the concentrations of chlorine and sodium in external environments can decrease ionic activity and bring about the reduction of large Na/Ca, Na/K, and Na/Mg ratios, but for primed seeds (cause of the presence of ascorbic acid as a small water soluble antioxidant and strong reducer in disinfecting oxygen reactive species and in particular H_2O_2) itself is oxidized and reduces superoxide compounds; for instance, in these seeds, some proteins and carbohydrates are decomposed as a result of hydrolyzing enzymes and reactions and ready for taking part in the germination process.

The issue can be an explanation for acceleration of germination and reduction of mean germination time which the results are in accordance with the results of Bikort et al (2005), Neuman and Shalta (2001), and Dowlatabadian et al (2008). Results from the mean comparisons of different levels of ascorbic acid showed that the acid increase leads to the increase of seed resistance against salt stress and improvement in the status of germination components' growth.

Based on the results, it was demonstrated that salt stress also results in peroxidase enzyme increase that the probably stress conditionslead to the increase of H_2O_2 in cells and since peroxidase is considered as one of the most specialized and main sweeping enzymes of H_2O_2, it will turn it into water and oxygen in peroxisome before any damages from H_2O_2homeostasis.It seems that the above enzymes all play a role through synthesis of the second cell wall. Also, salt stress probably happens with oxidative stress so that peroxidase is among the major compounds of disinfecting toxic oxygen radicals in the stresses resulting in the increase of this enzyme.

However, conditions are different in ascorbic acid priming because priming with ascorbic acid at the second level leads to the enzymes reduction. It seems that it happens because if in salt stress conditions with NaCl, ascorbic acid is placed in an acidic medium, it will positively be loaded and can combine with chlorine and if in basic medium, it will negatively be loaded. So, it can combine with sodium ion and probably result in compounds like sodium ascorbic acid and the like and reduce negative effects of sodium chloride and peroxidase is no longer produced massively. As a result, the amount of them decreases at stress time. Results were in accordance with results of Botella (1994), Dehkordi (2001) and Galardo et al (2001). Generally, NaCl resulted in the reduction of seed growth process, germination indices and increase in peroxidase activity. On the other hand, ascorbic acid priming led to the reduction of salt stress negative effects.

Table 1. comparing mean mutual effects of prolin and sodium chloride on the traits under study

Treatment	Trait	Germination percentage	Length of rootlet (mm)	Catalase (IMH2O2min)	Peroxidase (OD.g-1.FW.min-1)
A1	C1	a 92.83	a 67.11	c 44.1	cde 0.3
	C2	b 90	c 45.66	bc 1.98	bc 0.37
	C3	e 76.66	d 21.66	a 1.91	ab 0.41
A2	C1	a 93.36	a 63.33	d 1.51	cde 0.28
	C2	c 85.21	bc 51.55	e 1.31	e 0.23
	C3	d 79.94	c 46.22	de 1.34	de 0.26
A3	C1	b 90.08	b 55	f 0.56	bcd 0.33
	C2	cd 82.91	b 55	b 2.85	b 3.96
	C3	81.17 d	52.53 b	2.19 d	2.75 d

Treatments shown by at leas one common letter have no significance differenc

Table 2. table of analysis and variance of the traits under study

Changes source	df	Germination percentage	Length of rootlet	Catalase	Peroxidase
Ascorbic acid (A)	2	40.6781*	745.197**	0.055**	0.072**
Salt (B)	2	1230.944**	31.89**	0.250**	0.018**
A*B	4	89.325**	1086.010**	0.049**	0.024**
Error	18	10.488	41.23	0.023	0.012

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