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ORIGINAL ARTICLE

Effect of Salicylic Acid and Ascorbic Acid on Germination Indexes and Enzyme Activity of Sorghum Seeds under Drought Stress

Tabatabaei S. A.

Agricultural and Natural Resources Research Center of Yazd, Iran

*E-Mail: Omid0091@yahoo.com

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Seed priming methods have been used to increase germination characteristics under stress conditions. The effects of drought stress (0, -4, -8, -12 and -16 bar) and salicylic acid 25 ppm at 15 °C for 15 h and ascorbic acid 25 ppm at 15 °C for 15 h on germination percentage, germination index, means time to germination, normal seedling percentage and enzyme activity were assessed in the laboratory for sorghum seeds (*Sorghum bicolor L.*). Results showed that the highest germination percentage (83.33%), normal seedling percentage (69.67%), germination index (25.29) and the minimum means time to germination (2.87) were attained from priming with salicylic acid in control conditions. Therefore, seed priming significantly ($p \leq 0.01$) increased germination characteristics as compared to the unprimed under drought stress. Also, priming increased catalase and ascorbate peroxidase as compared to the unprimed seeds.

Key words: Sorghum bicolor L. Hormone-priming, Germination characteristics, Drought stress, Enzyme activity

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In arid regions, cereal production is widely limited by poor stand establishment (Jones and Wanbi, 1992). Particularly in drought-prone environments, cereal germination tends to be irregular and can extend over long periods (Bougne *et al.*, 2000). Sorghum bicolor is the fourth most important world cereal grain, following wheat, rice, and corn. Seed quality (viability and vigor) can have a profound influence on the establishment and the yield of a crop. Parera and Cantliffe (1994) reported that rapid and uniform field emergence is an

essential prerequisite to reach the yield potential, quality and ultimately profit in annual crops.

Seed characteristics are usually essential process in seedling establishment and plant development to obtain seedling numbers those results in higher seed crop (Almansouri *et al.*, 2001; Murungu *et al.*, 2003). Seed germination and establishment are the most sensitive stages to abiotic stresses (Patade *et al.*, 2011; Ansari *et al.*, 2012). Abiotic stress such as: drought stress, salt

stress and cold stress maybe delay seed germination and reduce the rate (Patade *et al.*, 2011; Rouhi *et al.*, 2011; Ansari and Sharif-Zadeh, 2012; Ansari *et al.*, 2012). Ashraf and Foolad (2005) reported that seed priming is one of the methods that can be taken to counteract the adverse effects of abiotic stress. The purpose of seed priming is to a partially hydrated with water, or various chemical solutions like polyethylene glycol (Osmo priming) or salts like CaCl_2 , CaSO_4 and NaCl (Halopriming) the seeds to a point where germination processes are begun but not completed (Asfrac and Foolad, 2005), followed by drying of seeds to the original moisture level. Seed priming techniques have been used to increase germination, improve germination uniformity, improve seedling establishment and stimulate vegetative growth in more field crops (Ansari and Sharif-Zadeh, 2012; Ansari *et al.*, 2012; Patade *et al.*, 2011; Foti *et al.*, 2008) under stressed conditions. Also, the priming strategies enhanced activities of free radical scavenging enzymes such as CAT and APX (Ansari and Sharif-Zadeh, 2012; Rouhi *et al.*, 2012). The study aimed was to determine the effect of salicylic acid and ascorbic acid on germination indexes and enzyme activity of sorghum seeds under drought stress.

MATERIALS AND METHODS

The study was conducted in the Faculty of Agricultural and Natural Resources Research Center of Yazd, Iran.

Drought stress at osmotic potentials of 0 (as control), -4, -8, -12 and -16 bar were adjusted using PEG 6000 before the start of the experiment.

Seeds of were pretreated with salicylic acid 25 ppm at 15 °C for 24 h and ascorbic acid 25 ppm at 15 °C for 15 h. sorghum seeds were exposure in 20 cm glass petri dishes containing 15 ml solution. The

imbibed seeds were then washed 4 times with tap water and dried on filter paper at $15\pm 1^\circ\text{C}$ for 24 h (Ansari and Sharif-Zadeh, 2012).

Standard germination test was carried out by place 50 seeds in 9 cm petri dishes at 25 °C. Seeds were observed daily until day 10th and germinated seeds were recorded. Investigated parameters were the germination percentage, germination index, normal seedling percentage and means time to germination.

All extraction procedures were carried out at 4 °C. The seed samples, weighting about 0.3 gr, were homogenized with 3 ml of tris (PH 7.8), followed by centrifugation of 20000 g for 20 min. The supernatants were used for determination of enzyme activity. The supernatants were used for determination of enzyme activity. Catalase (CAT, EC 1.11.1.6) activity was determined spectrophotometric ally following H_2O_2 consumption at 240 nm (Chiu *et al.*, 1995). Ascorbate peroxidase (APX, EC 1.11.1.7) activity was determined according to the procedures of Johnson and Cunningham (1972). The activities of APX and CAT were expressed per mg protein, and one unit represented 1 μmol of substrate undergoing reaction per mg protein per min.

All data were analyzed statistically by analysis of variance using SAS Software. Data for germination and normal germination percentages were subjected to arcsine transformation before analysis of variance was carried out with SAS software. Mean comparisons were performed using an ANOVA protected least significant difference (Duncan) ($P < 0.01$) test.

RESULTS AND DISCUSSION

Analyze of variance for studied traits under drought stress in sorghum seeds showed that Drought stress \times Priming interaction was

significantly ($P < 0.01$) for all traits (Table 1). Our results showed that the highest germination percentage (83.33%) (Fig. 1), germination index (25.29) (Fig. 2), normal seedling percentage (69.67%) (Fig. 4) and the minimum means time to germination (2.87) (Fig. 3) were attained from priming with salicylic acid in control conditions (0 bar).

In agreement with the results, earlier reports (Ansari *et al.*, 2012; Patade *et al.*, 2011), have shown negative affect stress conditions on germination characteristics. The results of our study suggested that hormone-priming cause improvement in the seed characteristics as compared to the unprimed. In agreement with the results, earlier reports (Ansari *et al.*, 2012; Rouhi *et al.*, 2011; Patade *et al.*, 2011) positive effects of priming in relation to seed performance, germination percentage and seedling indices.

Also, in the present investigation our results showed that hormone priming increases enzyme

activity (catalase and ascorbate peroxidase) as compared to the unprimed seeds (Fig. 5).

Ansari and Sharif-Zadeh (2012) reported that seed priming highly increased germination indexes, APX and CAT activities in mountain rye. Also, Rouhi *et al.* (2012) showed that germination characteristics and antioxidant enzyme activities (superoxide dismutase, catalase, and peroxidase) in treated seeds of Berseem clover (*Trifolium alexandrinum* L.) were significantly increased compared to those in control group. Oxidative stress blocks growth and development by decreasing cell division, therefore protection from oxidative stress is critical for seed germination. Recent studies show that the presence of several antioxidative and hydrolytic enzymes in dry cereal grains, and activities raised considerably after the start of seed imbibition (Chang *et al.*, 2000; Demeke *et al.*, 2001). Improvement in germination characteristics of primed seeds could be results of increasing the antioxidant profile of treated seeds (Ansari and Sharif-Zadeh, 2012).

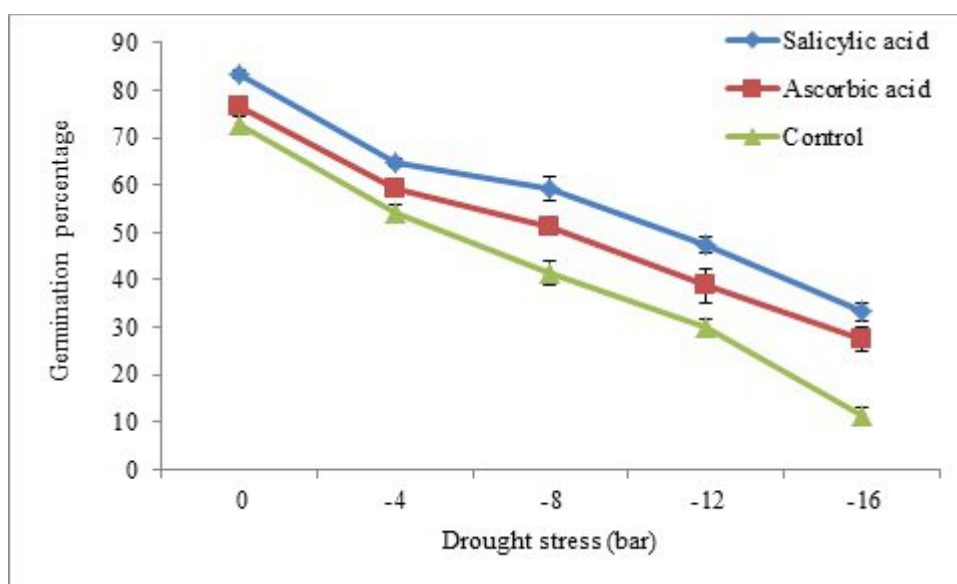


Figure 1. Effect of Drought stress × Priming interaction on germination percentage.

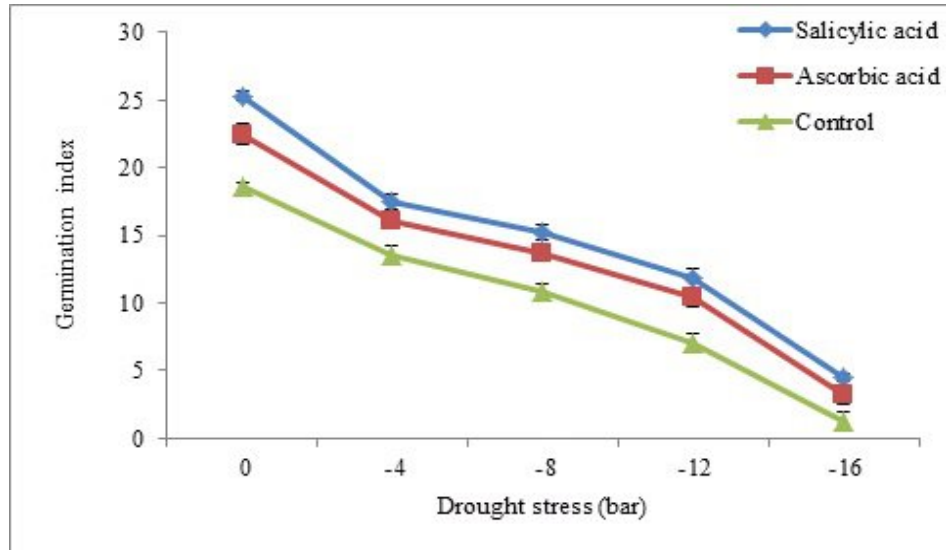


Figure 2. Effect of Drought stress × Priming interaction on germination index.

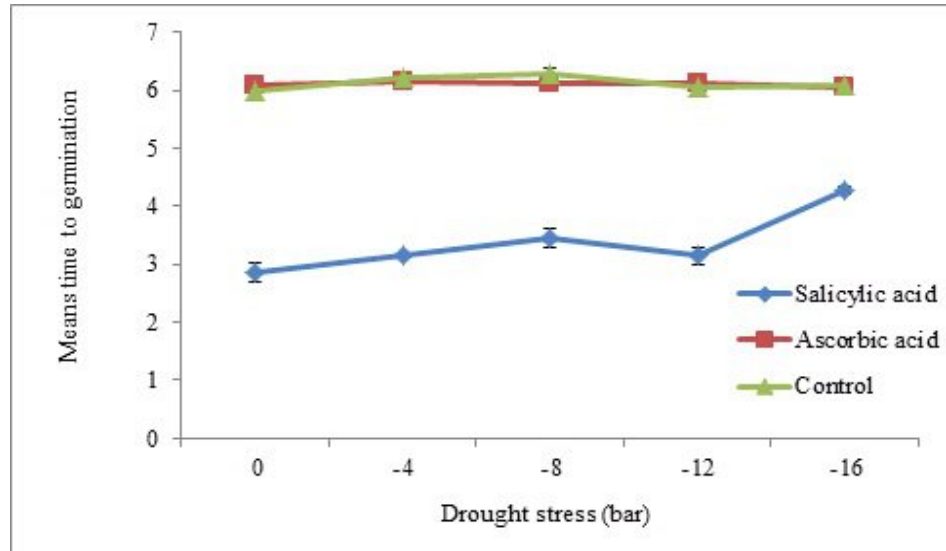


Figure 3. Effect of Drought stress × Priming interaction on means time to germination.

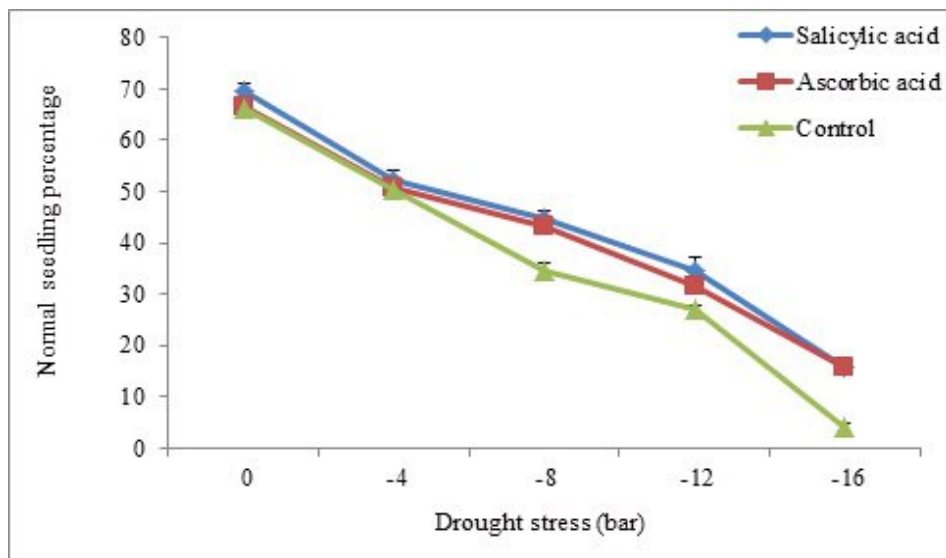


Figure 4. Effect of Drought stress × Priming interaction on normal seedling percentage.

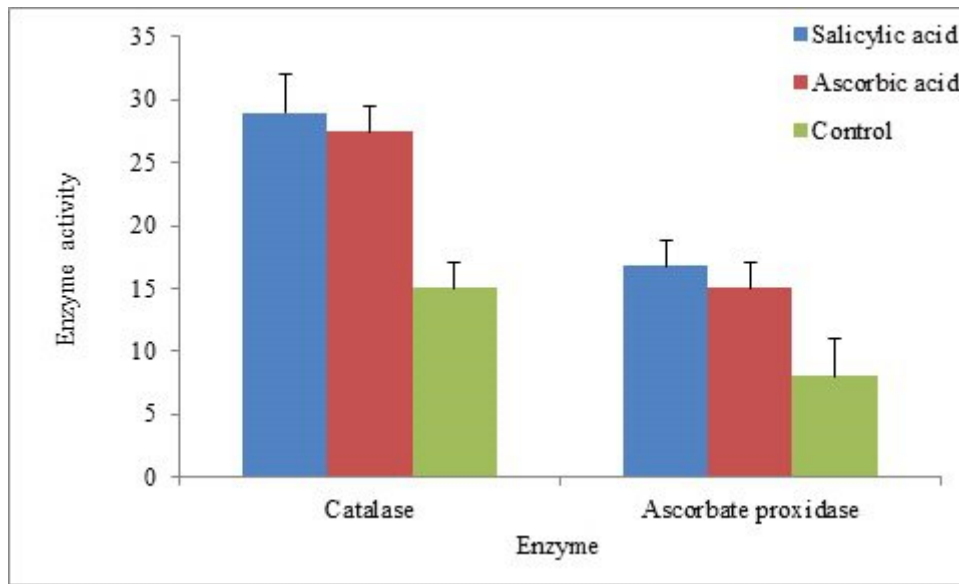


Figure 5. Effect of priming on enzyme activity.

Table 1. Variance analysis of the effect of priming on studied traits under drought stress in sorghum seeds.

S.O.V	df	Germination percentage	Germination index	Means time to germination	Normal seedling percentage
Drought stress (D)	2	932.62**	82.35**	37.39**	194.15**
Priming (P)	4	3715.42**	453.01**	0.31**	3955.2**
D*P	8	24.28**	1.28**	0.31**	22.18**
Error	30	5.68	0.19	0.01	3.02
CV%	-	4.76	3.49	1.98	4.29

CONCLUSIONS

The highest germination characteristics were attained from hormone-priming under control condition (0 bar). Hormone-priming increased germination characteristics as compared to the unprimed. Also, priming increased CAT and APX as compared to unprimed seeds.

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