



Effect of seed priming on germination characteristics, polyphenoloxidase, and peroxidase activities of four amaranth cultivars

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Abstract

Amaranth is a C₄ crop with increasing potential for cultivation in Iran. Amaranth is a very nutritious crop due to high quality amino acids and minerals in its seeds. Seed priming known as pre-sowing treatment which improve germination characteristics and results in uniform seed emergence. Peroxides (POD) and polyphenoloxidase (PPO) are two plant enzymes and play very important role in tolerance to environmental stress. Four amaranth cultivars from two genuses, namely, Amont, Trigin, Mercado, and Plainsman were used. Treatments consisted of four osmotic potentials (0, -10, -12 and -14 bars) and four treatments duration (3, 6, 9 and 12 hours). A randomized completely design with five replications were conducted. POD and PPO activities were determined in primed (-10 bar for 3 hours) and non-primed seeds after 8 hours of imbibitions. Results indicated that the best priming treatments for amaranth cultivars were -10 bars for 3 hours. According the results of this study, primed seeds significantly exhibited higher germination percentage, speed of germination, root length and seed vigor in all amaranth cultivars. Trigin cultivar showed the best performance among cultivars. Total seed protein, POD and PPO were also increased significantly by seed priming. Amont and Plainsman cultivars exhibited high protein content and POD activity. PPO activity increased by seed priming comparing to controls for Amont, Plainsman and Mercado cultivars, but for Trigin cultivar, no increase was detected. The highest increase in PPO activity was observed in Mercado cultivar.

Key words: Amaranth, seed priming, germination, enzyme activity.

Introduction

Amaranth is a C₄ crop with increasing potential for cultivation in Iran. Amaranth is a very nutritious crop and also known as alternative for cereals ^{14, 41}. The composition and nutritional properties of amaranth seeds lead to special attention over the past years ^{5, 30, 48}. Amounts of vitamin C, iron, carotene, calcium, folic acid and proteins are very notable. It was recommended by Aufhammer *et al.* ¹ that shorter the time to emergence could reduce the risk of upsilting and crusting of the soil surface before emergence. Seed priming describes the different germination enhancing pre-sowing treatments which do not result in radicle emergence ¹³. To do so, seeds are partially hydrated to a point where germination process begins, but radicle emergence does not occur. Seed priming has been reported to increase the yield of chickpea, maize, rice and wheat under semiarid conditions ^{19, 20, 39}. There are reports that hydration of seed up to, but not exceeding, the lag phase with priming permits early DNA replication ⁴, increased RNA and protein synthesis ^{12, 24}, greater ATP availability ³³, faster embryo growth ⁷, repair of deteriorated seed parts ^{26, 43} and reduced leakage of metabolites ⁴⁶ compared with control. Another effect of priming is the better performance of seeds under adverse conditions and environmental stress such as salinity ²⁷. Adverse environmental conditions, such as salinity, lead to secondary stresses like oxidative stress ⁵⁰. Del Ryo *et al.* ⁹ reported that the produced ROS (Reactive Oxygen Species) were decreased to low levels by antioxidant molecules. Varieties of general peroxidases (PODs; EC 1.11.1.7) are the main enzymes involved in the

detoxification of ROS ²². PODs are heme containing glycoproteins which participate in a great number of physiological processes, such as the biosynthesis of lignin and ethylene, defense against pathogens and wounding, auxin metabolism and stress response ²⁹. This type of POD catalyses the dehydrogenation of structurally diverse phenolic and endiolic substrates by HP2 and are thus often regarded as antioxidant enzymes, protecting cells from the destructive effect of derived oxygen species ⁴⁹. It was reported that plants with high levels of antioxidants, either constitutive or induced, have greater resistance to this oxidative damage ^{22, 35}. This was also reported by Meloni *et al.* ³⁴ who showed that salt-tolerant cotton cultivar may exhibit better protection against ROS by increasing the activity of antioxidant enzymes under salt stress. Polyphenol oxidase (PPO, E.C. 1.14.18.1) is an enzyme that is widely distributed in plants ³². PPO converts a variety of phenolic substrates to dark-coloured polyphenols (melanins). Most PPO activity of mature seeds is associated with the bran ²¹. The role of the polyphenol oxidases not only because of their contribution to flavor but also for their antioxidant activities are of great interest. This enzyme can play a significant role in overcoming various types of injuries in plants and were marked by many workers ^{23, 45}. Amaranth seed has limited food reserves and fast and uniform establishment is critical for successful production. There is little information available on the role of priming treatments of amaranth seeds and possible physiological processes that lead to the reported benefits of priming. The objectives of this study

were to determine if hydropriming and osmopriming with polyethyleneglycol (PEG) 6000 has positive effect on germination characteristics of amaranth seed cultivars and to determine antioxidant activities under osmopriming treatment.

Materials and Methods

Optimization of seed priming for amaranth cultivars:

Experiments were conducted in an incubator to determine the effects of hydro- and osmopriming on the germination characteristics of four amaranth cultivars from two adapted genotypes (*Amaranthus cruentus*, *Amaranthus hypochondriacus*). Investigated factors were cultivars, duration of seed priming and concentration of polyethylene glycol 6000 (PEG 6000). A factorial experiment with five replications was conducted. Seeds were soaked for 3, 6, 9 and 12 hours in distilled water for hydropriming and in solution of PEG 6000 with potential of -10, -12 and -14 bars for osmopriming. Osmotic potential of solutions were prepared using Kauffman formula³⁷. For priming treatments, 50 seeds were put in glass Petri dishes adding 5 ml of each solution. After finishing priming treatment, seeds were washed with distilled water three times and dried for 24 h in the incubator under dark condition. Germination test was conducted with primed and non-primed seeds with top of paper method (two filter papers) in 90 mm Petri dishes. The papers were saturated with distilled water and 25 seeds placed in each Petri dishes. Non-primed seeds were used as controls. The Petri dishes were closed and placed into incubator. All priming, drying, and germination experiments were conducted at 25°C. Germination experiment lasted 48 hours. To evaluate speed of germination we divided 48 hours of germination test to eight parts which each part represent six hours. Therefore, germinated seeds in every six hours were used to calculate speed of germination. Percentage of seed germination, germination speed and root length were recorded in all treatments. Weighted germination index (WGI) was used to evaluate speed of germination using the following equation:

$$WGI = \frac{(8 * m) + (7 * n_2) + (6 * n_3) + (5 * n_4) + (4 * n_5) + (3 * n_6) + (2 * n_7) + (1 * n_8)}{8 * N}$$

where n represents the germinated seeds after every six hours, and N represents the total seeds.

Root lengths were measured after seven days. Vigor index was measured by multiplying seedling length and final percentage of germination. Data analyzed using Minitab15 and MSTAT-C and arcsin data transformation was performed for variance uniformity in percentage of germination data. Then all treatments were compared using Duncan's test ($p < 0.01$) with the values of the least significant digits (LSD). In this study the real data were presented.

Determination of protein content and antioxidant activities:

Three replications of four amaranth seed cultivars were conducted in completely randomized design. Treatments consisted of primed (-10 bar for three hours) and non-primed amaranth seeds which were subjected to germination test for eight hours at 25°C as described in the previous part. After imbibitions time, seeds were removed from Petri dishes, put in liquid nitrogen and ground. Protein content was determined according to Bradford method³.

Enzyme extraction and assay: Extraction and peroxidase and polyphenol oxidase assay were determined by modified procedure of Mishra and Patra⁴⁰. The amaranth seed samples were homogenized with 10 ml of phosphate buffer pH 6.8 (0.1 M). The crude homogenate was taken for the assay of peroxidase and polyphenoloxidase activities after proper dilution.

Peroxidase assay (POD, EC 1.11.1.7): The assay mixture (6 ml) contained 300 µmol phosphate buffer (pH 6.8), 5 µmol pyrogallol, 50 µmol H₂O₂, and 1 ml enzyme extract diluted. The reaction was allowed to proceed for 5 min at 25°C after which the reaction was stopped by adding 0.5 ml 5% (v/v) H₂SO₄. After centrifugation for 15 min at 14,000g, the amount of purpurogallin formed was determined from the A at 420 nm.

Polyphenoloxidase assay (PPO, EC 1.14.18.1): Five ml assay mixture for polyphenoloxidase activity consisted of the same assay mixture as that of peroxidase without H₂O₂. The absorbancy of the purpurogallin formed was taken at 420 nm. Peroxidase and polyphenoloxidase activities were expressed in absorbancy units. Protein and enzyme assay was conducted using Shimadzu UV 180 spectrophotometer and each assay was repeated three times.

Results and Discussion

Optimization of amaranth seed priming: In order to determine the effects of priming treatments on germination characteristics, first analysis of variance was performed for germination data without control treatment. Significant three-way interactions (time, cultivar and osmotic potential) were found ($P < 0.01$, 252 d.f.) for all investigated characters except speed of germination. For hydropriming, germination percentage declined significantly for all cultivars when duration of treatment increased. Three hours of hydropriming is suggested for amaranth cultivars. Among cultivars, Trigin exhibited the highest reduction over hydro- and all PEG concentrations and durations (Table 1). Except for -10 bars, Plainsman cultivar exhibited the best germination performance and the lowest reduction after 12 hours of priming treatment. Considering treatment duration, four cultivars exhibited the highest percentage of germination at three hours (Table 1). Therefore, according to the results of this experiment, priming at

Table 1. Germination percentage of hydro- and osmoprimed seeds of four amaranth cultivars after 48 hours.

Priming duration (h)	Cultivar	Hydroprime	Osmotic potential (bar)		
			-10	-12	-14
3	Mercado	60.8	72.8	76.0	69.6
	Trigin	84.0	89.6	76.0	77.6
	Amont	70.4	73.6	65.6	65.6
	Plainsman	70.4	76.0	69.6	76.0
6	Mercado	40.8	68.8	60.0	73.6
	Trigin	45.6	81.6	73.6	77.6
	Amont	48.8	68.0	60.8	64.8
	Plainsman	37.6	68.8	64.0	79.2
9	Mercado	8.8	41.6	48.8	60.0
	Trigin	2.4	41.6	45.6	67.2
	Amont	9.6	49.6	49.6	65.6
	Plainsman	8.8	52.0	57.6	60.0
12	Mercado	8.0	48.0	44.0	44.8
	Trigin	3.2	40.0	28.8	47.2
	Amont	8.0	45.6	40.0	71.2
	Plainsman	13.6	36.8	56.0	68.8

LSD (0.05), 10.55.

-10 bars for three hours is considered the best treatment for amaranth cultivars.

For speed of germination, significant effects appeared in osmotic potential, duration of priming and interaction of osmotic potential and duration of seed priming. Increasing in duration of seed hydropriming reduced speed of germination (Fig. 1). Three hours was the best treatment for hydropriming. For osmopriming, in all cultivars, maximum speed of germination was at -10 bars and 3 hours. Among cultivars, Trigin exhibited the highest speed of germination (Fig. 1).

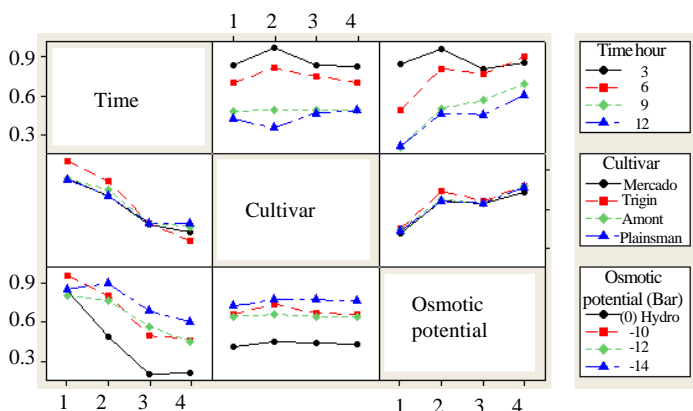


Figure 1. Interaction plot of treatment factors for speed of germination.

Osmopriming treatments induced longer root length compared to hydropriming (Table 2). Among osmopriming treatments, -10 bars for three hours exhibited more root length. Response of *Amaranthus cruentus* cultivars to priming treatments was better than *Amaranthus hypochondriacus* cultivar.

Table 2. Root length (mm) of hydro- and osmoprimed seeds of four amaranth cultivars after seven days.

Priming duration (h)	Cultivar	Hydroprime	Osmotic potential (bar)		
			-10	-12	-14
3	Mercado	40.63	46.29	40.57	45.67
	Trigin	44.94	47.04	43.48	44.48
	Amont	39.44	46.94	41.94	46.56
	Plainsman	44.50	44.72	43.39	44.13
6	Mercado	45.86	41.73	43.87	44.98
	Trigin	42.77	45.37	49.14	45.81
	Amont	40.79	45.92	45.09	41.58
	Plainsman	41.76	44.77	43.78	41.87
9	Mercado	39.57	43.55	43.50	42.41
	Trigin	43.55	47.03	47.36	47.68
	Amont	42.63	46.46	44.57	43.79
	Plainsman	41.03	45.35	44.49	40.83
12	Mercado	37.39	41.29	43.47	42.71
	Trigin	43.50	43.64	49.96	45.43
	Amont	42.11	43.68	40.77	44.24
	Plainsman	40.00	39.19	42.29	44.17

LSD (0.05) 3.276

Seed vigor also increased by seed priming (Table 3), but this increase was due to longer seedling lengths of primed seeds. Treatments with -10 bars and 3 h were the best treatments for increasing seed vigor in all amaranth cultivars. Mercado showed the highest seed vigor. After evaluation of priming treatments, comparison of priming treatments with control was conducted using randomized complete block design.

Table 3. Vigor index of hydro- and osmoprimed seeds of four amaranth cultivars after seven days.

Priming duration(h)	Cultivar	Hydro-prime	Osmotic potential (bar)		
			-10	-12	-14
3	Mercado	6652.33	7388.89	6708.56	6955.56
	Trigin	6897.22	7035.78	6335.78	6711.11
	Amont	6233.33	7111.11	6633.33	6933.33
	Plainsman	6920.44	6735.78	6411.11	6477
6	Mercado	6933.11	6522.22	6877.78	6934.11
	Trigin	6286.22	6396.89	6933.33	7022.22
	Amont	6427.56	6822.22	6922.22	6800
	Plainsman	6169.11	6726.67	6862	6524.67
9	Mercado	6177.78	6658.22	6864.44	6730.67
	Trigin	6730.89	6977.78	7166.67	7069.11
	Amont	6455.56	6881	7061.11	6477.78
	Plainsman	6351.22	6810.67	6688.67	6412.33
12	Mercado	6451.22	6243.89	6777	6791.33
	Trigin	6966.67	6913.44	7188.89	6780.78
	Amont	6491.33	6739.78	6033.33	6797.67
	Plainsman	6044.67	6270.22	6288.89	6766.67

LSD (0.05) 487.3

For all measured traits, a highly significant differences ($p < 0.01$, 268 d.f.) were detected between priming and control. Germination percentage (Fig. 2) and germination speed (Fig. 3) were significantly increased by osmopriming treatments in all amaranth cultivars after three and six hours. Comparing to control, hydropriming only for three hours exhibited higher germination (Figs 2 and 3).

Peroxidase and polyphenol oxidase activity and protein content of primed and non-primed amaranth cultivars: Two-way interaction (cultivar and priming treatments) was significant only for polyphenoloxidase activity ($p < 0.05$, 16 d.f.) and for total protein content and peroxidase activity main effects (priming and cultivar) were significant.

Significant increase in protein content due to seed priming was observed (Fig. 4). Priming increased total protein in amaranth seeds around 12%. Trigin cultivar had the least while Amont and Plainsman cultivars had the highest total protein content (Fig. 5).

An increase of 49% in peroxidase activity upon priming treatment was observed in amaranth seeds (Fig. 4). Two groups were found among cultivars for peroxidase activity. The first group with the least peroxidase activities included Mercado and Trigin. Amont and Plainsman with the highest enzyme activity made the second group (Fig. 5).

The results indicated that priming treatment increased PPO activity in four amaranth cultivars, however, the scope of this increase was different among them (Fig. 6). Mercado cultivar exhibited the maximum increase in PPO activity by around 40% and Trigin had the minimum increase by around 19%. Interestingly, all four cultivars showed similar PPO activity in control (Fig. 6).

Seed priming has typically been used to enhance germination⁴⁷. Ghana and Schillinger¹³ reported that priming media in laboratory enhanced germination during the first 24 to 48 h for winter wheat. Hardegree¹⁵⁻¹⁷ and Meyer *et al.*³⁶ have demonstrated that seed priming treatments can be used to improve the thermal germination response of native perennial grass species in both the laboratory and the field conditions. Bewly² suggested that seed germination has three steps. In the first step water will physically penetrate to seeds and in the second step metabolic activities started and enzyme

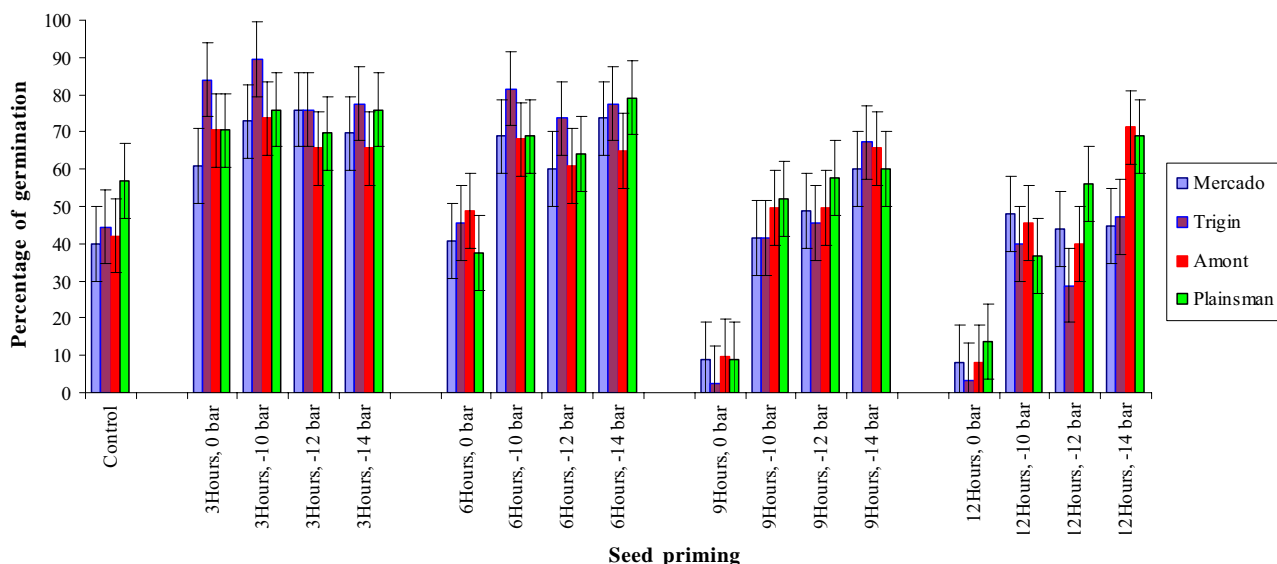


Figure 2. Comparison of hydro- and osmopriming with control on percentage of germination of four amaranth cultivars.

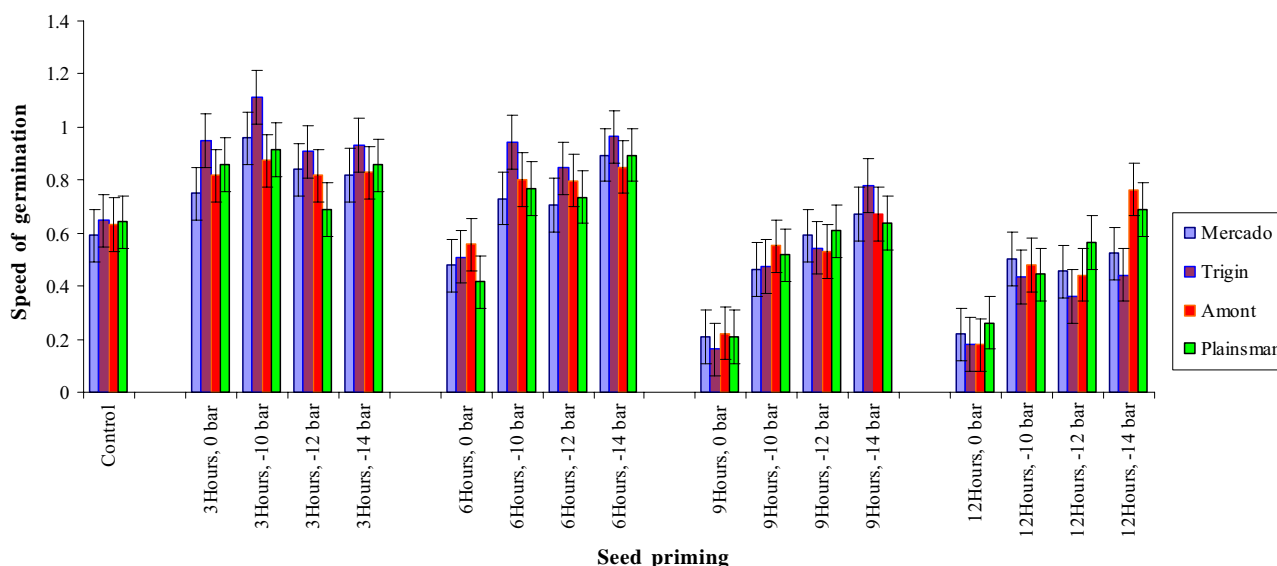


Figure 3. Comparison of hydro- and osmopriming with control on speed of germination of four amaranth cultivars.

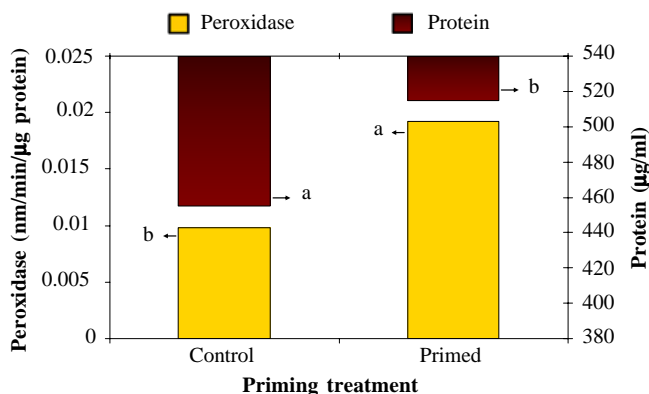


Figure 4. The effects of seed priming on total protein content and peroxidase activity.

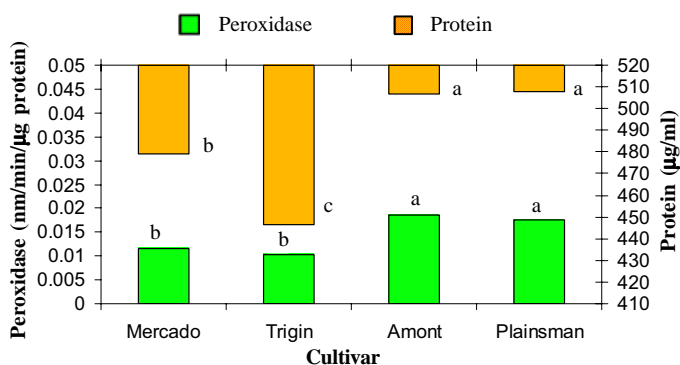


Figure 5. Total protein content and peroxidase activity in four amaranth cultivars.

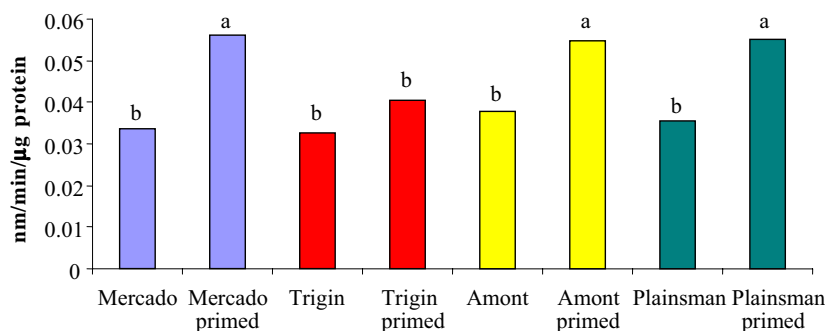


Figure 6. Effect of seed priming on polyphenoloxidase activity in amaranth cultivars.

synthesis and DNA repair happened. In these two steps, seeds are tolerant to drying. However, the third step is testa rupture and root start to be appears. This step is not tolerant to drying. Total germination percentage and germination rate, or derivative indices, are often used to make treatment comparisons and to rank relative germinability of seed populations under alternative environmental conditions^{6,44}. Both hydro- and osmopriming enhanced performance of amaranth seed cultivars. From the results of the present study for percentage of germination and rate of germination it is evident that pre-sowing treatment of amaranth seeds with distilled water for 3 hours and PEG 6000 at -10 bars for three hours was more effective than other treatments in amaranth cutlivars. Four cultivars showed no germination advantage, and sometimes a disadvantage, when seed was soaked in distilled water more than three hours. This poor performance may be due to the starting of step three in hydroprimed seeds². For osmopriming higher concentrations and longer treatment duration did not improve measured traits significantly. Kaur *et al.*²⁸ reported that in chickpea seeds, priming with PEG, mannitol (4%) and water for 24 hours produced stronger seedlings, however, increasing priming time from 24 h to 72 h did not further improve seedling growth. To examine the biochemical basis of osmopriming which might have resulted in increased germination traits, total protein content and activities of peroxidase (POD) and polyphenoloxidase (PPO) were determined in amaranth seeds after eight hours of imbibition. Total protein content and POD and PPO activities were greater in primed seeds. However, no significant two-way interactions between cultivar and priming was detected for POD (Figs 4- 6). 'Amont' and 'Plainsman' had higher protein content while 'Trigin' had less protein content. Seed priming highly increased POD and PPO activities in 'Mercado', 'Plainsman' and 'Amont'. For 'Trigin', priming could only improve POD activity (Figs 4-6). Among cultivars, Amont and Mercado exhibited the greater enzyme activity for POD and PPO, respectively. Oxidative stress inhibits growth and development by arresting cell division⁴², therefore protection from oxidative stress is crucial for seed germination. Numerous works reported the presence of several antioxidative and hydrolytic enzymes in dry cereal grains, and activities raised considerably after the start of seed imbibition^{7, 11, 38}. The enzymes of dry grains are characterized by high thermal and conformational stability²⁵. One of the best characterized antioxidant properties is peroxidase activity, which plays an important role in protecting seed from oxidative damages and maintaining seed viability. POD is well characterized antioxidative enzyme in roots of several plant species. Peroxidases participate in lignin biosynthesis, cell wall cross-linkage, IAA degradation and disease resistance, and convert H₂O₂ to water.

Conclusions

Seed priming has positive effects on germination characteristic of amaranth cultivars such as speed of germination and root length. Therefore, it could be used as pre-sowing treatment in field conditions. Antioxidant enzymes activities were increased in primed seeds. It is suggested higher activity of antioxidant enzymes could increase tolerance of primed seeds to environmental stresses such as salinity.

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