



Effect of Magneto-priming by tryptophan and ascorbic acid on germination attributes of barley (*Hordeum vulgare*, L.) under salinity stress

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Abstract

The present study investigated whether magneto-priming with tryptophan or ascorbic acid could alter the responses of barley seeds germinated under water salinity levels (2000, 4000, 6000 and 8000ppm). Magnetic seeds were irrigated by solutions containing tryptophan or ascorbic acid. Non-priming seeds were taken as control. Seed germination (%), germination index (GI), germination speed index (SGI), germination rate (GR, day), mean germination rate (MGR, day), seedling shoot length (cm), seedling root length (cm), seedling length (cm), seedling fresh weight (g), seedling dry weights, (g) seedling vigor 1 (SV1) and seedling vigor 2 (SV2) as affected by magneto-priming treatments were studied. Data showed that germination characters improved with magneto-priming treatments under different salinity levels. Tryptophan and ascorbic acid as magneto-priming gave higher values as to seed germination and seed vigour under different salinity levels. It also significantly increased some activities of enzymes, peroxidase, Polyphenoloxidase and chitinase. During seedling growth under salinity conditions, the primed seeds significantly increased accumulation enzymes with salinity levels until 6000ppm after that it was decreased. This suggests that magneto priming seed with at a suitable concentration can improve germination and seedling growth under high-saline soils.

Keywords: magnetic field, tryptophan, ascorbic acid, salinity stress, germination, enzymes, barley

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INTRODUCTION

Salt tolerance at the germination stage is important, because salinity is usually more severe at the soil surface, especially in cases with a high level of ground water (Mastsumoto 1989). Salinity is reported to not only/delays, but also decreases seed germination of most crops. Lower levels of salinity delay germination, whereas higher levels reduce the final percentage of seed germination, (Ghoulam and Fares 2001). In addition, salinity imposes other stresses such as ion toxicity, on plants, as a result of ion entry in excess of appropriate concentrations and nutrient imbalances, osmotic stress and oxidative damage (Zhang et al. 2006). El-Hamamsy and Behairy (2015) they found seedling vigor traits decreased with increasing of NaCl concentrations and the influence were significantly at the salt stress levels. Seed germination is, known to be controlled by a number of, physiological mechanisms. These mechanisms are necessary for, the growth and development of the embryo. Seed germination is also the most sensitive stage to a biotic stress, thus under environmental, stress seed of many species cannot

germinate. Seed priming method is a technique by which seed germination and seedling growth can be improved under different adverse conditions/including salinity stress. Seed priming is a pre-sowing strategy, for influencing seedling development, by modulating pre-germination metabolic activity prior to emergence of the, radicle and generally enhances germination rate and plant performance. Priming is soaking of seeds in a solution, of any priming agent followed by drying of seeds that initiates, germination related processes without radical emergence (McDonald, 2000). With priming, the process of germination is encouraged by soaking seeds in solutions containing of different compounds, such as salts, metals, growth regulators or phytohormones. Hussain et al. (2016) time and then, seeds are air-dried. Same authors found that primed-seeds gave better germination, and high growth rate even under stress conditions. In this regard, priming was

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found to be effective, for the production vigorous seedlings and substantially increased the yield, of many crop species (Nawaz et al. 2013). Hozayn et al. (2018) found that magneto-priming treatments markedly enhanced all tested germination indices in comparison with control treatment (unprimed seeds) and corresponding control at various salinity stress concentrations in addition to improvements were recorded in antioxidants enzymes activity (POD, PPO and CHIA), Field emergence (FE; %) and seedling dry weight (g pot⁻¹) compared to corresponding control. Earlier researches reported that seed priming advanced the time to 50% germination by nearly 70 h and increased crop establishment by 14% in comparison to unprimed seeds (Murungu et al. 2004) and increased. The yield of barley by 40% (Harris et al. 2005). Seed priming treatments resulted in positive, effects on many field crops, such as wheat, sugar beet, maize, soybean and sunflower (i.e., Parera and Cantliffe 1994, Saglam et al. 2010). However, there are reports that, seed priming permits early DNA replication, increase RNA and protein synthesis, enhances, embryo growth, repairs deteriorated seed parts and reduces leakage, of metabolites. Seed priming is seen as a viable, technology to enhance rapid and uniform emergence, high vigor and better yields in some field crops, (Basra et al. 2002, Chiu et al. 2002). Magneto-priming is a non-invasive priming treatment of dry seeds, which used for enhancing seed vigor, seedling growth and field emergence under environmental stresses (Bhardwaj et al. 2012, Bilalis et al. 2013, Shine et al. 2011). Proline and arginine as amino acids have a particular function in plant responses to stresses such as salt stress. The major objective of this present study was to evaluate effect of tryptophan and ascorbic acid as magneto priming on germination traits under salinity stress conditions compared with non-magneto priming in barley.

MATERIALS AND METHODS

Laboratory and field emergence experiments were conducted at the Laboratory of Seed Technology Department, Field Crops Research Institute, Agricultural Research Center, and at Screen House of Field Crops Research Department, National Research Centre, Giza, Egypt. The study aims to evaluate the effect of magneto-priming with Tryptophan and Ascorbic under different salinity stress levels (control, 2000, 4000, 6000 and 8000 ppm) on germination traits, seedling growth and vigor, antioxidants enzymes activity and field performance characteristics of barley (*Hordeum vulgare*, L.). The experiment laid out in completely randomized design (CRD) with four replications. Barley seeds (var. Giza-123) were obtained from Central Administration for Seed Production (CASP). Salinity was artificially created in the sterilized Petri dishes. Four appropriate amounts

of artificial sea water were used by dissolving known weight of natural salt crust in distilled tap water, to manufacture four levels of salinity treatments (2000, 4000, 6000 and 8000 ppm). The source of salt crust was from the salterns of Rashid, El- Beheira Governorate, Egypt.

The dry seeds were passed through the funnel of magnetic device (350 mT; 0.5 inch and made from Russia), then magnetized and they were soaked in magnetized solutions of Tryptophan 0.01mM and Ascorbic 0.1 mM, seeds were left for four hours. Finally, treated seeds were exposed to surface-dried with paper towels to match their initial moisture content at 15°C for 24h in the incubator under dark condition. For equal control, amount seeds from the same lot were kept under non-magnetic field condition. The seeds were ready for germination test in the laboratory and screen house experiment.

Germination test was performed according to ISTA rules, (1999), where 25 barley seeds were sown in each sub-replication in sterilized Petri dishes covered at the bottom with two sheets of Whitman filter paper, then placed in an incubator at 20±2°C. Total numbers of normal seedlings were counted daily and the germination percentage was calculated at 7th day. Seed germination (G%), shoot, root and seedling length (cm), seedling fresh and dry weight (g), seed germination Index (GI), germination rate (GR), speed germination index (SGI), mean germination time (MGT), seedling vigor-I (SVI) and seedling vigor -I (SVII) were calculated.

The following were utilized:

Seed germination (%): (The number of germinated seeds/The total number) X 100

Germination Rate (GR): It was defined according to the following formula of Bartlett (1937). $GR = \frac{a + (a + b) + (a + b + c) + \dots + (a + b + c + m)}{n(a + b + c + m)}$

Where a, b, c are No. of seedlings in the first, second and third count, m is No. of seedlings in final count, n is the number of counts.

Mean Germination Time: (MGT): $MGT = \frac{\sum Dn}{\sum n}$, where (n) is the number of seeds, which were germinated on day, D is number of days counted from the beginning of germination (Ellis and Roberts 1981).

Speed Germination Index (SGI):

$SGI = \frac{(\text{No. of germinated seed/days of first count}) + (\dots/\dots) + (\text{No. of germinated seed/days of final count})}{n}$. Seeds were considered germinated when the radical was at least 2 mm. long (AOSA 1983).

Seedling root and shoot length (cm): It was measured of ten normal seedling at 14 days after planting.

Seedling fresh and dry weight (g): Ten normal seedlings 7days after planting were measured to determine fresh weight then the seedlings were dried in hot-air oven at 85° C for 12 hours to obtain the seedlings dry weight (g).

Table 1. Effect of seed magneto-priming on seed germination (G; %), germination index (GI) and germination speed index (SGI), germination rate (GR; day) and mean germination time (MGT; day) of barely seeds germinated under different salinity levels

Treatment		Seed germination (%)	Germination index (GI)	Germination rate (GR; day)	Mean germination time (MGT; day)	Speed germination index (SGI)
Magneto-Priming	Salinity (ppm)					
Without priming	Control	90.0	2.7	0.9	2.0	6.6
	2000	83.3	2.1	0.9	2.2	6.3
	4000	76.7	2.0	0.8	2.4	5.2
	6000	71.7	1.8	0.8	2.5	4.7
	8000	60.0	0.5	0.5	4.3	1.0
Tryptophan	Control	93.3	2.3	1.0	1.1	8.00
	2000	90.0	2.2	1.0	1.3	7.3
	4000	85.0	2.1	1.0	1.3	7.2
	6000	80.0	1.8	0.9	1.3	6.6
	8000	65.0	1.8	0.9	1.4	6.4
Ascorbic	Control	91.7	2.5	0.9	1.6	8.3
	2000	91.7	2.3	0.8	1.7	6.8
	4000	80.0	2.1	0.8	1.8	6.1
	6000	80.0	1.8	0.7	1.9	5.4
	8000	70.0	1.3	0.6	2.3	3.7
F test		**	**	**	**	**
LSD 5%		9.13	0.32	0.09	0.37	1.43
Magneto-priming	control	76.3	1.7	0.8	2.7	4.8
	Tryptophan	82.7	2.0	1.0	1.3	7.1
	Ascorbic	82.7	1.97	0.8	1.8	6.1
F test		**	**	**	**	**
LSD 5%		4.08	0.14	0.04	0.17	0.64
Salinity levels (ppm)	Control	91.6	2.3	0.9	1.6	7.6
	2000	88.3	2.2	0.9	1.7	6.8
	4000	80.6	2.1	0.9	1.8	6.2
	6000	77.2	1.8	0.8	1.9	5.6
	8000	65.0	1.2	0.7	2.6	3.7
F test		**	**	**	**	**
LSD 5%		5.27	0.18	0.05	0.22	0.83

Seedling vigor: It was calculated following Abdul Baki and Anderson (1973) as;

Vigour index I = Germination (%) x Seedling length (Root +Shoot)

Vigour index II = Germination (%) x Seedling dry weight (Root +Shoot)

Statistical analysis: the data were statically analyzed by an analysis of variance (ANOVA) of completely randomized design (MSTAT-C v. 3.1. 1988). Least Significant Difference (LSD) was applied to compare mean values.

Field emergence experiment: It was studied in screen house, tested characters were field emergence (%), seedling dry weight and seedling vigor.

RESULTS

Seed magneto-priming and salinity stress and its interaction were significant for all studied traits.

Seed Germination Traits

Results presented in **Table 1** showed significant effect of tryptophan or ascorbic acid as Magneto-priming on seed germination (%), Germination Index (GI), Germination Speed Index (SGI), Germination Rate GR (day) and Mean Germination Time MGT (day) of barley seeds germinated under different salinity levels as compared with non magnetic- priming (control). The data showed that treated seed by two magnetic priming improved the parameters mentioned above compared to un-treated seed under corresponding salinity level. The improvement ranged between 5.3 and 6.5% in G%, 14.4

and 12.2% for GI, 28.0 and 4.1 % in SGI, 20.0 and 2.6% for GR (day) , Similar trend was observed in MGT, where it was faster by -51.7 and -23.9% by using tryptophan priming and ascorbic priming treatment compared to untreated seed with corresponding salinity levels, respectively.

Seedling Parameters

Seedling traits (i.e., shoot, root and seedling length, seedling fresh and its dry weight and vigor) were positively significant affected by two priming magnetic seed treatment compared with untreated seed under different salinity levels (**Table 2**). Treated seed caused an increased between 12.9 – 13.8 % in shoot length, 13.7 -16.6% in root length and 13.8- 14.7% for seedling length regarding magneto-priming with tryptophan or ascorbic, respectively compared with control (non magnetic priming).The same trend was in seedling weight and seedling vigour which was increase by 16.0% in seedling fresh weight and 22.2% in seedling dry weight.

The data showed that significant increases were recorded in seedling dry weight (g) when seed treated with two magnetic priming compared to untreated seed under different salinity levels (**Table 2**). The increasing ranged from 13.8 – 34.3% by using tryptophan magnetic priming and from 9.2 – 49.9% by using ascorbic magnetic priming in seedling dry weight, respectively.

Significant difference in seedling vigour 1 (SV1) and seedling vigour 2 (SV2) regarding tryptophan or ascorbic acid as Magneto-priming treated and untreated barley seeds under various salinity levels. Treated seed by

Table 2. Effect of seed magneto-priming on seedling parameters of barley seeds germinated under different salinity levels

Treatment		Seedling length (cm)			Seedling weight (g)		Seedling vigor	
Magneto-Priming	Salinity (ppm)	Shoot	Root	Seedling	Fresh	Dry	SV1	SV2
Without priming	Control	10.2	9.5	19.7	0.58	0.09	1773.0	7.9
	2000	10.0	8.5	18.5	0.51	0.08	1541.7	6.7
	4000	7.5	6.8	14.3	0.50	0.07	1092.6	5.2
	6000	6.5	6.1	12.6	0.47	0.06	899.5	4.4
	8000	5.6	5.0	10.6	0.33	0.05	636.0	3.0
Tryptophan	Control	11.3	10.7	21.9	0.66	0.01	2043.9	9.3
	2000	11.0	9.2	20.2	0.64	0.01	1813.5	8.6
	4000	9.2	8.6	17.7	0.61	0.09	1504.5	7.8
	6000	8.3	8.0	16.3	0.57	0.09	1304.0	6.8
	8000	7.1	7.5	14.6	0.38	0.06	949.0	3.8
Ascorbic	Control	11.1	9.9	21.0	0.75	0.11	1925.0	10.3
	2000	10.7	9.0	19.7	0.74	0.11	1801.3	10.3
	4000	8.6	7.9	16.5	0.52	0.08	1316.0	6.3
	6000	8.7	8.3	17.0	0.49	0.07	1356.0	5.7
	8000	8.2	7.8	15.9	0.37	0.05	1113.0	3.4
F test		**	**	**	**	**	**	**
LSD 5%		1.30	0.96	1.71	0.05	0.05	192.22	0.91
Magneto-priming	Control	7.9	7.2	15.1	0.48	0.07	1188.5	5.4
	Tryptophan	9.4	8.8	18.1	0.57	0.09	1523.0	7.3
	Ascorbic	9.4	8.6	18.0	0.57	0.09	1502.3	7.2
F test		**	**	**	**	**	**	**
LSD 5%		0.58	0.43	0.76	0.024	0.024	85.96	0.41
Salinity levels (ppm)	Control	10.9	10.0	20.9	0.66	0.100	1914.0	9.2
	2000	10.5	8.9	19.4	0.63	0.01	1718.8	8.5
	4000	8.4	7.7	16.1	0.55	0.08	1304.4	6.4
	6000	7.8	7.5	15.3	0.51	0.07	1186.5	5.7
	8000	7.0	6.8	13.7	0.36	0.05	899.3	3.4
F test		**	**	**	**	**	**	**
LSD 5%		0.75	0.56	0.99	0.030	0.030	110.98	0.52

Table 3. Effect of magneto priming on enzymes of seeds germinated under different salinity levels

Treatment		Enzymes (g/fresh weight)		
Magneto-Priming	Salinity (ppm)	Peroxidase	Polyphenol-oxidase	Chitinase
Without priming	Control	0.34	0.44	1.23
	2000	0.59	0.68	1.27
	4000	0.72	0.97	1.55
	6000	0.72	0.98	1.64
	8000	0.57	0.69	0.81
Tryptophan	Control	0.29	0.36	1.54
	2000	0.56	0.77	1.55
	4000	0.92	0.96	1.65
	6000	0.98	1.22	1.97
	8000	0.61	0.68	0.98
Ascorbic	Control	0.46	0.54	1.36
	2000	0.55	0.64	1.39
	4000	0.71	0.95	1.45
	6000	0.89	1.33	1.67
	8000	0.88	0.92	1.00
F test		**	**	**
LSD 5%		0.09	0.03	0.04
Magneto-priming	Control	0.59	0.75	1.30
	Tryptophan	0.67	0.79	1.54
	Ascorbic	0.70	0.88	1.37
F test		**	**	**
LSD 5%		0.04	0.01	0.02
Salinity levels (ppm)	Control	0.36	0.45	1.38
	2000	0.57	0.70	1.40
	4000	0.78	0.96	1.55
	6000	0.86	1.18	1.76
	8000	0.69	0.76	0.93
F test		**	**	**
LSD 5%		0.05	0.02	0.02

magnetic priming gave more value compared with untreated seed under corresponding salinity levels. The treated seed increased for SV1 by 18.2 to 18.6 and for SV2 was 22.0 to 23.1% for tryptophan and ascorbic priming treatment under different salinity levels.

Enzymes Activity

Salinity had pronounced effect on enzymes activity. Data in (Table 3) revealed that the magneto-priming treatments increased enzyme activity over the control. The high values for peroxidase were seen at 0.70, 0.67 and 0.59, and for polyphenoloxidase were 0.88, 0.79 and 0.75 for, chitinase were 1.37, 1.54 and 1.30 with treatment ascorbic and tryptophan and control, respectively.

Priming with tryptophan and ascorbic had the benefit in enzyme stimulation. Consequently, magneto priming improved seed germination and seedling establishment in studied plants. Although magnetic priming caused an increase in seed germination (%) at all salinity levels. It seemed that priming increased the activity of peroxidase and polyphenol oxidases by increasing the respiration rate that improved the germination traits. The germination of primed seeds started earlier than control seeds and it was accelerated under salinity stress seedling emergence. Seed priming had more germination rate than control and produced more dry matter under the salinity stress. As priming is simple and cheap, therefore we can offer this method to the farmers, so they can get better crop stand and synchrony of emergence in medicinal plants under the environmental stresses.

Field Emergence Experiment

At field emergence, Table 4 showed significant effects of salinity irrigation water, priming-seeds and its interaction treatments on field emergence of barley seeds. Regarding priming-seed treatments, significant increases in field emergence of barley seeds were

Table 4. Effect of magneto-priming on field emergence (%), seedling dry weight (g pot⁻¹) and seedling vigor(SVII)parameters of barley seeds under different salinity levels

Treatment		Field emergence (%)	Seedling dry wt. (g pot ⁻¹)	Seedling vigor (SVII)	
Magneto-Priming	Salinity (ppm)				
	Control	87.7	2.5	7.4	
	2000	83.0	2.1	6.6	
	4000	76.0	2.0	6.4	
	6000	64.0	1.6	6.0	
Without priming	8000	51.0	1.0	4.2	
	Control	90.0	3.41	8.1	
	2000	88.0	2.89	7.1	
	4000	83.0	2.77	6.8	
	6000	81.0	2.51	6.2	
Tryptophan	8000	77.0	2.32	5.7	
	Control	91.0	3.3	8.0	
	2000	89.0	3.1	7.6	
	4000	83.0	2.8	6.9	
	6000	79.0	2.5	6.3	
Ascorbic	8000	56.0	1.6	4.5	
	F test	***	***	***	
	LSD 5%	2.89	0.21	0.52	
	Magneto-priming	Without	72.3	1.8	6.1
		Tryptophan	83.8	2.8	6.8
Ascorbic		79.6	2.7	6.7	
F test	***	**	***		
LSD 5%	1.67	0.12	0.30		
Salinity levels (ppm)	Control	89.6	3.1	7.8	
	2000	86.7	2.7	7.1	
	4000	80.7	2.5	6.7	
	6000	74.7	2.2	6.1	
	8000	61.3	1.6	4.8	
F test	***	*	***		
LSD 5%	1.29	0.09	0.23		

obtained due to sowing magneto-priming seeds with tryptophan and ascorbic compared to control treatment. The increment reached to 15.9 and 10.1% in field emergence, 52.7 and 47.3% in seedling dry weight, 10.6 and 8.8% in seedling vigour for tryptophan and ascorbic magneto, respectively. Significant differences were obtained in recorded barley traits at field emergence due to the interaction between salinity water treatments and priming-seed treatments. Tryptophan magneto priming gave the high values of all recorded parameters, followed by ascorbic magneto priming while, sowing control seeds gave the low values in tested parameters compared to others treatments.

DISCUSSIONS

Seed germination is define to be, controlled by a number of physiological mechanisms. These mechanisms are necessary, for the growth embryo and development of the embryo. The data recorded show that the reduction for all variables increases with the increase in concentration, of the salting. Also, plants of antioxidant, tryptophan and ascorbic acid are effective in stimulating, barley seed germination. And using magnetic priming seeds, one can overcome the deleterious effect of salinity stress on seed germination.

Seed priming method is a technique by which, germination traits (such as seedling length, germination index, vigor, and weight seedling), and seedling, growth

can be improved under different adverse, conditions including salinity stress. With priming, the process of seed germination is encouraged, by soaking seeds in solutions containing of different compounds such as, salts, growth regulators, or phytohormones (Hussain et al. 2016) for certain time and then seeds are, air-dried.

The seed priming is a pressing strategy for influencing, seedling development by modulating pre-germination metabolic activity, prior to emergence of the radicle and generally enhances, germination rate and plant performance. Seed priming is soaking of seeds in a solution, of any priming agent followed by drying of seeds that, initiates germination related processes without radical emergence (McDonald 2000). Priming of seeds with different materials may helpful to alleviate the deleterious effects of, salinity and enhance seed germination in salt stress/environment by (Mohammad et al. 2010).

Salinity stress higher than 2.5 dS m probably, decreased the cell division and expansion and consequently reduced, plant growth and development. Mer et al. (2000) observed that radicle length decreased, in wheat, barley, pea and cabbage by increasing salinity. They noted that the decrease in growth, of young seedling by increasing salinity was mostly, because of decrease in water absorption, by radicle and by accumulation of soluble salts in the cell.

By increasing salinity, fresh and dry, weight of plumule decreased, which might be attributed to decrease in remobilization, of the seed reserves from cotyledons to the embryonic axis. The factors that affected the growth rate, of embryonic axis also had affect on the reserves remobilization, and transfer from cotyledons to the embryonic, axis (Akita and Cabuslay 1990). Consequently priming with tryptophan and ascorbic, improved seed germination and seedling establishment in studied plants. Although priming with tryptophan and ascorbic caused an increase, in germination of barley seeds at all salinity levels, but this increase, was greater at lower levels. It seemed that priming with tryptophan and ascorbic increased the activity of some enzymes and by increasing, the respiration rate improved the germination rate, and percentage. The germination of primed seeds started, earlier than control seeds and under salinity stress, seedling emergence, was accelerated. Seed priming with tryptophan and ascorbic had more seed germination rate than control, and produced more dry matter under the salinity stress. Jin et al. (2009) reported that both sensitive and tolerant barley genotypes had significantly higher POD activities in the salt-stressed plants than the control. Moreover, the salt-tolerant genotypes showed a higher enzymatic activity than .the sensitive ones under salinity stress. They concluded that the rate of POD activity be based on both genotype and salinity level. Yildiz and Terzi (2013) found that highly

significant correlation between increased salinity levels (0, 100, 200 and 300 mM) and increased POD activity and the tolerant cultivar of barley showing more increase than the sensitive one.

CONCLUSION

Tryptophan and ascorbic acid as magneto-priming improves seed germination and seedling growth of barley seeds under different salinity levels stress to 6000ppm then decreased after that with highly salinity. Also, priming increased enzymes activity, therefore can be said that improvement the germination

characteristics of primed seeds could increase the tolerant of salinity of treated seeds.

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