



Effect of Hydro-priming and Hormonal Priming on Germination Traits of Chickpea Cultivars

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ABSTRACT: Priming of seeds in osmoticums such as ascorbic acid, salicylic acid and in water is a simple and safe technique for enhancing seed germination. Therefore, the laboratory assay was conducted in seed Laboratory of Islamic Azad University of Sanandaj. The experiment was conducted as a factorial design based on completely randomized design with three replications. Factor A included six priming treatments of: 50 ppm Salicylic acid (SA) , 100 ppm SA, 50 ppm Ascorbic acid (AsA), 100 ppm AsA, hydro-priming and control (no priming) and factor B included the four chickpea cultivars (Kaka, Jam, Piruz and ILC462). Results showed that the maximum rate of shoot length, root length, shoot and root dry weight and shoot and root fresh weight in all chickpea cultivars was recorded by 100 AsA priming treatment. Application of higher dose (100 ppm) of SA and AsA resulted in the highest rate of germination in all chickpea cultivars. Germination percentage was significantly increased in all priming treatments in comparison with no priming or control.

Key words: Ascorbic acid, Germination, Salicylic acid, Seed priming.

INTRODUCTION

Emergence and rapid germination are important determinants of successful stand establishment of seedling. The chickpea (*Cicer arietinum* L.) as a healthy vegetarian food has an important role in human food and domestic animal feed. Chickpea is increasingly being sown in the spring rather than the autumn in Iran. However, drought stress and weed interference are considered as important problems for spring sowing of chickpea. In low temperature regions, chickpeas are drilled into cool soils. Temperatures of 10°C, commonly found in spring planting, also suppress chickpea germination.

Soaking of seeds in water or an osmotic solution permits partial seed hydration, so that pre-germination metabolic activities proceed but primary root protrusion is prevented. Such treatments, which are usually followed by drying of the seeds, are known as priming. Priming of seeds in osmoticums such as ascorbic acid, salicylic acid and in water (hydro-priming) has been reported to be a simple and safe technique for increasing the capacity of seed to osmotic adjustment and enhancing seed germination (Afghani Asl and Taheri, 2012; Ghasemi and Hosseini, 2012; Nautiyal *et al.*, 2013). Taylor *et al.* (1998) used a broader term “seed enhancement” which includes pre-soaking

hydration, coating technologies and seed conditioning. It is seen as a viable technology to enhance rapid and uniform emergence, higher vigor and better yields mostly in vegetable and flower species and some field crops.

Different osmotica can be used in seed priming and these have got different characteristics and levels of efficacy. Some of the osmotica that can be used include ascorbic acid (AsA) and salicylic acid (SA). AsA is one of the most important antioxidants abundantly occurring in plants (Ahmad *et al.*, 2012). Despite its role in scavenging reactive oxygen species, AsA is also involved in regulating photosynthetic capacity by controlling stomatal movement and is also an important co-factor of some enzymes or protein complexes are involved in the regulation of photosynthesis (Ahmad *et al.*, 2012). Seed priming with SA increased germination under low temperature condition (Sedghi *et al.*, 2010) and improved chilling tolerance faster, synchronous emergence of seed by activation of antioxidants, maintenance of tissue water contents and reduced membrane permeability (Rajabi Khamseh *et al.*, 2013). Seed priming with ascorbic acid (Sharafizad *et al.*, 2013) and salicylic acid induced salinity tolerance (Gautam and Singh, 2009; Jamshidi Jam *et al.*, 2012).

Thus, it seems that ascorbic acid, salicylic acid and water are promising materials for seed treatments. In the present study, the effects of seed priming with different concentration of ascorbic acid, salicylic acid and hydro-priming were investigated on chickpea cultivars.

MATERIALS AND METHODS

The laboratory tests were conducted in the Laboratory of Islamic Azad University of Sanandaj. This experiment was conducted as a factorial layout based on completely randomized design (CRD) with three replications. Factor A included the six priming treatment (50 ppm SA, 100 ppm SA, 50 ppm AsA, 100 ppm AsA, hydro-priming and control) and factor B included the four chickpea cultivars (Kaka, Jam, Piruz and ILC462).

For priming, chickpea seeds were soaked in aerated solution of respective osmoticum having concentration 50 and 100 ppm of SA and AsA and water for 24 h at room temperature. Untreated dry seeds considered as the control. After 24 h priming treatment in a germination box at 25°C, the seeds were washed with distilled water for 2 min and surface dried on absorbent paper. Then, seeds were surface sterilized using 0.05% sodium hypochlorite to remove microbes. Then, they were placed on a piece of clean germination paper, allowing dehydration in a drying oven at 25°C to retrieve the original seed moisture before priming treatment. The growth conditions of seeds in the germination box were 30°C of temperature, 40 J m⁻² s⁻¹ of light intensity and 12 h d⁻¹ of photoperiod. Germinating seeds were counted every day during 1-7 days after treatment. Ten days after treatment, seedling height and root length were investigated, and the dry weights of seedling shoot and root were measured after pre-drying at 90°C for 10 min and continuous drying to constant weights at 70°C. According to the methods described by Yuan-Yuan *et al.* (2010) the percent of germination calculated through the number of germinated seeds with the first 7 days / total number of seeds × 100.

To measure the emergence rate, the plots were daily visited and the emerged seedlings were recorded. The emergence rate was calculated through the following equation

$$\sum_{i=1}^i \frac{n_i}{d_i}$$

where n_i is the number of emerged seedlings on day i , and d_i is the number of days after sowing. Vigor index (VI) was calculated through the seedling length mean multiplied by emergence percentage divided by 100 (Yuan-Yuan *et al.*, 2010). The data collected in this study were subjected to analysis of variance (ANOVA) using PROC GLM of SAS statistical program and the least significant difference (LSD) was used to compare means of traits ($p < 0.05$).

RESULTS AND DISCUSSION

A. Germination rate

The results of analysis of variance showed that the priming treatments, chickpea cultivars and interaction of them had a significant effect on germination rate. The highest and lowest germination rate were in Kaka, jam, ILC462-AsA100 and jam, ILC462-SA and Kaka in control, in piruz Hydro-priming treatments, respectively (Table 1). The germination rate was reduced through increasing SA concentration. By reduction SA and AsA concentration from 100 to 50 ppm, the germination rate decreased. In all cultivars, the higher dose (100 ppm) of AsA and SA resulted in the highest rate of germination in all chickpea cultivars. Koocheki and Azizi (2006) indicated that priming *Teucrium ploiium* seeds with GA (100, 250, 500, 1000 and 1500 ppm) for 72 hours led to increased emergence rate and percentage. Similarly, Mazaherie Tirani, and Manouchehri (2005) reported that high concentrations of SA reduced the *Brassica napus* L. seed germination compared to hydro-priming and this is in accordance with the findings of present study. Also the hydro-priming treatment increased the emergence percentage compared to control treatment.

Table 1: Analysis of variance and the effects of priming on germination characteristics chickpea.

Germination percentage	Germination rate	Shoot dry weight	Mean square				Shoot length	Root length	Degrees of freedom	Sources of variation
			Root dry weight	Shoot weight	Root weight	Shoot weight				
^{ns} 8/18	0/01986*	0/00011**	0/00108*	0/001737**	^{ns} 0/00028	6/3676**	^{ns} 0/525	3	Cultivar	
1018/96**	0/99514**	0/00100**	0/00531**	0/004805**	0/04937**	18/2532**	6/604**	5	Priming	
8/18*	0/02264**	0/000103**	0/00125**	0/000899**	^{ns} 0/00053	1/1807**	1/570**	15	Priming cultiva Error	
3/88	0/00701	0/000027	0/00028	0/000336	0/00034	0/3015	0/325	48		
2/28	14/32	25/12	28/74	20/00	7/84	13/01	7/87		Coefficients of variation(%)	

ns, *, ** were significant and non-significant at 5% and 1%.

Table 2: Comparison of the interaction between priming and number on chickpea root length.

Radicle length(cm)				
kaka	piruz	ILC482	jam	Priming treatments
b8/0	b7/5	b7/4	b7/7	50 AsA
a11/7	a9/4	a11/8	a12/4	100 AsA
c6/3	c5/8	cd6/0	cd6/1	50 SA
d4/8	c5/8	e4/7	e4/6	100 SA
b7/9	b8/2	bc6/8	bc7/0	Hydro-priming
c6/3	c6/2	d5/8	d5/6	control
1/2649	1/2124	0/7878	0/6536	LSD(0.05)

* The numbers in each column with the same letter are based on a significant difference (LSD) test at the 5% level.

Table 3: Comparison of the interaction between priming and cultivar of shoot chickpea.

Shoot length (cm)				Priming treatments
kaka	piruz	ILC482	jam	
b5/8	ab5/1	b4/5	b4/6	50 AsA
a7/3	a5/4	a6/1	a6/1	100 AsA
cd3/8	c3/5	de2/5	cd2/7	50 SA
d3/0	b4/4	e2/1	d2/0	100 SA
c4/5	a5/6	cd3/2	c3/5	Hydro-priming
c4/3	bc4/2	bc4/0	c3/2	control
1/0982	1/1014	1/0011	0/629	LSD(0.05)

The numbers in each column with the same letter are based on a significant difference LSD test at the 5% level.

Table 4: Comparison of the effect of priming on the weight of chickpea root.

Root weight (g)	Priming treatments
b0/253	50 AsA
a0/356	100 AsA
c0/206	50 SA
c0/211	100 SA
cd0/196	Hydro-priming
d0/182	control
0/0151	LSD(0.05)

Average of similar letters according to LSD test at the 5% level are not significantly different

Table 5: Comparison of the interaction between priming and cultivar weight of shoot chickpea.

Shoot weight (g)				
kaka	piruz	ILC482	jam	Priming treatments
ab0/112	c0/079	ab0/106	ab0/108	50 AsA
a0/125	a0/118	a0/128	a0/134	100 AsA
bc0/090	bc0/088	d0/051	d0/048	50 SA
bc0/085	abc0/108	cd0/064	cd0/063	100 SA
abc0/099	abc0/106	bcd0/080	bc0/079	Hydro-priming
c0/074	ab0/113	bc0/091	cd0/053	control
0/0384	0/0448	0/0224	0/0163	LSD(0.05)

The numbers in each column with the same letter are based on a significant difference LSD test at the 5% level.

Table 6: Comparison of cultivar interaction between priming and root dry weight chickpea.

Root dry weight (g)				
kaka	piruz	ILC482	jam	Priming treatments
b0/058	ab0/063	abc0/057	bc0/062	50 AsA
a0/093	a0/077	a0/084	b0/081	100 AsA
b0/060	ab0/058	bc0/052	a0/142	50 SA
bc0/045	ab0/056	cd0/048	c0/049	100 SA
bc0/033	b0/041	ab0/077	bc0/070	Hydro-priming
c0/026	b0/036	d0/023	d0/016	control
0/0114	0/0173	0/0153	0/0541	LSD(0.05)

The numbers in each column with the same letter are based on a significant difference LSD test at the 5% level are not

Table 7: Comparison of cultivar interaction between priming and dry weight of shoot chickpea.

Shoot dry weight (g)				
kaka	piruz	ILC482	jam	
bc0/0246	a0/0277	a0/0300	a0/0389	50 AsA
a0/0360	a0/0297	a0/0314	a0/0394	100 AsA
b0/0250	ab0/0236	b0/0088	b0/0092	50 SA
d0/0159	ab0/0242	b0/0083	b0/0103	100 SA
cd0/0163	bc0/0190	b0/0130	b0/0123	Hydro-priming
d0/0149	c0/0145	b0/0142	b0/0071	control
0/0106	0/0073	0/0114	0/0066	LSD(0.05)

The numbers in each column with the same letter are based on a significant difference LSD test at the 5% level are not

Table 8: Comparison of the interaction between priming and cultivar rate of germination of grain.

Germination rate				
kaka	piruz	ILC482	jam	
c0/50	c0/50	b0/50	b0/50	50 AsA
a1/00	b0/67	a1/00	a1/00	100 AsA
c0/50	c0/50	b0/50	b0/50	50 SA
b0/83	a1/00	a1/00	a1/00	100 SA
c0/50	d0/33	c0/33	c0/33	Hydro-priming
d0/23	d0/22	c0/33	c0/25	control
0/2107	0/2107	0/0000	0/0000	LSD(0.05)

* The numbers in each column with the same letter are based on a significant difference LSD test at the 5% level.

Table 9: Comparison of the interaction between priming and cultivar arc sinuses square root of grain seed germination rates.

Seed germination rates				
kaka	piruz	ILC482	jam	
a90/00	a90/00	a90/00	a90/00	50 AsA
a90/00	a90/00	a90/00	a90/00	100 AsA
a90/00	a90/00	a90/00	a90/00	50 SA
a90/00	a90/00	a90/00	a90/00	100 SA
a90/00	a90/00	a90/00	a90/00	Hydro-priming
b71/01	b70/54	b62/63	b65/53	control
1/6102	5/4817	3/0805	2/6417	LSD(0.05)

The numbers in each column with the same letter are based on a significant difference LSD test at the 5% level.

B. Seedling shoots length and weight

The results of analysis of variance showed that the priming treatments and chickpea cultivars had a significant effect on shoot length; also, interaction of priming \times cultivars had a significant effect on shoot weight. Results showed that the highest shoot length was cultivars for 100 ppm AsA treatment and the lowest length Piruz cultivar was attributed to 100 ppm SA treatment. Kaka cultivar had the longest shoot compared to other cultivars. The highest shoot weight was for Jam-AsA100 treatment and the lowest length, was attributed to piruz-SA100 treatment. These results are similar with observations of Ghoohestani *et al.* (2012), who showed that shoot fresh and dry weight and root fresh weight of sugar beet were increased in seedlings raised from seeds primed with 50 ppm salicylic acid, which confirms the results of Eisavand *et al.* (2011) in carrot and Ahmad *et al.* (2012) in maize plants in response to salicylic acid treatment. SA in plants generating a significant impact on plant growth and development, photosynthesis, transpiration, ion uptake and transport and also induces specific changes

in leaf anatomy and chloroplast structure. It is supposed that the protective and growth promoting effect of SA are due to increased level of cell division within the apical meristem of seedling root, which caused an increase in plant growth. SA is recognized as an endogenous signal, mediating in plant defense, against environmental stress (Jamshidi Jam *et al.*, 2012). They also reported that pre-treatment with salicylic acid lead to increase dry and fresh weight of leaves.

C. Radicle length and weight

The results showed that the priming treatments, chickpea cultivars of priming had a significant effect on radicle length and weight. The longest AsA and shortest SA radicle were observed in Jam treatments, respectively. Also, the highest AsA 100 and lowest control radical weight were treatments, respectively. Eisavand *et al.* (2011) showed the increase in the respiration activities, ATP production, induced RNA activity and protein synthesis in the primed seeds enhanced length and weight of primed sunflower seed. Also found that the root length of cucumber and pepper increased due to hydro-priming effects.

Ahmad *et al* (2012) observed that germination percentage, root and shoot length, root-shoot ratio and catalase activity decreased with increasing salinity, while seeds treated with 20 ppm ascorbic acid.

D. Root and Shoot dry weight

The results of analysis of variance showed that the priming treatments and chickpea cultivars had a significant effect on shoot dry weight and radicle weight; also, Results showed that The highest shoot dry weight was various for 100 ppm ASA-jam treatment and the lowest weight control -Kaka was attributed treatment; also, Results showed that The highest root dry weight was cultivars for 100 ppm ASA-Kaka treatment and the lowest weight control-Jam was attributed treatment. Kodabakhash *et al* (1389) have reported that treatment of chickpea by hydro-priming increased the number of root nodules, and fresh and dry weight of chickpea seedlings (Table 7).

E. Germination percentage

The results of analysis of variance showed that the priming treatments, chickpea cultivars and interaction of them had a significant effect on germination percentage (Table 1). Germination percentage was significantly improved by the application of all priming treatments as compared with no priming or control (Table 8, 9).

CONCLUSION

Priming can be useful for chickpea seedling, because this technique increases seed and seedling quality. This is of importance as primed seeds germinate faster and this will increase their competition power against the weeds. The priming by AsA and hydro-priming was less effective than salicylic acid priming. This effect may however be modified by variables such as the size and the nature of chickpea seeds. For interested researchers, however, studying the very low concentration of AsA and SA will be a window for research.

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