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Influence of hydropriming on seed germination behavior of canola cultivars as affected by saline and drought stresses

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ABSTRACT

In order to determine the impact of hydropriming on germination characteristics of three canola cultivars under drought and salinity stresses, an experiment was conducted in 2012 at research laboratory in Bu-Ali Sina University as a factorial experiment in CRD with 3 replications. In this experiment, three factors were examined including canola cultivars (Hayola 401, Hayola 308 and RGS 003), seed hydropriming and similar osmotic potential levels by PEG-6000 and NaCl (-2, -4,-6, -8 and -10 bars) plus zero osmotic potential using by distilled water as third factor. Results revealed that in both stress, all measured traits except allometric coefficient (root:shoot length ratio) were decreased by reducing osmotic potential. Also at -8 and -10 bars of PEG all traits except germination percentage and germination index were zero. Hydropriming increased germination percentage especially under drought stress. The Hayola 401 displayed highest increase in germination percentage by hydropriming as compared with its no-primed treatment (24.6 % vs 11.5 and 11.18 % in RGS003 and Hayola 308 respectively). In all cultivars and osmotic potentials, hydropriming increased coefficient of velocity of germination about 6.5 and decreased mean germination time by 5.5 %. Highest mean germination time was achived in Hayola 308 at -10 bars in PEG (3.06 days). Germination index fluctuations by drought stress were more than salinity stress that indicate drought stress in lower potentials had been more harm to germinability than salinity stress. Also root and shoot length were reduced more in PEG than NaCl solution. However hydropriming in RGS003 and Hayola 308 at -6 bars in PEG caused increase in root length by 148 and 102 % respectively. Allometric coefficient of primed seeds of all cultivars in both stress medium increased up to -4 bars. Vigor index was better in NaCl than in PEG at the equivalent osmotic potentials and in Hayola 401 at -10 bars of NaCl the vigor index was increased about 231% compared with its no-primed.

Key words: Hydropriming, canola, germination, osmotic potential, NaCl, PEG.

INTRODUCTION

Canola *(Brassica napus* L.*)* is one of the most important oil seed crops in Iran that its production has been notably extended in recent years [30]. Canola cultivars have been developed as both spring and winter annuals and their tolerance to drought stress is less than small-grain crops [28]. Poor seedbed, low quality seeds, environmental stresses such as high and low temperatures and salinity reduce good seedling establishment. [48,49]. Seed quality (viability and vigor) can have a profound influence on the establishment and the yield of a crop [3]. Water stress is another critical environmental factor that restricts seed germination [12]. Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment [4]. Under these stresses there is a decrease in water uptake during imbibitions and furthermore salt stress may cause excessive uptake of ions [32]. Priming is one of the physiological methods, which improves seed performance and provides faster and synchronized germination [46]. Seed priming accelerates seed germination and seedling establishment under both normal and stressful environments [6]. Various pre-hydration or priming treatments have been employed to increase the speed and synchrony of seed germination [6] and it is a commercially used technique for improving seed

germination and vigor [50]. Increased salinity is a severe problem to crop production while pre-sowing seed treatments can effectively induce salt tolerance in plants [1]. This method has been suggested to improve germination performance under saline or water stress [12]. Priming may be helpful in reducing the risk of poor stand establishment under drought and salt stresses and allow more uniform growth under conditions of irregular rainfall and drought on saline soils [35]. Singh and Rao (1993) stated that KNO3 effectively improved germination, seedling growth and seedling vigor index of canola cultivars with low germination [43]. Many results have been earlier reported for improving germination and seedling vigor in wheat cultivars by seed priming under saline conditions [9, 21, 23]. Significant increase in the number of mitochondria in response to priming was reported in osmoprimed leek cells [5], although these were not correlated to respiration levels [50]. Priming also repairs any metabolic damage incurred by the dry seed, including that of the nucleic acids, thus fortifying the metabolic machinery of the seed [50]. The beneficial effects of priming have also been demonstrated for many field crops such as soybean, sugar beet, and canola [25, 41, 44]. Rao *et al*. (1987) reported that primed *Brassica* seeds might reduce the risk of poor stand establishment in cold and moist soils. Guzman and Olave (2006) reported that seed priming with nitrate solutions resulted in an improved germination rate, radical growth and germination index. Kaya *et al.* (2006) reported that seed priming increased germination and seedling growth of sunflower under drought stress. Acceleration of germination in primed seeds can be due to the increasing activity of the degrading enzymes, such as α- amylase, synthesis of RNA and DNA, the amount of ATP and the number of mitochondria [2]. The present study was conducted to evaluate the seed priming effects on germination and seedling growth of three canola cultivars under drought and saline stresses.

MATERIALS AND METHODS

The experiment was carried out at the physiology laboratory in faculty of agriculture of Bu-Ali Sina University, Iran. The canola cultivars were Hayola 401, Hayola 308 and RGS 003. The experiment was a factorial with three factors arranged in a completely randomized design with three replications. The first factor was cultivars, the second was seed treatments (untreated and hydroprimed) and the third factor was the osmotic potential levels by using NaCl as salinity stress and polyethylene glycol 6000 (PEG) as drought stress separately in 5 similar osmotic potentials (-2, -4, -6, -8 and -10 bars) plus distilled water (0 bar). Solutions with different osmotic potentials were prepared by adding NaCl or PEG to distilled water according to Van't Hoff (Lang, 1967) and Michel – Kaufmann (Michel and Kaufmann, 1973) equations, respectively. For hydropriming, canola seeds were immersed in distilled water at 25°C for 6 hours. The treated seeds were dried back to their original moisture content during the next 48 hours at room temperature in shade. Primed and non-primed seeds were placed in 9 cm glass petri dishes. Fifty seeds were placed in each petri dish. The petri dishes were moistened with 10 ml of distilled water, PEG-6000 and NaCl solutions. Seeds were allowed to germinate at 25 ± 1°C and germinated seeds were recorded every 24 hours for 7 days. Germination was considered when the radicles were greater than or equal to 2 mm in length. Root and shoot length were measured after the 7th day after starting experiment and they were averaged of 10 randomly selected seedlings in each experimental unit.

mean germination time (*MGT*) was calculated based on the equation 1 of Ellis and Roberts (1981).

Equation 1: $MGT = \frac{\sum \boldsymbol{ni\ di}}{\sum \boldsymbol{ni}}$

n_i and d_i are respectively number of germinated seeds and the number of days from from the beginning of germination experiment in i th counting.

Coefficient of velocity of germination (*CVG*) and germination index (*GI*) were calculated according to the equations 2 and 3, respectively [37]:

Equation 2: $CVG = \frac{\sum_{i=1}^{k} fi}{\sum_{i=1}^{k} \text{fi xi}} \times 100$

where *fi* is number of seeds newly germinating on day *i*; x_i is number of days from the beginning of germination experiment, and *k* is the last day of germination.

Equation 3: $GI = \sum_{i=1}^{k} |(8 - Di)Gi|/s$

Where *k* is number of germination counting (days); 8 is total number of days spent in the germination test plus 1; *Di* is number of days until the *i*th reading; *Gi* : number of normal seeds germinated in the *i*th day, and *S*: total number of seeds used in the test.

The vigor index (*VI*) was calculated according to equation 4[31].

Equation 4: *VI* = [(root length + shoot length) × germination percentage]

Germination percent, root and shoot lengths were measured in the 7th day of the beginning of germination experiment. Allometric coefficient was calculated by root to shoot lengths ratio. Data analyses were carried out using SAS and MSTATC and the comparison of means was performed by LSD test at 5%.

RESULTS AND DISCUSSION

Germination percentage

Results of variance analysis showed that cultivar, hydropriming, osmotic stress and the all double interactions were significant for this trait (Table1). The Hayola 401 displayed highest increase in germination percentage by hydropriming as compared with its no-primed treatment (24.6% vs 11.5 and 11.18% in RGS003 and Hayola 308 respectively) (Table 2). Different responses of canola cultivars to seed priming was reported by Ghassemi-Golezani *et al*. (2010). This cultivar had lower germination percentage in both hydroprimed and no-primed seeds as compared with other cultivars. Germination percentage was higher in hydroprimed seeds compared with no-primed seeds in all of the osmotic stress levels, and hydropriming resulted in the increased germination percentage in both NaCl and PEG stress environments, especially under lower osmotic potentials (Table3). According to Table 3 it can be seen that in lower osmotic potentials, the PEG had worse effect on germination percentage than NaCl due to the more adverse effects of drought stress than ionic toxicity of salinity stress[32].

Table 1: Variance analysis of studied traits

S.O.V	df	Mean Squares							
		Germination Percentage	Coefficient Velocity of Germination	Mean Germination Time	Germination Index	Root Length	Shoot Length	Allometric Coefficient	Vigor Index
Cultivar (C)	2	670.46**	16.21^{ns}	0.04^{ns}	2.45**	1.63**	0.223**	1.01**	83908.06**
Prime (P)	1	5642.67**	174.03**	0.569**	11.518**	12.64**	0.624**	6.14**	380728.5**
Stress (S)	10	9354.97**	220.3**	0.816**	33.426**	177.48**	44.46**	111.8**	2871807.6**
C × P	2	297046**	18.8^{ns}	0.058^{ns}	1.578**	0.091^{ns}	0.0004^{ns}	3.889**	6906.4*
C × S	20	106.75**	12.49*	0.048**	0.4201**	1.45**	0.348**	1.002**	25268.3**
P × S	10	135.46**	7.16^{ns}	0.033^{ns}	0.254^{ns}	1.24**	0.067**	2.156**	26360.4**
C × P × S	20	72.78^{ns}	7.85^{ns}	0.031^{ns}	0.3766*	0.25**	0.048**	2.416**	4067.2**
Error		50.11	6.35	0.023	0.187	0.083	0.0081	0.142	1463.4
C.V. (%)		9.4	5.5	6.8	9.8	9.05	6.6	15	9.7

*ns, * and **: not significant, significant at the 5 and 1% levels of probability, respectively.*

Table 2: Effect of cultivar and hydropriming on germination percentage

Canola Cultivar		RGS003	Hayola 308	Hayola 401	LSD $_{5\%}$
Germination Percentage	No-Primed	72.82	72.06	63.3	5.93
	Primed	81.21	80.12	78.88	

Table 3: Effect of osmotic stress (by PEG and NaCl) and hydropriming on germination percentage

Osmotic Potential		0	-2 $_{PEG}$	-4 $_{PEG}$	-6 $_{PEG}$	-8 $_{PEG}$	-10 $_{PEG}$	-2 $_{NaCl}$	-4 $_{NaCl}$	-6 $_{NaCl}$	-8 $_{NaCl}$	-10 $_{NaCl}$	LSD $_{5\%}$
Germination Percentage	No-Primed	80.67	93.67	90.44	78.67	52.89	7.33	82.22	81.44	74.22	74.44	47.33	6.75
	Primed	89.44	95.44	93.89	87.55	68.67	24	92	88.78	86.67	87.33	67	

The positive effects of hydropriming are probably due to the its stimulatory property at the early stages of the germination process by mediation of cell division in germinating seeds [47]. Bajehbaj (2010) showed that in primed seeds of sunflower cultivars by KNO3 solution, germination percentage and seedling growth increased under salinity stress.

Coefficient of velocity of germination

The effects of hydropriming and osmotic stress on coefficient of velocity of germination were statistically significant at 1% , also cultivar factor had an interaction with osmotic stress at 5% (Table 1). The lowest amount of

coefficient of velocity of germination was obtained from -10 bars osmotic potential of PEG solution in all cultivars (Table 4). Difference between 0 and -10 bars potential of NaCl was not significant for Hayola 401 and RGS003 cultivars (Table 4). Hydropriming increased coefficient of velocity of germination in all of the cultivars and stress levels, from 44.61% in no-primd to 47.5% in hydroprimed seeds . It has been declared that priming had been resulted in more germination speed of melon and sunflower especially in saline and drought stresses [24, 47]. Singh *et al*. (1999) reported that seed osmopriming of muskmelon with PEG resulted in higher amylase and dehydrogenase activity and germination rate in saline condition.

Mean germination time

Results of variance analysis displayed that mean germination time was affected by osmotic stress and seed hydropriming. In addition the interaction of cultivar and stress was significant at 1% probability level (Table 1). Cultivars showed different reaction to osmotic stress levels. There was significant difference between Hayola 308 and other cultivars at -10 bars potential of PEG solution (Table 4). Hydropriming reduced mean germination time in all of the cultivars and stress levels from 2.28 to 2.16 days (about 5.5%). There are some studies that reported seed priming could reduce germination time [25, 34, 51]. Bailly *et al.* (2000) reported that seed osmopriming treatment of sunflower increased strongly superoxide dismutase and catalase activities as an antioxidant system for better germination. Also priming with PEG in wild rye resulted in higher superoxide dismutase and peroxidase activity that ultimately resulted in lower germination time [22].

Germination index

The main effects, the interaction of all three factors and dual interactions apart hydropriming in osmotic stress were significant (Table 1). For all cultivars in no-primed treatment, the highest and lowest germination index were obtained at -2 and -10 bars potential respectively with PEG (Table 5). In other words, germination index fluctuations by drought stress were more than salinity stress which is consistent with the results of Murillo-Amador *et al.* (2002). This means that drought stress in lower potentials had been more harm to germinability than salinity stress. It seems that entering of sodium and chloride ions into embryo cells have facilitated osmotic adjustment under salt stress[15]. Meanwhile germination index decreased by reducing osmotic potential and hydropriming increased it in all osmotic stress levels (Table 5). Priming may increase the activity of antioxidants like glutathione and ascorbate in germinating seeds, then it can lessen side effects of stress conditions and lead to more germination rate via reduction of lipid peroxidation activity [40].

Root and shoot lengths

The main effects, the interaction of all three factors and dual interactions except hydropriming in cultivar were significant at 1% on the root and shoot lengths (Table 1). According to table 5, reduction in osmotic potential decreased root and shoot length but this reductions were higher in PEG solutions (Table 5). At the -8 and -10 bars in PEG solution, the growth of root and shoot after germination were stopped. Murillo-Amador *et al*. (2002) reported that seedling growth of cowpea was inhibited by both NaCl and PEG but higher inhibition occurred due to PEG. Similar result was found by Demir and Van De Venter (1999) in watermelon. Hydropriming in RGS003 and Hayola 308 at -6 bars osmotic potential of PEG caused to increase of root length by 148.5 and 102%, respectively. Also in Hayola 401, root length was increased by hydropriming about 94% at -6 bars osmotic potential of NaCl (Table 5). Rao *et al*. (1978), reported that priming increased the root length of lettuce seedlings. Liu *et al*. (1997) found priming induced nuclear DNA synthesis in the radicle tip cells of tomato seeds. Similar results were also reported for pepper [42], maize [18] and leek seeds [10]. Also, has been reported that increased activity of acid phosphatase and phytase in primed seeds have increased the root growth[33].

Hydropriming enhanced length of shoot in osmotic stress conditions as compared to no-primed treatment (Table 5). Hydropriming in Hayola 401 increased shoot length by 106% at -10 bars osmotic potential of NaCl.

Allometric coefficient

Analysis of variance for allometric coefficient revealed that the main and all interaction effects, were significant at 1% (Table 1). According to Table 5 it can be seen that in Hayola 308 and Hayola 401 cultivars in no-primed treatment, root to shoot length ratio increased up to -4 bars osmotic potential of PEG but in RGS003 this ratio reduced. However in all cultivars under drought stress, hydropriming increased the root to shoot ratio compared with no-primed treatment especially at -4 bars of PEG and NaCl in RGS003 cultivar. Pace *et al*.(1999) reported that in cotton, root growth was not decreased in the drought-treated plants compared with the controls, when the root to shoot ratio was more in the drought treated plants than the controls. This response may permit plants to survive drought by accessing water from deeper in the soil profile. In salinity treatments, only at the potential of -2 bars, root to shoot ratio in all cultivars increased and in other osmotic potentials, however the ratio was decreased due to decrease absorption of sodium and chloride ions into the seedlings[32].

Table 4: Effect of osmotic stress (by PEG and NaCl) and cultivar on germination percentage, coefficient of velocity of germination and mean germination time

Osmotic Potential		0	-2 PEG	-4 PEG	-6 PEG	-8 PEG	-10 PEG	-2 NaCl	-4 NaCl	-6 NaCl	-8 NaCl	-10 NaCl	LSD 5%
Germination Percentage	RGS003	87.17	94.67	92.17	86.33	64.33	18	89.33	87.33	78.66	85.33	63.83	8.23
	Hayola308	90	97	94.33	79.33	55	12.67	93	89	85	81.33	60	
	Hayola401	78	92	90	83.67	63	16.33	79	78.67	77.67	76	47.67	
Coefficient Velocity of Germination (%)	RGS003	46.92	48.15	45.88	45.31	42.97	37.45	48.36	48.27	48.78	47.94	46.03	2.87
	Hayola308	47.13	47.91	47.65	44.59	44.71	32.83	48.78	47.89	48.16	48.63	43.62	
	Hayola401	44.22	44.28	45.88	45.54	45.71	36.99	45.91	47.79	48.27	45.64	45.04	
Mean Germination Time (days)	RGS003	2.14	2.077	2.183	2.208	2.333	2.735	2.068	2.077	2.05	2.088	2.183	0.17
	Hayola308	2.125	2.088	2.098	2.025	2.245	3.057	2.048	2.088	2.077	2.058	2.3	
	Hayola401	2.285	2.323	2.187	2.203	2.192	2.71	2.192	2.095	2.073	2.198	2.228	

Table 5: Effect of osmotic stress (by PEG and NaCl), hydropriming and cultivar on germination index, root and shoot length, allometric coefficient and vigor index

Cultivar	Osmotic Potential	Germination Index		Root Length (cm)		Shoot Length (cm)		Allometric Coefficient		Vigor Index	
		No-Primed	Primed	No-Primed	Primed	No-Primed	Primed	No-Primed	Primed	No-Primed	Primed
RGS003	0	5.009	5.116	7.4	7.81	2.19	2.4	3.383	2.95	825.63	837.36
	-2 PEG	5.532	5.651	7.12	7.75	1.34	1.2	6.047	6.143	791.56	861.42
	-4 PEG	5.195	5.593	2.45	3.15	0.27	0.29	5.17	11.59	149.56	328.24
	-6 PEG	4.806	5.298	0.33	0.82	0.23	0.27	1.46	2.703	45.62	89.45
	-8 PEG	3.311	4.11	0	0	0	0	0	0	0	0
	-10 PEG	0.325	1.829	0	0	0	0	0	0	0	0
	-2 NaCl	5.058	5.59	8.27	8.57	4.55	4.9	1.82	1.837	1085.33	1265.2
	-4 NaCl	5.198	5.168	4.73	7.3	2.81	3.07	1.687	4.327	650.72	914.93
	-6 NaCl	4.681	4.76	1.6	2.52	1.28	1.5	1.247	1.683	218.72	327.01
	-8 NaCl	5.149	4.894	1.17	1.33	0.8	0.93	1.47	1.423	163.12	199.29
	-10 NaCl	3.673	3.727	0.27	0.37	0.16	0.33	1.707	1.193	25.52	46.49
Hayola 308	0	5.119	5.441	6.97	7.27	3.32	3.03	2.103	2.393	891.96	861.13
	-2 PEG	5.775	5.678	5.73	7.03	0.89	1.13	6.78	6.243	640.3	793.64
	-4 PEG	5.644	5.486	2.83	3.38	0.35	0.5	8.417	7.153	298.15	367.03
	-6 PEG	4.472	4.739	0.46	0.93	0.24	0.32	1.94	2.913	53.15	104.56
	-8 PEG	2.935	3.494	0	0	0	0	0	0	0	0
	-10 PEG	0.325	0.914	0	0	0	0	0	0	0	0
	-2 NaCl	5.465	5.629	7	7.43	4.38	4.37	1.597	1.703	1039.36	1117.2
	-4 NaCl	5.216	5.374	6.4	7.77	3.41	4.2	1.937	1.853	856.49	1093
	-6 NaCl	4.9	5.465	3.1	3.4	1.53	1.65	2.043	2.073	373.55	450.81
	-8 NaCl	4.8	4.912	1.24	1.37	0.63	0.7	1.957	1.953	150.09	170.87
	-10 NaCl	3.102	3.539	0.23	0.26	0.19	0.22	1.163	1.253	20.02	34.41
Hayola 401	0	3.974	5.085	6.8	7.41	3.07	3.11	2.217	2.38	684.73	912.2
	-2 PEG	5.24	5.413	6.79	7.37	0.92	1.01	7.38	7.02	700.95	779.09
	-4 PEG	5.201	5.35	2.39	3.78	0.34	0.42	7.033	7.67	241.43	383.21
	-6 PEG	4.688	5.113	0.41	0.93	0.25	0.27	2.056	3.41	59.13	107.12
	-8 PEG	3.348	4.047	0	0	0	0	0	0	0	0
	-10 PEG	0.814	1.091	0	0	0	0	0	0	0	0
	-2 NaCl	4.393	4.873	6.33	7.17	4.73	5.23	1.34	1.37	782.6	1082.47
	-4 NaCl	4.162	5.131	5.4	6.33	3.1	3.17	1.747	2	600.87	823.13
	-6 NaCl	3.919	5.392	1.25	2.43	1.1	1.3	1.14	1.53	155.37	243.37
	-8 NaCl	3.509	5.407	0.75	1.02	0.71	0.81	1.067	1.263	88.63	167.03
	-10 NaCl	2.044	3.591	0.17	0.31	0.16	0.33	1.14	1.017	11.68	38.68
	LSD 5%	0.69		0.46		0.14		0.63		60.92	

Vigor index

Analysis of variance for vigor index like allometric coefficient, revealed that the main and all interaction effects, were significant at 1% and 5% (Table 1). By increasing the severity of osmotic stress, vigor index decreased in all of the cultivars (Table 5). Basra *et al*. (2003) found that germination and seedling vigor of wheat under saline stress were reduced due to entering Na^+ and/or Cl^- in to the embryo cells.

For Primed and no-primed seeds, vigor index was better in NaCl than in PEG at the equivalent osmotic potentials like effects on root and shoot length traits (Table 5). Also hydropriming treatment caused significant increase in vigor index especially in Hayola 401 at -10 bars of NaCl that vigor index was increased about 231% compared with its no-primed. Elouaer and Hannachi (2012) reported that osmopriming of sufflower by NaCl and KCl have improved vigor index parameter. It is evident that priming can increase free radical scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase in seeds [11, 13].

CONCLUSION

Generally hydropriming improved the seed germination behavior of canola cultivars under drought and salinity stress. However hydropriming had better effects under drought stress (PEG) up to -6 bars osmotic potential, due to more increase in root length, allometric coefficient, germination index and vigor index (except Hayola 401 at -10 bars in NaCl) in three studied cultivars. Meanwhile the cultivar Hayola 401 showed a better response to hydropriming treatment than the other two cultivars.

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