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The Effect of Priming on Seed Performance of Canola (*Brassica napus* L.) Under Drought Stress

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Abstract: In order to investigate the effect of priming on canola seed performance under drought stress a study was carried out at the Seed Research Laboratory of Razi University, Kermanshah, Iran. The experiment was a factorial with three factors arranged in a completely randomized design with three replications. The first factor was seed priming (control (untreated), KNO_3 and distilled water), the second was drought stress levels (0.0, -0.3, -0.6, -0.9, -1.2 and -1.5 MPa) and the third was canola cultivars (Okapi and Talayeh). Results indicated that for both cultivars germination percentage (GP), mean germination rate (MGR), radicle length (RL), plumule length (PL) and seedling dry weight (SDW) were reduced when drought stress level were increased from 0 to -1.5 MPa. Although, the cultivars showed different responses to the increased drought stress level. For all of the traits under study, the best results due to the priming treatments were obtained at the drought stress levels higher than -0.6 or -0.9 MPa. So that, at the -1.5 MPa of drought stress level, GP, MGR, RL, PL and SDW were improved 128.62, 200, 223.08, 350 and 69.94%, respectively by KNO_3 and 21.44, 71.43, 219.23, 100 and 41.18%, respectively by distilled water when compared with control. However, seeds primed with KNO_3 showed better performance than those primed with distilled water. In general, this study revealed that seed priming especially with KNO_3 improved canola seed performance under drought stress condition. However, the improvements were more obvious at the higher levels of drought stress.

Key words: Canola · Drought stress · Seed priming

INTRODUCTION

Canola is one of the most important oil seed crops which its production has been notably extended during recent years in Iran. Canola seeds are common planted in seedbeds having unfavorable moisture (because of the lack of rainfall at planting time). Drought stress is responsible for both inhibition and delayed seed germination and seedling establishment of canola in many areas of Iran. This stress adversely affects growth and development of crop and results into low canola yield and economic return. Under this stress condition there is a decrease in water uptake both during imbibition and seedling establishment [1]. It has also been shown that the inhibition of radicle emergence is mainly because of a decrease in water potential gradient between the external environment and the seeds [2]. Kaya *et al.* [3] also found that seed germination of sunflower delayed under drought condition.

Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops, especially under stress conditions [4-6]. According to Sivritepe and Dourado [7] seed priming is one of the physiological methods, which improves seed performance and provides faster and synchronized germination. Singh and Rao [8] stress that potassium nitrate effectively improved germination, seedling growth and seedling vigor index of the seeds of sunflower varieties with low germination. Olouch and Welbaum [9] suggested that priming can be a valuable process for improving germination and uniformity of heterogeneously matured seed lots. Rao *et al.* [10] also reported that primed Brassica seeds may reduce the risk of poor stand establishment under unfavorable condition. Guzman and Olave [11] reported that seed priming with nitrate solutions resulted in an improved germination rate, radicle growth and germination index. Kaya *et al.* [3] suggested that seed priming increased germination and seedling growth of sunflower under drought stress.

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The present study was conducted to evaluate the seed priming effect on germination and seedling growth of two canola cultivars under drought stress.

MATERIALS AND METHODS

The experiment was carried out at the Seed Research Laboratory of Faculty of Agriculture of Razi University, Kermanshah, Iran. The canola cultivars used were Okapi and Talayeh (the canola cultivars which are widely planted in the region). The experiment was a factorial with three factors arranged in a completely randomized design with three replications. The first factor was seed priming (control (untreated), KNO_3 and distilled water), the second was drought stress levels (0.0, -0.3, -0.6, -0.9, -1.2 and -1.5 MPa) and the third was canola cultivars (Okapi and Talayeh).

For distilled water treatment, canola seeds were immersed in distilled water at 25°C for 18 h under dark conditions. For KNO_3 treatment, the seeds were immersed in 500 ppm KNO_3 solution at 25°C for 2 h under dark conditions [8]. Thereafter, the seeds treated with KNO_3 solution rinsed with distilled water. Following this, the primed seeds were dried between two filter papers. Primed and non-primed seeds were placed in 9 cm glass petri dishes on a layer of filter paper (Whatman # 41). Twenty five seeds were placed in each petri dish. The petri dishes were moistened with 10 ml of PEG-6000 solution at water potentials of 0.0 (distilled water), -0.3, -0.6, -0.9, -1.2 and -1.5 MPa [12]. The petri dishes were placed in a germinator at 20±1°C.

Seed germination was recorded daily up to day 7 after the start of the experiment. A seed was considered germinated when radicle emerged by about 2 mm in length. Then the mean germination rate was calculated according to the following equation [13]:

$$MGR = \Sigma n / \Sigma Dn$$

Where MGR is the mean germination rate, n is the number of seeds germinated on day and D is the number of days from the start of test.

Moreover, germination percentage was determined in the end of test. To determine the radicle and plumule length after the 7th day, radicles and plumules produced in each petri dish were separated from the seeds, their lengths were measured, then dried at 70°C to a constant weight and seedling dry weights were determined. Data analyses were carried out using SAS software [14].

RESULTS AND DISCUSSION

Analysis of variance (Table 1) indicated that all of the traits under study including germination percentage (GP), mean germination rate (MGR), radicle length (RL), plumule length (PL) and seedling dry weight (SDW) were significantly influenced by drought stress (at the 0.01 level of probability). The evaluated traits except PL were also significantly affected by seed priming and cultivar (Table 1). Moreover, the two-way interaction effects (seed priming × drought stress and drought stress × cultivar) were statistically significant for the studied traits except the PL, whereas, seed priming × cultivar interaction was only significant for GP and MGR (Table 1). However, the three-way interaction effect (seed priming × drought stress × cultivar) was not significant for all of the traits under study (Table 1).

All of the traits under study were reduced when drought stress level were increased from 0 to -1.5 MPa (data not shown). This is in agree with Murillo-Amador *et al.* [15] in cowpea, Demir and Van De Venter [16] in watermelon, they affirmed that drought may influence germination by decreasing the water uptake. El-Midaoui *et al.* [17] reported that root and shoot growth significantly decreased by osmotic stress at -0.6MPa and above induced by PEG 6000. Murillo-Amador *et al.* [15] also found that seedling growth of cowpea was inhibited by both NaCl and PEG, but higher inhibition occurred due to PEG.

The cultivars under study showed different responses to the increased drought stress level (Table 2) so that, increased drought stress level from 0 to -1.5 MPa reduced GP, MGR, RL and SDW by 71.92, 84.13, 67.44 and 84.38%, respectively for Talayeh. Whereas, these reductions were by 56.60, 79.41, 69.08 and 53.50%, respectively for Okapi. However, at -1.5 MPa of drought stress level both cultivars failed to produce the plumule (Table 2). Murillo-Amador *et al.* [5] found that germination and emergence rate of two cowpea cultivars were delayed by PEG solution with differences between cultivars. Sadeghian and Yavari [18] also reported that seedling growth severely diminished with increased drought stress and genetic differences were found in sugar beet.

In present study, both KNO_3 and distilled water treatments improved canola seed performance. The improvements were more notable at the higher levels of drought stress (Table 3). For all of the traits under study,

Table 1: Analysis of variance of the traits under study.

	Mean Square				
Source of Variance	GP	MGR	RL	PL	SDW
Seed priming (S)	553.926 **	0.057 **	7.296 **	3.726 ns	961.75 **
Drought stress (D)	27268.410**	0.634 **	378.371 **	29.741 **	2460.53 **
Cultivar (C)	2169.037 **	0.422 **	11.807 **	2.379 ns	207.93 *
S×D	662.815 *	0.437 *	322.079 **	2.150 ns	317.75 *
S×C	362.815 *	0.044 *	3.606 ns	2.495 ns	78.39 ns
D×C	381.481 *	0.027 *	91.312 *	2.428 ns	407.32 **
S×D×C	154.459 ns	0.004 ns	1.380 ns	2.360 ns	37.62 ns
Error	86.667	0.006	1.214	2.222	72.54

Abbreviations: GP, germination percentage; MGR, mean germination rate; RL, radicle length; PL, plumule length; SDW, seedling dry weight.ns, * and **: Non significant and significant at the 0.05 and 0.01 level of probability, respectively.

Table 2. Means comparison of the drought stress level × cultivar interaction for the traits under study.

	Trait									
Drought stress	Germination percentage		Mean germination rate		Radicle length (cm)		Plumule length (cm)		Seedling dry weight (mg)	
level (MPa)	Talayeh	Okapi	Talayeh	Okapi	Talayeh	Okapi	Talayeh	Okapi	Talayeh	Okapi
0	95.8 ab	100 a	0.63 a	0.68 a	2.15 b	2.62 a	2.22 c	2.99 a	70.44 bc	89.11 a
-0.3	89.7 b	89.9 ab	0.61 a	0.61 a	2.12 b	2.40 a	1.92 d	2.64 b	61.33 cd	80.67 b
-0.6	48.2 c	88.7 b	0.46 bc	0.51 bc	2.00 b	2.13 b	0.84 e	0.87 e	43.55 de	73.00 bc
-0.9	43.5 c	77.9 b	0.37 d	0.45 c	1.07 c	1.16 c	0.46 f	0.86 e	31.00 e	66.56 c
-1.2	29.4 d	66.4 bc	0.24 e	0.26 e	0.80 d	0.84 d	0.00 e	0.29 f	22.22 f	51.00 d
-1.5	26.9 d	43.4 c	0.10 f	0.14 f	0.70 d	0.81 d	0.00 e	0.00 e	11.00 g	41.44 de
LSD (0.05)	10.09		0.07279		0.1655		0.285		8.004	

The same letters at each column indicate an insignificant difference at the 0.05 level of probability. LSD, least significant difference

Table 3: Means comparison of the drought stress level × seed priming interaction for the traits under study

	Trait														
Drought stress	Germination percentage			Mean germination rate			Radicle length (cm)			Plumule length (cm)			Seedling dry weight (mg)		
level (MPa)	KNO3	DW	C	KNO3	DW	C	KNO3	DW	C	KNO3	DW	C	KNO3	DW	C
0	90.00 abc	92.00 abc	92.00 abc	0.53 b	0.56 ab	0.62 a	3.75 a	3.51 b	3.35 b	0.50 a	0.35 cd	0.31 d	83.17 a	82.83 a	83.33 a
-0.3	98.00 ab	100.00 a	97.33 ab	0.50 bc	0.55 ab	0.57 ab	2.61 c	2.37 cd	2.17 cd	0.45 b	0.31 d	0.30 d	76.17 ab	87.83 a	79.00 ab
-0.6	96.67 ab	98.00 ab	90.67 abc	0.56 ab	0.57 ab	0.63 a	2.26 d	2.05 d	1.60 de	0.40 c	0.32 d	0.30 d	69.33 b	71.87 b	66.13 b
-0.9	90.67 abc	86.67 bc	80.67 c	0.44 c	0.41 c	0.42 c	1.22 e	1.14 ef	0.99 fg	0.36 cd	0.27 de	0.16 f	44.92 cd	39.78 d	36.84 d
-1.2	31.33 d	22.00 ef	11.33 ef	0.33 cd	0.28 d	0.23 d	0.85 gh	0.84 gh	0.39 h	0.30 d	0.20 e	0.10 g	29.99 de	27.00 e	25.93 e
-1.5	21.33 de	11.33 ef	9.33 f	0.21 de	0.12 ef	0.07 f	0.84 gh	0.83 gh	0.26 h	0.09 g	0.04 h	0.02 h	26.89 e	24.00 e	17.00 f
LSD (0.05)	10.71			0.089			0.202			0.05			9.8		

Abbreviations: KNO3, potassium nitrate; DW, distilled water; C, control (untreated).

The same letters at each column indicate an insignificant difference at the 0.05 level of probability. LSD, least significant difference.

the best results due to the priming treatments were obtained at the drought stress levels higher than -0.6 or -0.9 MPa (Table 3). So that, at the -1.5 MPa of drought stress level, GP, MGR, RL, PL and SDW were improved 128.62, 200, 223.08, 350 and 69.94%, respectively by KNO_3 treatment and 21.44, 71.43, 219.23, 100 and 41.18%, respectively by distilled water treatment when compared with control (untreated) (Table 3). In other words, the positive effects of priming treatments were increased at high levels of drought stress (Table 3). Kaya *et al.* [3] also found both hydro- and osmopriming gave better performance than control (untreated) under drought stress with clear effectiveness of seed priming in improving the germination percentage at low water potential.

Overall, for most traits under study, seeds primed with KNO_3 showed better performance than those primed with distilled water. This was more obvious especially at the higher levels of drought stress (Table 3). Demir and Van De Venter [16] reported the beneficial effects of KNO_3 on seed germination of watermelon. In their study, hydropriming shortened mean germination rate, however, final germination was higher from KNO_3, suggesting nontoxicity of KNO_3 due to ion accumulation

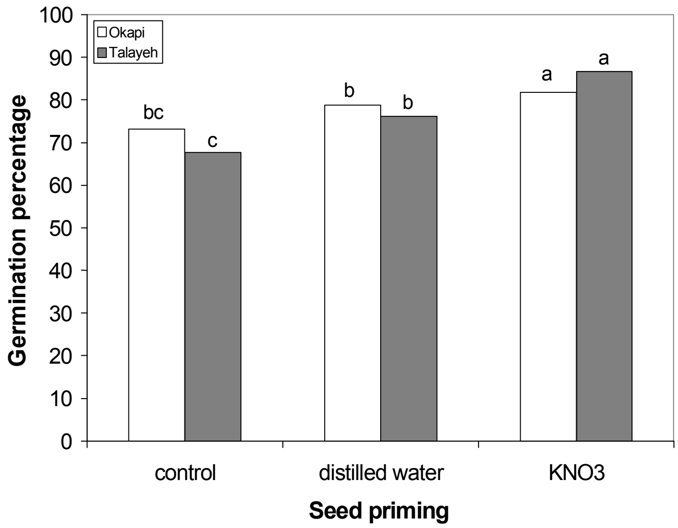

Fig. 1: The effect of different seed priming treatments on germination percentage of two canola cultivars.

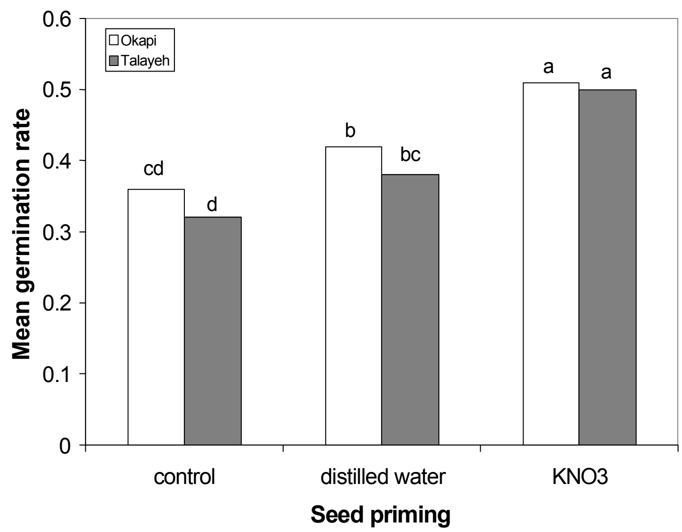

Fig. 2: The effect of different seed priming treatments on mean germination rate of two canola cultivars.

in the embryo. According to Mohammadi [19] different seed treatments led to improved seed germination percentage, germination rate and seedling growth of soybean. Among the seed treatments, KNO_3 showed the better results for all of the traits under study. Similar findings also suggested by Ghassemi-Golezani *et al.* [20]. This can be attributed to more nitrogen and potassium accumulation in seeds treated with KNO_3 [21, 22]. For both cultivars, seed priming enhanced GP and MGR (Fig. 1 and 2). Although, Talayeh responded better to seed priming, especially when seeds primed with KNO_3 (Fig. 1 and 2). For Talayeh, seed priming with KNO_3 enhanced GP and MGR by 28.06 and 56.25%, respectively, as compared with control, whereas, these enhancements were 11.76 and 41.67%, respectively for Okapi. Ghassemi-Golezani *et al.* [20] also reported that rapeseed cultivars respond differently to seed priming.

In fact, improved seed performance induced by seed priming may be due to altered physiological condition of the embryo. It may be also due to libration of enzymes, thus rapidly increasing in the production of soluble food nutrients, the whole system is already in motion so that when the seeds are sown developmental processes go on more rapidly than in case of non-primed seeds [23]. There are several indications that many physiological mechanisms are involved in seed priming, the repair of the age related cellular and sub-cellular damage that could accumulate during seed development [24, 25] and an advancement of metabolic events during the prolonged lag phase-II imbibition that repairs the radicle protrusion

[26]. Some morphological changes also occur in the primed seeds which are helpful in the later growth of embryo, e.g. a portion of the seed endosperm is hydrolyzed during priming that permits faster embryo growth [25]. 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 [27].

In present study, correlation analysis of the traits indicated the positive and significant correlations (at the 0.01 level of probability) between GP with MGR and SDW ($r = 0.87$ and 0.72, respectively). Moreover, there was a positive and significant correlation between MGR and SDW ($r = 0.77$). It is concluded that improved MGR of canola seeds due to seed priming can be lead to the higher seedling dry weight produced.

CONCLUSION

Overall, the present study revealed that canola seed performance was diminished as drought stress level was enhanced. Although, the canola cultivars showed different responses to drought stress. Seed priming especially with KNO_3 improved canola seed performance under drought stress condition. The improvements were more obvious at the higher levels of drought stress. Therefore, seed priming can be used as an efficient method to improve seed performance of canola under drought stress condition.

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