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Effect of ethanol, methanol, zinc, manganese, and boron seed priming on seed mineral concentration at 8 weeks after planting and the end of growth in Canola (*Brassica napus* L.) under water deficit stress

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Abstract

For studying the effects of seed priming with ethanol, methanol, Zn, B and Mn on seed mineral concentration at 8 weeks after planting and the end of growth in Canola (Brassica napus L.), 2 separate laboratory and greenhouse experiments were conducted. Laboratory experiment was performed as a Completely Randomized Design with 6 priming treatments: (Non-primed, 2% ethanol, 2% methanol and 0.5% each ZnSo4, H3BO3, and MnSO4.H2O solutions) in three replicates. Greenhouse experiment was conducted as a factorial experiment form by Completely Randomized Design with 4 levels of irrigation: (100%, 75%, 50%, and 25% FC) with 3 replications. The detailed results of the study proved that Zn, B, and Mn application through canola seed treatments could be used to increase this mineral concentration in seeds under normal irrigation (100% FC) at 8 weeks after planting and the end of growth compared with the other treatments. Based on the results, the Zn concentration showed an increase of 79% (8WAP) and 40% (EOG), due to Zn-seed priming. Also, the B concentration showed an increase of 2/5 times (8WAP) and 2/2 times (EOG), due to B-seed priming and Mn-seed priming increased 2/5 times (8WAP) and 93% (EOG) Mn concentration of the seeds.

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Introduction

Water stress affects almost every developmental stage of the plant. However, damaging effects of this stress was more noted when it coincided with various growth stages such as germination; seedling shoot length, root length and flowering (Khayatnezhad, *et al.*, 2010). Seed priming is a technique that controls seeds hydration and drying to their original moisture content (Farooq *et al.* 2012). During the seed hydration period, a variety of physiological and biochemical changes take place in seeds. This technique has become a common seed treatment that can increase rate, percentage and uniformity of germination or seedling emergence, mainly under unfavourable environmental conditions. Rapid seed germination and stand establishment are critical factors for crop production under stress conditions (Askari Nejad, 2013). There are mainly 3 methods of micronutrient application in crops: application to soil, foliar sprays, and seed treatment (Johnson *et al.* 2005). Each method may affect plant growth differently. The use of nutripriming has been reported to be a better strategy in overcoming micronutrient deficiencies. In nutripriming, micronutrients are used as osmotica (Singh, 2007). Primed seeds usually have better and more synchronized germination (Farooq *et al.* 2009) owing simply to less imbibition time and build-up of germination-enhancing metabolites (Farooq *et al.* 2006). Ethanol and methanol has also been reported to have stimulatory effects on the germination of seeds of many plant species (Farooq *et al.* 2007). Canola is an important oil crop often cultivated in arid and semiarid regions of the world such as Iran where water deficit stress and multiple micronutrient deficiencies occur in its soils that are becoming more prevalent as cropping intensity increases. Therefore, the objectives of this study were to explore the beneficial effects of ethanol, methanol and some of micronutrients as priming reagent on the seed mineral concentration under water deficit stress in Canola.

Materials and methods

The experiment was conducted at the Research Station of the Islamic Azad University, Tabriz Branch, north-western Iran, during the 2011. The canola cultivar used was Okapi.

Laboratory experiment

The laboratory experiment was carried out in a Completely Randomized Design in 3 replicates. The seeds were soaked in 2% ethanol, 2% methanol and 0.5% each ZnSo4, H3BO3, and MnSO4.H2O solutions for 12h at 27±2 0C.

Greenhouse experiment

Primed seeds were sown in pots with 35cm diameter containing loamy soil. In each pot 10 seeds were planted 2 cm in depth. The factorial experiment form by Completely Randomized Design was done with 4 levels of irrigation: 100%, 75%, 50% and 25% Field Capacity with 3 replications. Water deficit stress was imposed from stem elongation to physiological maturity. The mineral concentration was determined in 8 weeks after planting and the end of growth using the Atomic Absorption Spectrophotometry method (Hanlon, 1998).

Statistical analysis

Data analyses were carried out using MSTAT-C software. The means of the treatments were compared using the LSD (Least significant difference, $P<0.05$) values.

Results and discussion

The analysis of variance showed that seed priming and water deficient stress had a significant effect on the Zn, Mn, and B seed concentration in 8 weeks after planting and the end of growth ($p<0.01$), (tab.1).

The mean comparison of data showed that seed priming with Zn in 100 % FC had the highest (69/07 mg kg^{-1}) and seed priming with Mn in 25 % FC had the lowest (38/57 mg kg^{-1}) Zn concentration in the seed at 8WAP. (tab2). Furthermore, seed priming with ethanol in 100 % FC had the highest (71/47 mg kg^{-1}) and seed priming with Mn in 50 % FC had the lowest (41/82 mg kg^{-1}) Zn concentration in the seed at the EOG (tab2). Taylorson and Hendricks (1979) suggested that the stimulatory effect of ethanol might

involve modification of the properties of a membrane(s). Ethanol might also be involved metabolically in the stimulation of germination, as a respiratory substrate. It might accelerate germination by promoting the uptake of oxygen and increasing levels of fructose 2, 6-bisphosphate which has been suggested to stimulate glycolysis in seeds (Farooq *et al.*, 2007).

Table 1. The analysis of variance of measured traits in greenhouse experiment.

S.O.V	df	Zn 8 WAP	B 8 WAP	Mn 8 WAP	Zn EOG	B EOG	Mn EOG
WDS	3	618**	1730**	0/003**	423**	1696**	0/026**
Priming	4	202**	1159**	0/028**	289**	1233**	0/027**
WDS× Priming	12	294**	83/81**	0/067**	281**	87/71**	0/064**
Error	38	1/12	1/39	0/001	1/126	4/49	0/001
CV	-	1/9	2/41	5/08	1/75	3/85	4/94

* and ** significant at 5% &1% respectively, WDS: Water deficit Stress, 8WAP: 8weeks after planting, EOG: End of growth.

Table 2. Mean comparison of interaction between priming and water deficit stress based on LSD**.**

WDS	Priming	Zn mg kg^{-1} 8 WAP	B mg kg^{-1} 8 WAP	Mn mg kg^{-1} 8 WAP	Zn mg kg^{-1} EOG	B mg kg^{-1} EOG	Mn mg kg^{-1} EOG
25% FC	Ethanol	59/50	27/40	0/5610	67/50	33/40	0/6777
	Methanol	40/73	31/40	0/5333	46/73	37/73	0/6600
	Zn	57/47	27/50	0/6103	61/47	33/83	0/7400
	B	59/47	53/50	0/4707	61/47	61/50	0/5973
	Mn	38/57	24/70	0/7200	61/30	28/70	0/8733
50% FC	Ethanol	61/47	55/20	0/6937	57/47	61/20	0/8203
	Methanol	66/33	61/63	0/4627	69/53	67/30	0/5927
	Zn	66/13	42/13	0/5413	54/30	49/13	0/6613
	B	40/43	71/60	0/5100	43/43	77/93	0/6437
	Mn	60/40	42/50	0/5727	41/82	49/17	0/6860
75% FC	Ethanol	46/43	62/57	0/6633	54/43	68/23	0/7900
	Methanol	62/53	53/43	0/5720	73/07	58/43	0/6887
	Zn	65/40	42/60	0/6830	69/40	48/27	0/8063
	B	60/60	69/40	0/5117	62/60	76/07	0/6417
	Mn	68/20	42/17	0/5100	69/20	48/17	0/6367
100% FC	Ethanol	46/47	53/60	0/7013	71/47	59/60	0/8280
	Methanol	69/07	57/30	0/5437	71/33	63/30	0/6703
	Zn	48/30	48/47	0/5510	70/13	53/80	0/6743
	B	39/57	61/70	0/4810	44/67	67/03	0/6077
	Mn	60/30	51/30	1/052	61/40	56/63	0/1/145
LSD	-	1/751	1/950	0/0521	1/751	3/497	0/0521

The results showed that the B-seed priming in 100% FC had the highest effect on the B concentration of seed in 8WAP (61/7 mg kg^{-1}) whereas Mn-seed priming in 25% FC showed the lowest effect (24/7 mg kg^{-1}), (tab.2). In wheat, rice, and chickpea, priming with 0.008 M boric acid increased B seed concentration compared with the control (Johnson *et al.* 2005).

Also, B-seed priming in 75% FC with 76/07 mg kg^{-1} had the highest effect on the B concentration of seed in the end of growth and methanol-seed priming in 25% FC with 33/4 mg kg^{-1} had the lowest effect on B concentration of seed in the EOG. Based on the results, the priming with Mn in 100% FC had the highest effect (1/052 mg kg^{-1}) on the Mn concentration of the seed in 8WAP while methanol-priming in 50% FC had the lowest effect (0/462 mg kg^{-1}), (tab.2). Furthermore, Mn-seed priming in normal irrigation had the highest effect (1/145 mg kg^{-1}) on the Mn concentration of the seed in EOG while methanol-priming in 50% FC had the lowest effect (0/592 mg kg^{-1}), (tab.2).Priming wheat seeds in $MnSO_4$ solutions (0.1M for 12h) significantly improved grain Mn contents (Nazir *et al.* 2000).

Conclusion

In conclusion, this study showed that Zn, B, and Mn application through canola seed treatments could be used to increase this mineral concentration in seeds at 8 weeks after planting and the end of growth in the normal irrigation (100% FC) condition.

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