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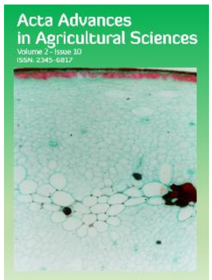

Research Article

Micronutrient priming improves germination and seedling establishment in lentil

Ali Asghar Aliloo [1*], Shirin Alahyari [1] and Seyed Bahman Mosavi [2]

[1] *Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Maragheh, Maragheh, P.O. Box 55181-83111, Iran.*

[2] *Department of Soil Science, Faculty of Agriculture, University of Maragheh, Maragheh, P.O. Box 55181-83111, Iran.*

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* Corresponding Author;
E. Mail: aliloo@maragheh.ac.ir, aliasghar.aliloo@gmail.com

ABSTRACT

Seed priming is one of the most effective methods of micronutrient delivery in dryland agriculture. However, possible effect(s) of the method must be evaluated on seed performance during germination and emergence. So, in this study, the influence of nutrient priming of lentil seeds was investigated by solutions of potassium iodide (KI), copper iodide (CuI), zinc iodide (ZnI_2) and zinc sulfate ($ZnSO_4$) under laboratory, greenhouse and field conditions. Experiments showed that the used compounds had no significant negative effects on seeds performance. Also, the responses of the seeds to applied micronutrients varied in different environments. The speed of germination for primed seeds was higher than that of unprimed seeds, especially at laboratory tests. When germination and emergence circumstances severed, the emergence percentage dramatically decreased from controlled conditions to field environments. It was concluded that seed treatment with the micronutrients, except for KI treatment, had a potential to improve seedling emergence and stand establishment under field conditions; therefore, the application of mentioned compounds could safely be used for different purposes.

Global production of lentil is about 3.6 million tons (Fao, 2013). Globally lentil productivity in recent decade reached to 950 kg/ha by 2004–2006, however, these yields are still low in comparison with other crops yield because some plant factors limited yield potential of lentil landraces, which are also vulnerable to an array of stresses (Cokkizgin *et al.*, 2005). Yield limiting factors include lack of seedling vigor, slow leaf area development, high rate of flower drop, low pod setting, poor dry matter, low harvest index, lack of lodging resistance, low or no response to inputs and exposure to various biotic and abiotic stresses (Erskine, 2009).

Plant establishment is a key factor in building plant density in the field (Jabbaria *et al.*, 2013). There are many contributions that affect seed germination and seedling emergence. In harsh environments, generally, those yield affecting parameters can be easily disturbed and consequently the crop yield will be decreased. The crop yield is directly related to plant density and any negative deviation from the optimum rate causes yield loss (Dong *et al.,* 2010). The response of seed lots during seed germination and seedling emergence to adverse field conditions is unequal and research showed that seed size (Madanzia *et al.,* 2010) and seed vigor (Adetimirin, 2008) have significant role in those differences.

Seed priming or seed pre-treatment has been accepted as success means to alleviate seed vigor in horticultural and agronomical seeds (Farooq *et al.,* 2005). Many studies have been shown that primed seeds have improved some key field parameters including; field emergence percentage, emergence rate, phytochrome-induced dormancy, and increase the rate of germination at any particular temperature (Copeland and McDonald, 2001). Positive changes in those parameters could lead plant or crop to high performance. In seed priming generally seed are soaked in different osmotic and non-osmotic solutions for a determined time during which germination processes begin while radicle emergence has been prevented by osmotic potential or dehydration (McDonald, 2000). In a seed, the natural stages of germination occur up to the point of radicle emergence. Radicle emergence requires high seed water content (Bradford, 1995). So when we prime seeds, we limit their water content and the metabolic steps necessary for germination can occur without the irreversible act of radicle emergence. With primed seeds, plant stands emerge more rapidly and uniformly (Afzal *et al.,* 2009).

Plant physiological processes are susceptible to water deficit (Taiz and Zeiger, 2014), hence in semi-arid areas water shortage cause a minimum growth of crop plants. Soil physicochemical properties also are affected by water stress and under these conditions mineral availability fall to a lower status (Misra and Tyler 2000). Many reports show the minerals with low diffusion capacity are more constraints. For instance, the low availability of phosphorus, zinc and copper is common in soils with low moisture contents (Khan *et al.,* 2004). Therefore, this study was aimed to use different nutrient seed priming treatments on lentil seeds to evaluate possible nutrient deficiency effects on germination, emergence and establishment of lentil in the laboratory, greenhouse and field conditions.

Materials and Methods

Seeds of *Lens culinaris* var. Kimya were obtained from international dry-land agricultural research institute, Maragheh, Iran. Seeds were cleaned and sorted by hand then the initial moisture content was calculated (ISTA, 2009). The standard germination revealed that seed lot viability was near to 99%. Seeds were kept at 4 ºC in a refrigerator until the experiments were performed.

Seed priming

The effects of seed priming was evaluated by seed soaking in solutions of KI, CuI, ZnI_2 and $ZnSO_4$ with 100 ppm concentration for 6 hours. All priming treatments accompanied with control were performed at 20±2°C under light conditions. After priming, seeds were removed and rinsed under tap water for 3 min and then dried to the original moisture content (~10%) over a lab table. Primed seeds in all experiments were used immediately after preparation.

Laboratory germination and seedling vigor

Four replicates of 25 seeds were germinated between double layered rolled germination papers. The rolled paper were put into plastic bags to avoid possible moisture losses. Seeds were allowed to germinate at 20±1°C in the light for 14 days. Germination was considered to have occurred when the radicles were 2 mm long. Germination was recorded every 24 h for 14 days. Mean germination rate (R) was calculated according to Ellis & Roberts (1980).

$$R = \sum Ni / \sum (Di * Ni)$$

Where *Ni* is the number of seeds germinated on day *D*, *Di* is the number of days counted from the beginning of the test.

The vigor index was calculated according to following formula,

Vigor index (VI) = [Mean germination rate × germination percentage]

The time to 50% germination (T50) was calculated according to the following formula of Farooq *et al*. (2005),

$$\mathrm{T50} = \mathrm{ti} + \frac{\left[\left(\frac{N}{2} - \mathrm{ni}\right) - (\mathrm{tj} - \mathrm{ti})\right]}{\mathrm{nj} - \mathrm{ni}}$$

where N is the final number of emerged seeds and n, nj are the cumulative number of seeds germinated by adjacent seed count at times ti and tj respectively, when ni < N/2 < nj.

Greenhouse emergence test

Greenhouse experiments were carried out using three different seed beds (media) including; soil (silty loam), silt (0.002-0.05 mm) and sand (0.05- 2.00 mm) with three replications. Plastic pots (size 30×30×40 cm) were filled with the same weight of media and then their water contents were reached to field capacity. 50 Seeds (primed seeds and control) were planted in each pot. Pots were covered with plastic bags to avoid water losses. During experiments, the temperature fluctuated between 25 and 30 ºC. Number of emerged seedlings in pots within each plot was counted in daily intervals until seedling establishment became stable.

Field emergence

Field experiments were conducted in 2012 at the Research Farm of the Faculty of Agriculture, Maragheh University, Iran. The seeds were then sown at a depth of about 4 cm with a density of 80 seeds/m² (25 cm × 5 cm) during the early May (when soil temperature was above 8 ºC). The soil texture of the field was sandy loam. Number of emerged seedlings in an area of 1 m² within each plot was counted in daily intervals until seedling establishment became stable.

Statistical analysis

The experiments were carried out using a completely randomized design (CRD), a factorial experiment based on a completely randomized design and randomized complete block design (RCBD) with four replicates for laboratory, greenhouse and field experiments, respectively. The data were analyzed using SAS software version 9.1. Duncan's multiple range tests at 5% level of significance, was used to separate means.

Results

Laboratory experiments

The results showed that effects of seed treatments were not significant on germination percentage, mean

Table 1. *Effects of seed treatments on germination of lentil seeds in the laboratory*

Treatment	Germination percentage	Mean germination rate	Vigor index	T50
CuI	100 ± 0.00 a	0.30 ± 0.05a	31.5 ± 3.54 a	2.25 ± 0.05a
KI	98.52 ± 0.14 a	0.27 ± 0.07a	27.3 ± 10.57a	2.50 ± 0.01ab
ZnI_2	95.74 ± 4.51 a	0.29 ± 0.11a	28.1 ± 9.26 a	2.50 ± 0.01ab
$ZnSo_4$	94.86 ± 1.05 a	0.32 ± 0.04a	31.6 ± 2.85 a	2.00 ± 0.21a
Control	97.21 ± 0.02 a	0.22 ± 0.19a	22.2 ± 5.73 a	3.25 ± 0.08b
LSD $_{(0.05)}$	*14.5018*	*0.0878*	*10.1567*	*0.2412*

Means (Mean ± SE) with the same letter are not significant at p<0.05.
The time to 50% germination (T50).

***Table** 2. Effects of seed treatments and media on germination and emergence of lentil see*

Treatment	Emergence percentage	Mean emergence rate	Vigor index	T50
Media				
Sand	47.3 ± 10.83 b	0.09 ± 0.03b	4.54 ± 2.11 b	8.25 ± 0.41b
Silt	36.8 ± 5.54 b	0.16 ± 0.07a	6.66 ± 1.34 b	6.42 ± 0.29b
Soil	91.6 ± 1.62 a	0.20 ± 0.02a	18.9 ± 1.97 a	4.86 ± 0.43a
LSD $_{(0.05)}$	*13.2584*	*0.1281*	*4.0025*	*1.5369*
Seed treatments				
CuI	53.5 ± 14.31 a	0.16 ± 0.07 a	9.77 ± 1.11 a	6.11± 2.14 a
KI	53.3 ± 13.82 a	0.17 ± 0.01 a	9.07 ± 1.25 a	7.87± 1.68 a
ZnI_2	64.8 ± 11.93 a	0.14 ± 0.06 a	10.34± 1.31 a	6.37± 1.05 a
$ZnSo_4$	62.2 ± 13.10 a	0.16 ± 0.03 a	10.52± 1.09 a	5.22 ± 2.76a
Control	58.8 ± 10.52 a	0.16 ± 0.09 a	10. 51± 1.82a	6.57 ± 3.01a
LSD $_{(0.05)}$	*12.0081*	*0.0921*	*1.8541*	*2.9952*

Means (Mean ± SE) with the same letter are not significant at p<0.05.
The time to 50% emergence (T50).

Table 3. *Effects of seed treatments on germination and emergence of lentil seeds in field condition*

Treatment	Germination percentage	Mean germination rate	Vigor index	T50
CuI	51.11 ± 4.58 a	0.42 ± 0.19a	22.13 ± 5.95a	9.00 ± 3.25a
KI	42.44 ± 5.14 a	0.33 ± 0.08a	11.03 ± 3.54a	10.66 ± 1.81 a
ZnI_2	56.00 ± 3.97 a	0.33 ± 0.07a	21.32 ± 9.85a	11.66 ± 3.57 a
$ZnSo_4$	47.33 ± 8.25 a	0.38 ± 0.68a	26.99 ± 11.2a	9.66 ± 5.14 a
Control	48.22 ± 7.64 a	0.36 ± 0.25a	16.51 ± 2.04a	9.33 ± 4.98 a
LSD $_{(0.05)}$	*26.1423*	*0.8854*	*14.2674*	*5.2176*

Means (Mean ± SE) with the same letter are not significant at p<0.05.
The time to 50% emergence (T50)

germination rate and vigor index ($p \leq 0.05$). However, seed treatments significantly influenced T_{50} (table 1). Seed treatments with CuI and KI slightly improved germination percentage compared to control and seeds under CuI treatment completely germinated (table 1). Although the effects of seed treatments on mean germination rate and vigor index were not significant but all treatments improved this trait in comparison with control. Among treatments, $ZnSo_4$ treatment had highest mean germination rate and vigor index (table 1). In comparison with control all priming treatments improved T_{50}, and the most effective treatment was ZnSo4 priming.

Greenhouse experiments

Effects of different seed beds (media) on seed emergence, seedling emergence, vigor index and T_{50} were significant at $p \leq 0.01$. However, seed priming had not remarkably influenced studied traits. Also, interaction between media and seed priming treatments were not significant ($p \leq 0.05$). Emergence percentage in soil media was 91.6% while the value decreased to below 50% for sand and silt substrates (table 2). Similarly, the highest value of the mean emergence rate, vigor index and T_{50} obtained from the soil and followed by silt and sand media, respectively. With respect to table, seed treatments with zinc compounds, especially in $ZnSo_4$ treatment, improved establishment indexes compared with other treatment.

Field experiment

Results pertaining to seedling emergence percentage and mean emergence rate in this state revealed that there were not statistical differences between treatments.. Although, little enhancement was seen by CuI treatment for both seedling emergence percentage and mean emergence rate (table 3).

Discussion

The seed vigor may be affected by internal and external factors. Besides environmental circumstances, seed treatments consider as one of the main external factors for some purposes such as bacterial inoculation, fungal inoculation, seed disinfection, priming and bio-fortification (McDonald, 2000). This study examined the nutrient priming effects on the seed vigor indexes. The studies revealed that the application of the micro-nutrients at priming processes had positive effects on seed vigor in comparison with control, particularly in $ZnSo_4$ treatment. When the seed germination condition was changed from laboratory to the field condition, the germination percentage and emergence were declined continuously. In this study, mean germination percentage for laboratory, greenhouse and field was 97%, 58% and 48%, respectively. Therefore, the standard germination test is not a reliable test for prediction of lentil seed performance at field conditions because germination in field conditions exposes seed to unfavorable conditions and the commonly used standard germination test cannot predict field emergence. This finding is accommodated with Perry (1987).

Emergence percentage and vigor indexes were significantly greater in soil media than those of the sand and silt media, noticeably (Table 2). The result suggested that the plant was susceptible to the soil texture thereby in textures with low water content capacity, the emergence percentage was dramatically decreased. It has been proved that species response to soil properties during germination and emergence is a species specific phenomenon (Schütz *et al.*, 2002; Penman *et al.*, 2008). Germination and seedling survival may differ among soil types, since moisture availability may be a function of soil type (Scheffer, 1998). Moisture requirements during germination and seedling growth, may therefore play a significant role in determining the plant establishment.

The fast rate of germination was obtained by pre-treatments (table 1), probably due to faster water uptake and earlier initiation of metabolic processes at lab condition. Previous study about lentil seeds (Ghassemi-Golezani *et al.,* 2008) was clarified that hydro priming decreased imbibition time for germination, compared to control but maximum values of moisture content among the seed treatments did not change. During the pre-treatment, the seeds start to complete leg phase of germination hence primed seeds inter rapidly into phase III of imbibition once rehydrated during sowing (Bradford *et al.,* 1990). The laboratory test also revealed that among studied indexes, T_{50} is more sensitive index than the other vigor indices to discriminate minimum differences. The highest vigor index was obtained by $ZnSo_4$ treatment in both greenhouse and field studies which had not remarkably differences with other treatments. These results are in line with fidings of Wang (2009) who suggested that Zn involves in physiological processes during early seedling development, possibly in protein synthesis, cell elongation membrane function and resistance to abiotic stresses. Seed priming with ZnSO4 has improved seed germination, crop emergence, stand establishment, and subsequent growth and yield of *Echinacea purpurea* L. (Babaeva *et al.,* 1999), *Phaseolus vulgaris* L. (Kaya *et al.,* 2007), *Hordeum vulgare* L. (Ajouri *et al.,* 2004). Seed priming noticeably improved Zn uptake, the plant biomass and water use efficiency in water deficit conditions. Imran *et al.* (2013) reported that nutrient seed priming with Fe, Zn and Mn micronutrients improved early seedling development and root growth of maize exposed to low root zone temperatures.

Results showed that seed priming with Cu compound did not statistically affect emergence percentage and its rate. However, this treatment increased emergence percentage, rate of emergence and vigor index by 5%, 15% and 25%, respectively in comparison with control. The findings seem to be consistent with Malhi (2009) who showed that CuEDTA priming on wheat seeds did not improve emergence percentage. In another experiment on Maize, Foti *et al.,* (2008) reported that CuSO4 seed priming improved stand establishment by 43% compared with the untreated seeds. These data must be interpreted with caution because iodine salts, particularly KI, are poisonous to the plants thereby we used these compounds at low concentration.

Conclusion

To sum up, seed treatment with micronutrients, except for KI, had the potential to improve seedling emergence and stand establishment in field condition. Commonly, the method is a cost effective and economically more practicable than soil and foliar applications, especially for micronutrient delivery in dry land agriculture where both environmental conditions and technical problems limit the yield quantity and quality.

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