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Effects of Seed Priming in Glumed and Glumeless Barley Cultivars

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Abstract

Micronutrients are vital for plant growth and human health. Soil and foliar applications are the most prevalent methods of micronutrient addition but the cost involved and difficulty in obtaining high quality micronutrient fertilizers are major concerns with these in developing countries. Micronutrient seed treatments, which include seed priming and seed coating, are attractive and easy alternatives. In this study, Turkish glumed barley cultivar Aydanhanım and glumeless cultivar Özen were primed with $ZnSO_4$ to improve seed germination speed, germination percantage and important components that affect yield. The results showed positive effect of osmopriming compared to control on seedling growth. The early and maximum germination was achieved on osmoprimed seeds. The results showed that the osmopriming could effect germination ratio of *Hordeum vulgar* L. positively under arid Central Anadolu conditions and could be positively manipulated for increased yields of *Hordeum vulgare* L.

Keywords: *Hordeum vulgare* L., $ZnSO_4$, Seed, Germination speed, Germination percantage

Introduction

For optimal growth and development, 17 essential elements are required by crop plants. These elements, when required in relatively high amounts, are called macronutrient elements or, in trace amounts, micronutrient elements. While micronutrients are required in relatively small quantities for plant growth, they are as important as macronutrients. If any element is lacking in the soil or not adequately balanced with other nutrients, growth suppression or even complete inhibition may result (Mengel *et al.,* 2001). Micronutrients often act as cofactors in enzyme systems and participate in redox reactions, in addition to having several other vital functions in plants. Most importantly, micronutrients are involved in the key physiological processes of photosynthesis and respiration (Marschner, 1995; Mengel *et al.,* 2001) and their deficiency can impede these vital physiological processes thus limiting yield gain. Plant response to Zn deficiency occurs in terms of decrease in membrane integrity, susceptibility to heat stress, and decreased synthesis of carbohydrates, cytochromes, nucleotides, auxin and chlorophyll. Further, Zn containing enzymes are also inhibited at low Zn, which include alcohol dehydrogenase, carbonic anhydrase, Cu-Zn-superoxide dismutase, alkaline phosphatase, phospholipase, carboxypeptidase, and RNA polymerase (Ahmad & Prasad, 2011).Zinc (Zn) deficiency is a major yield-limiting factor in several Asian countries (Wissuwa *et al*., 2006; Rehman H *et al*., 2012). Turkish soils are also deficient in these nutrients and low availability of Zn in soils is one of the widest ranging abiotic stresses and must be supplemented through proper crop nutrients

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management. A major proportion (about 80 %) of the released Zn is fixed in the soil in a non-exchangeable form (Singh *et al*., 2004; Alloway, 2008). In crop plants, micronutrients may be applied to the soil, foliar sprayed or added as seed treatments. foliar sprays have been more effective in yield improvement and grain enrichment; but high cost has restricted its wider adaption, particularly by resource-poor farmers (Johnson *et al.,* 2005). Moreover, foliar application occurs at later growth stages when crop stands are already established. Seed treatment is a better option from an economical perspective as less micronutrient is needed, it is easy to apply and seedling growth is improved (Singh *et al.,* 2003).Seed treatment with micronutrients seems pragmatic, inexpensive and an easy method of micronutrient delivery especially by small landholders in developing countries (Farooq *et al.,* 2012).Seeds may be treated with micronutrients either by soaking in nutrient solution of a specific concentration for a specific duration (seed priming) or by coating with micronutrients. Seed invigoration is a relatively new term and has been interchangeably used for both methods of seed treatment (Farooq *et al.,* 2009). In recent years, a lot of work has been done on the invigoration of seeds to improve the germination rate and uniformity of growth and reduce the emergence time of some field crops (Basra *et al.*, 2003), improve seedling vigour, better stand establishment (Ali *et al.*, 2007; Arif *et al.*, 2005) increasing yield (Yilmaz *et al.*, 1998) and for overcoming micronutrient deficiencies (Harris *et al.*, 2007). In micronutrient seed priming (nutripriming), micronutrients are used as osmotica (Imran *et al.,* 2004; Singh 2007). Primed seeds usually have better and more synchronized germination (Farooq *et al.,* 2009) owing simply to less imbibition time (Brocklehurst and Dearman, 2008; McDonald, 2000; Taylor *et al.,* 1998) and build-up of germination-enhancing metabolites (Basra *et al.,* 2005; Farooq *et al.,* 2006). Several reports indicated the potential of nutripriming in improving wheat yields (Marcar and Graham, 1986; Wilhelm *et al.,* 1988), rice (Peeran and Natanasabapathy, 1980) and forage legumes (Sherrell 1984). Harris et al. (2007) showed that enhancing Zn seed content by priming seeds with solutions of $ZnSO_4$ was highly cost effective in increasing maize yield. seed with 0.05% ZnSO4 solution increased germination and field emergence by 38 and 41%, respectively (Babaeva *et al.*, 1999). Likewise, in common bean (*Phaseolus vulgaris* L.), seed priming with Zn significantly improved yield and related traits (Kaya *et al.,* 2007). In barley (*Hordeum vulgare* L.), seed priming with Zn improved germination and seedling development (Ajouri *et al.,* 2004). Ozturk *et al*. (2006) found that Zn in newly-developed radicles and coleoptiles during seed germination was much higher (up to 200 mg kg-1) thus highlighting the involvement of Zn in physiological processes during early seedling development, possibly in protein synthesis, cell elongation membrane function and resistance to abiotic stresses (Cakmak, 2000). Harris and his team, in their preliminary trials, demonstrated that seed priming with ZnSO4 (0.4%) was effective to meet Zn requirements of wheat with a mean yield (mean of eight on-farm trials) increase of 615 kg ha-1 (21%) compared with crops from nonprimed seeds. Seed priming was also cost effective compared with soil application with benefit:cost ratio of 8 and 360 from soil application and seed priming, respectively (Harris *et al.,* 2005). The effects of hydro-priming plus KNO_3 and osmo-priming by PEG4000 on seed quality of Thai barley for malt production had significantly high seed could be effectively applied to improve barley seed quality in the Thai malt industry (Junhaeng, *et al.*, 2016).

Materials and Methods

The seeds of Turkish glumed barley cultivar Aydan Hanım and glumeless cultivar Özen were used in this experiment. Seeds of barley (*Hordeum vulgare* L.) were obtained from *Central Research Institute for Field Crops, Ankara, Turkey and the* experiments were conducted in the gene bank laboratories, Ankara Turky. The seeds were pretreated with solution of sodium hypochlorite to control possible fungal contamination during priming. Seed samples were divided into six sub-samples. One of the sub-samples was considered as control (unprimed) and the other five sub-samples were prepared for priming treatments.

Seed priming: The sub-samples were primed by soaking the seeds in five different dosage of $ZnSO_4$. All priming treatments were performed at room temperature. After priming, samples of seeds were removed and rinsed three times in distilled water and then dried to the original moisture level.

Germination Rate and Germination Percantage: Three replicates of 100 seeds were germinated between double layered rolled germination papers. The rolled papers with seeds were put into plastic bags to avoid moisture loss. Seeds were allowed to germinate at 20±1°C in the dark for 7days. Germination was considered to have occurred when the radicles were 2 mm long. Germinated seeds were counted every 24 h for 7 days. The seeds were counted on 4th day as per ISTA rules and calculated in percent to determine germination speed.

Germination percentage: Germinated seeds were recorded every 24 h for 7 days. Mean germination percentage was calculated according to Bewley and Black (1994) to assess the rate of seed germination:

Germination percentage = $\sum n_i$ / N X 100

where n_i is the number of seeds germinated on day i, N is the total seed in the test. At the end of germination test (7 days), radicles and shoots were cut from the cotyledons and their length was measured and fresh weight of them were weighed, then dried in an oven at 70±2°C for 48 h. The dried radicles and shoots were weighed to the nearest g and the mean radicle and shoot dry weights and consequently mean seedling dry weight were determined.

Seedling emergency rate (%): It was determined by testing the seeds in three replications. Every replication made use of 300 seeds and were surface sterilised with sodium hypochloride. Special tray (30x30 cm) were prepared and were filled with sterilised moistened sand upto 2 cm. After placing primed seeds in these trays, the same sand was used to cover them for 5-7 times. Seedling emergence rate (%) was determined on 7th day and seedling emergence capacity (%) was determined on 10th day.

Result and Discussion

Germination and seedling vigor: Seed quality parameters including germination rate, germination percentage length of root, length of shoot, shoot and root fresh weight, shoot and root dry weight, seedling emergency rate and seedling emergency capacity of barley were significantly increased as an affect by priming (Table 1, 2).

The results showed that under laboratory conditions , the cv Özen was better in terms of the Germination rate that ranged 96.00 to 100.00 %. Maximum Germination rate was obtained using 4 mM $ZnSO_4$ and minimum germination speed was obtained on the control treatment seeds. However, maximum germination rate with ZnSO4 did not show any significant difference statistically among different doses of Zn SO4. The cultivar had germination rate of 94.00 to 100.00% Maximum germination rate was recorded on 10 Mm $ZnSO_4$ priming and the minimum germination rate was recorded on control treatment seeds. However, maximum germination rate recorded on 4 mM to 10 mM doses remained statistically nonsignificant and were at par.

Germination percantage as shown in (Table 3) was 89.32 to 98.66 % for cv Özen. Maximum germination percentage for the cv Özen was recorded on 4 mM $ZnSO_4$ priming and the minimum germination percentage was noted on control application seeds. However, 4 mM and 6 mM primed seeds showed statistically similar germination percentage. Germination percentage of cv. Aydan hanım had range of 94.66 to 99.33%. Maximum germination percentage was recorded on 10 mM $ZnSO_4$ priming and the minimum seed priming was noted on control application seeds. However, germination percentage on all of the primed seeds was statistically at par.

The shoot length of of cv Özen seedlings (Table 3) ranged 7.99 cm to 10.20 cm. Maximum shoot length was recorded on 4 mM $ZnSO_4$ primed seeds. The minimum shoot length was recorded on control application seeds. However, no statistical difference was recorded on the shoot length recorded on 2 mM, 4 mM ve 6 mM primed $ZnSO_4$ seeds. The seedlings of Cv Aydanhanım had shoot length of 10.02 cm to 11.32 cm. The longest shoots were recorded on 8 mM $ZnSO_4$ priming seeds and the minimum length was recorded on control priming application seeds However, the shoot length recorded on 2 mM, 6 mM, 8 mM ve 10 mM ZnSO4 primed seeds showed statistical similarities. Minimum shoot length was recorded on 4 mM ZnSO4 primed seeds and control application seeds and were statistically at par.

The results (Table 4) showed that root length was better in cv Özen and range 10.73-13.72 cm. The maximum root length was noted on 4 mM ZnSO4 primed seeds and the minimum root length was noted on control (application) seeds. However, the root length recorded on 4 mM, 8 mM and 10 mM ZnSO4 primed seeds remained statistically similar. The root length on cv Aydınhanım ranged 14.75 to 17.64 cm. The maximum root length was recorded on 8mM ZnSO4 primed seeds and the minimum root length was noted on control application seeds. However, the root length recorded on 2, 4, 6 and 8 mM primed seeds remained statistically at par.

The results (Table 4) showed that fresh weight of shoots ranged 0.54 - 0.69 g in cv. Özen. Maximum fresh shoot weight was noted on 4mM $ZnSO_4$ primed seeds; whereas, the minimum shoot fresh weight was noted on on control application seeds. However, maximum shoot fresh weight was noted on 4, 6, 8and 10 mM $ZnSO_4$ primed seeds that were similar in statistical terms. Fresh shoot weight of cv. Aydanhanım ranged 0.39 to 0.71 g. Maximum fresh shoot weight was noted on 2mM $ZnSO_4$ primed seeds and the minimum fresh shoot weight was noted on control application seeds. However, the fresh shoot weight noted on 2Mm, 6mM, 8 mM and 10 mM $ZnSO_4$ seeds remained statistically at par.

The fresh root weight of cv Özen ranged 0.36 g ile 0.59 g. The maximum fresh root weight was noted on 4mM $ZnSO_4$ treated seeds. Minimum fresh root weight was noted on control application seeds. However, maximum fresh root weight noted on 4 mM and 10 mM $ZnSO_4$ primed seeds remained statistically at par. Fresh root weight of cv Aydanhanım ranged 0.40 g to 0.56 g. Maximum fresh weight was noted on 8 mM $ZnSO_4$ primed seeds and the minimum fresh root weight was noted on control application seeds. However, the fresh root weight obtained on 2Mm, 6mM, 8 mM and 10 mM seeds remained statistically at par. Minimum fresh root weight was noted on 4Mm primed and control application seeds.

The results (Table 5) shows that seedling emergency rate of cv. Özen ranged 80-88%. Maximum seedling emergency rate was noted on seeds treated with 4mM $ZnSO_4$ primed seeds and the minimum seedling

Table 1: Analysis of variance (ANOVA) of the effects of seed priming on barley seed germination and vigor in laboratory

Source of Variation	Df	Germination Rate	Germination Percentage	Length of root	Length of shoot	Shoot fresh weight
Variety	1	1.77*	30.25**	16.68**	136.34**	0.002 *
Priming	5	4.11**	8.05*	1.51*	4.35*	0.033*
Variety x kimyasal	5	0.64*	3.38*	1.35*	2.31*	0.016*
Error	24	0.50	1.55	0.41	1.47	0.010

Table 2: Analysis of variance (ANOVA) of the effects of seed priming on barley seed germination and vigor in laboratory.

Source of Variation	**Df**	**Shoot dry Weight**	**Root fresh Weight**	**Root dry Weight**	**Seedling emergency rate (%)**	**Seedling Weight Capacity (%)**
Variety	1	1.611E-5	0.002ns	0.00**	13.44*	0.250ns
Priming	5	5.691E-5	0.016*	9.04E-5*	32.26**	37.294**
Variety x kimyasal	5	2.646E-5	0.023*	5.61E-5*	8.44*	8.717*
Error	24	2.599E-5	0.007	3.72E-5	3.58	3.556

emergency rate was noted on control application seeds. However, maximum seedling emergency rate noted on 4, 6 and 8 mM $ZnSO_4$ primed seeds remained statistically at par. Aydanhanım showed seedling emergency rate of 77.32 to 94%. Maximum seedling emergency rate was noted on 8 mM $ZnSO_4$ primed seeds and the minimum seedling emergency rate was noted on control application seeds. However, the maximum seedling emergency rate noted on 4 and 8 mM primed seeds remained statistically at par. The minimum seedling emergency rate was noted on 2 mM primed seeds and control application seeds that remained statistically at par.

The results (Table 5) shows that seedling emergency capacity (%) of cv. Özen ranged 82.66 to 94%. The maximum seedling emergency capacity was noted on 4mM ZnSO4 primed seeds and minimum seedling emergency capacity was noted on control application seeds. However, the maximum seedling emergency capacity noted on 4 and 6 mM ZnSO4 primed seeds remained statistically at par. Cv. Aydanhanım showed seedling emergency capacity of 80.00 to 96.00%. The maximum seedling emergency capacity was noted on 8 mM ZnSO4 primed seeds and the minimum seedling emergency capacity was noted on control application seeds. However, the maximum seedling emergency

Table 3: Means of seed germination speed, germination percentage and length of shoot parameters of barley as affected by $ZnSO_4$ priming

Seed Priming ($ZnSO_4$)	Germination rate		Germination Percentage		Length of shoot	
	Özen	Aydanhanım	Özen	Aydanhanım	Özen	Aydanhanım
2 mM	100.00 a	98 b	90.00 c	97.33 ab	9.95 ab	10.64 ab
4 mM	100.00 a	98.66 ab	98.66 a	98.66 a	10.20 a	10.24 b
6 mM	99.32 a	98 b	96.00 ab	96.66 ab	9.82 ab	10.72 ab
8 mM	98.00 a	98 b	94.00 b	97.33 ab	9.18 bc	11.32 a
10 mM	98.66 a	100 a	94.00 b	99.33 a	8.93 c	11.30 a
Control	96.00 b	94 c	89.32 c	94.66 b	7.99 d	10.02 b

Different letters in each column indicate significant difference at $P \leq 0.05$

Table 4: Means of length of root, shoot fresh weight and root fresh weight parameters of barley as affected by $ZnSO_4$ priming

Seed Priming ($ZnSO_4$)	Length of root		Shoot fresh weight		Root fresh weight	
	Özen	Aydanhanım	Özen	Aydanhanım	Özen	Aydanhanım
2 mM	11.76 bc	16.12 abc	0.65 ab	0.71 a	0.39 c	0.494 ab
4 mM	13.72 a	16.00 abc	0.69 a	0.56 b	0.59 a	0.40 b
6 mM	11.28 bc	16.52 ab	0.61 ab	0.66 ab	0.39 c	0.51 ab
8 mM	12.68 ab	17.64 a	0.59 ab	0.68 ab	0.46 bc	0.56 a
10 Mm	12.72 ab	15.24 bc	0.63 ab	0.62 ab	0.55 ab	0.47 ab
Control	10.73 c	14.75 c	0.54 b	0.39 c	0.36 c	0.40 b

Different letters in each column indicate significant difference at $P \leq 0.05$

capacity noted on 4 and 8 mM primed seeds remained statistically at par.. The minimum seedling emergency capacity noted on control application seeds and 2Mm ZnSO4 primed seeds remained statistically similar. Arshad *et al*. (2002) reported that the maximum field emergence of Raya (*Brassica carinata* L.) was noted in ZnSO4 followed by MnSO4. In barley (*Hordeum vulgare* L.), seed priming with Zn improved germination and seedling development (Ajouri et al., 2004).

Priming seeds with ZnSO4 resulted in advanced metabolic processes and higher germination rate and percentage, compared with unprimed seeds (Table 3-4-5). This suggests that there is no toxic effect of ZnSO4, due to ion accumulation in the embryo (Demir and Venter, 1999). Pre-sowing treatment with inorganic salts not only promotes seed germination of most crops, but also stimulates faster growth, metabolic processes and, hence, ultimate crop yield (Sallam, 1999). (Shekafande and Takhti , 2012) reported that the maximum germination percentage, stem length, root length, stem fresh and dry weight and root fresh weight of *Ziziphus Spina-Christi* was noted in 8dS/m of $ZnSO_4$.

Table 5. Means of seedling emergency rapid and seedling emergency capacity of barley as affected by $ZnSO_4$ priming

Seed Priming ($ZnSO_4$)	seedling emergency rate (%)		seedling emergency rate (%)	
	Özen	Aydan Hanım	Özen	Aydan Hanım
2 mM	81.33 b	80.00 d	83.32 d	81.32 d
4 mM	88.00 a	91.32 ab	94.00 a	92.66 ab
6 mM	86.66 a	86.00 c	91.32 ab	86.66 c
8 mM	86 .00 a	94 .00 a	88.66 bc	96.00 a
10 mM	80.66 b	88.66 bc	84.66 cd	90.00 bc
Control	80.00 b	77.32 d	82.66 d	80.00 d

Different letters in each column indicate significant difference at $P \leq 0.05$

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