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Seed priming and seedling establishment of barley (*Hordeum vulgare* L.)

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Abstract

This research was designed to compare the effects of hydropriming, osmopriming (10% PEG, 20% PEG, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$) and nutrient priming (in $ZnSO_4$ solutions with 10, 50 and 100 mM Zn, in KH_2PO_4 with 10, 50 and 100 mM P together with their combinations) on seedling vigor of barley. Laboratory tests were conducted as CR design and field experiment was carried out as RCB design at Dryland Agricultural Research Institute (DARI) in Iran. Analysis of variance of laboratory data showed that all traits such as percentages of viable seeds and germination, electrical conductivity (EC) of seed leachates, germination rate, root and shoot dry weight and seedling dry weight were significantly affected by seed priming. Nutrient priming in P solutions was superior, compared to other priming technics. This priming media improved root and shoot dry weight by 25 and 12.5% over unprimed seeds, respectively. The superior treatments in laboratory including hydropriming, 10% PEG, 5 mM $CaCl_2$, 10 mM Zn, 50 mM P, 100 mM P, 10 mM Zn + 50 mM P and 10 mM Zn + 100 mM P solutions were applied on seeds which subsequently sown in the field. Priming treatments had significant ($p \leq 0.05$) effects on mean seedling emergence percentage and rate in the field. Maximum seedling emergence percentage and rate were achieved with 100 mM P and 10 mM Zn + 100 mM P primings, respectively. Therefore, these priming techniques could be used to improve seedling establishment of barley in the field.

Key words: Seed priming, seed vigor, seedling, barley.

Introduction

In arid regions, cereal production is widely limited by poor stand establishment and nutrient deficiencies [18]. Cereal germination tends to be irregular and can extend over long periods [7], particularly in drought-prone environments. The adverse effects of drought stress can be alleviated by various measures, including seed priming (pre-sowing seed treatment) [5]. The general purpose of seed priming is to partially hydrate the seed to a point where germination processes are begun, but not completed. Treated seeds are usually re-dried to primary moisture before use, but they would exhibit rapid germination when re-imbibed under normal or stress conditions [5]. Such controlled imbibition of seed followed by dehydration was shown to improve germination and early seedling growth under salt and drought stress, compared to seedlings grown from untreated seed. Various pre-hydration or priming treatments have been employed to increase the speed and synchrony of seed germination [8].

In the past two decades, intensive research has been conducted to improve seed germination and seedling emergence under saline and drought conditions by pre-treating seed with solutions of different organic and inorganic salts and water. For example, it was determined that rice seed treated with mixed salt solution, germinated significantly more rapidly than unprimed seed under salt-stress conditions [9]. For the first time, Strogonov [28] proposed that salt tolerance of plants could be enhanced by treatment of seed with salt solution prior to sowing. Osmoconditioning of Bermuda grass (*Cynodon dactylon*) seeds, using PEG followed by immediate sowing, improved germination and seedling growth under saline conditions [2].

Seed dressing of limiting nutrients (nutrient priming) has been advocated as a low-cost and highly effective approach since the 1970s [24, 25]. In nutrient priming, seeds are pre-treated (primed) in solutions containing the limiting nutrient instead of being soaked simply in water. Early season P supply is critical for yield formation[12]. Phosphorus can be applied in moderate quantities at sowing to sustain early growth vigor, but P only moves 3 to 5 cm from the application point [15]. An alternative approach is to prime cereal seeds with P [23]. Al Mudaris and Jutzi [3] and Asgedom and Becker [4] proposed nutrient priming to be a novel approach that combines the positive effects of seed priming with an improved nutrient supply. In the case of barley production on calcareous soils in China, seed soaking in P solution stimulated seedling growth, and plants used the added P as early as three days after application [32]. Under Zn-deficient soil conditions, plants grown from high Zn seeds produced more root and shoot biomass, enabling the plants to take up soil Zn more efficiently in later growth stages [11]. Surface dressing of seeds with Zn significantly increased wheat yield in Turkey [31]. However, the use of pre-soaked seeds in highly drought-prone environments can considerably decrease the risk of crop failure.

Low-input production of barley on the predominantly calcareous soils in most countries of West Asia and North Africa (WANA) such as Iran, is affected by drought, cold and low availability of P and Zn. While increased seedling vigor via seed priming may improve barley plant establishment, possible benefits are likely to be limited when water, P and Zn are deficient. Therefore, this research was aimed to evaluate the effects of hydro-, osmo- and nutrient priming on seedling vigor and establishment of barley.

Materials and Methods

Seeds of traditional facultative barley variety (Abidar) were obtained from Dryland Agricultural Research Institute (DARI), Marageh, Iran. These seeds were divided into 21 sub-samples. A sub-sample was kept as control (unprimed) and each of other 20 sub-samples was subjected to a priming treatment in distilled water (hydropriming), 10% PEG, 20% PEG, 2.5 mM $CaCl_2$ and 5 mM $CaCl_2$ (Osmopriming) and in $ZnSO_4$ solutions with 10, 50 and 100 mM Zn, in KH_2PO_4 with 10, 50 and 100 mM P and in $ZnSO_4$+ KH_2PO_4 solutions with their combinations (nutrient priming) for 12 hours under laboratory conditions. Primed seeds dried back to primary moisture and treated with difencolazole 3% DS at the rate of 2‰. Laboratory tests were conducted with CR design to determine percentage of viable seeds and germination, electrical conductivity (EC) of seed leachates, germination rate, root and shoot dry weight and seedling dry weight for each primed sample. Twenty five seeds from each priming treatment were placed on wet filter paper and germinated in incubator at 10°C for 12 days, using four replications[17]. Germination (protrusion of radicle by 2 mm) was recorded in daily intervals.

Superior treatments at laboratory including hydropriming, 10% PEG, 5 mM $CaCl_2$, 10 mM Zn, 50 mM P, 100 mM P, 10 mM Zn + 50 mM P and 10 mM Zn + 100 mM P solutions were selected and applied on seeds similar to laboratory procedure. Primed seeds dried back to primary moisture and then were sown in the field for evaluation of priming effects on seedling vigor and establishment. Field experiment was carried out with RCB design at DARI (Latitude 37°15′ N, Longitude 46°15′ E, Altitude 1725 m above sea level with long-term rainfall 342 mm) in autumn 2006. Before planting, 60 kg h^{-1} N (from urea) was applied to the soil. Seeds were sown by Winterstiger planter in 4-6 cm depth with a density of 450 seeds/m^2 on 22 September 2006. Plots consisted of 12 rows, with 7 m in length, spaced 20 cm apart.

Data were subjected to normal distribution tests and analysis of variance and Duncan's Multiple Range Test (DMRT) for comparison of means were performed, using Genstat, SPSS and MSTATC softwares.

Results

Analysis of variance of laboratory data showed that all traits were significantly affected by priming methods (Table 1). Maximum germination percentage and rate belong to 50 mM P (Table 2), but this was not statistically higher than 10% PEG, 20% PEG, 2.5 mM $CaCl_2$, 10 and 100 mM P and 10 mM Zn + 100 mM P. The lowest germination rate was achieved from 100 mM Zn + 10 mM P. Electrical conductivity (EC) of leachates from non-primed seeds was the highest and increased rapidly during 12 h of the imbibition. Hydropriming, osmopriming with 10% and 20% PEG and nutrient priming with 10 mM Zn and 10 mM Zn + 100 mM P solutions

Table 1. Analysis of variance of the effects of different priming methods on seeds quality of barley.

S.O.V	d.f	Viable seeds percentage	Germination percentage	EC (µs/cm/g)	Germination rate	Root dry weight (g/root)	Shoot dry weight (g/shoot)	Seedling dry weight (g/seedling)
Treatment	20	129.56 **	385.63 **	68724 **	0.0063064 **	0.0097783 **	0.0033298 **	0.023545 **
Error	63	11.87	20.87	5648	0.0003895	0.0001308	0.0001202	0.0003795
C.V%	-	3.6	5	23	6.3	8.9	6.8	6.8

** Significant difference at pd≤0.01.

Table 2. Means of barley seed quality parameters, affected by different priming methods.

Seed priming method	Mean of traits						
	Viable seeds, %	Germination %	EC (µs/cm/g)	Germination rate	Root dry weight (g/root)	Shoot dry weight (g/shoot)	Seedling dry weight (g/seedling)
T1 = Control (no priming)	100 a +	99 a	548.1 g	0.3188 cde	0.1386 c	0.1820 abcd	0.3306 cd
T2 = Hydropriming	98 ab	95 abc	170 ab	0.3310 cd	0.1515 bc	0.1623 efg	0.3141 cd
T3 = 10% PEG	99 a	97 a	158 ab	0.3318 cd	0.1640 ab	0.1682 cdef	0.3322 bc
T4 = 20 % PEG	100 a	100 a	130.5 a	0.3380 bcd	0.1604 ab	0.1710 bcde	0.3302 bcd
T5 = 2.5 mM $CaCl_2$	100 a	99 a	237.6 abc	0.3415 bc	0.1356 cd	0.1743 bcde	0.3135 cd
T6= 5 mM $CaCl_2$	100 a	96 ab	519 fg	0.3345 cd	0.1582 ab	0.1674 def	0.3314 bc
T7= 10 mM Zn	99 a	95 abc	188.8 ab	0.2860 fgh	0.1460 bc	0.1604 efgh	0.3064 cd
T8 = 50 mM Zn	92 cde	85 de	335.5 cde	0.2593 hij	0.0622 f	0.1433 hi	0.2055 gh
T9 = 100 mM Zn	87 e	73.5 f	350.6 cde	0.2543 ij	0.0248 g	0.1210 jk	0.1458 i
T10 =10 mM P	100 a	99 a	156.2 ab	0.3653 ab	0.1703 a	0.1857 abc	0.3560 ab
T11 =50 mM P	100 a	99 a	319.8 cde	0.3760 a	0.1766 a	0.1962 a	0.3728 a
T12 =100 mM P	100 a	97 a	428.2 efg	0.3428 bc	0.1706 a	0.1989 a	0.3692 a
T13 =10 mM Zn + 10 mM P	98 ab	95 abc	170.5 ab	0.3260 cd	0.1633 ab	0.1681 cdef	0.3314 bc
T14 =10 mM Zn + 50 mM P	98 ab	94 abc	273.3 bcd	0.3208 cd	0.1611 ab	0.1710 bcde	0.3321 bc
T15 =10 mM Zn + 100 mM P	99 a	97 a	366.2 de	0.3345 cd	0.1724 a	0.1865 ab	0.3589 ab
T16 =50 mM Zn + 10 mM P	93 bcd	85 de	340.2 cde	0.2458 ij	0.0592 f	0.1321 ij	0.1913 h
T17 =50 mM Zn + 50 mM P	95 abc	88 cd	432.6 efg	0.2908 efg	0.1182 e	0.1507 fgh	0.2689 f
T18 =50 mM Zn + 100 mM P	93 bcd	89 bcd	439.3 efg	0.3310 cd	0.1371 cd	0.1621 efg	0.2992 de
T19 =100 mM Zn + 10 mM P	78 f	62 g	377.1 de	0.2325 j	0.0201 g	0.1146 k	0.1347 i
T20 =100 mM Zn + 50 mM P	89 de	79 ef	402.3 ef	0.3078 def	0.1210 de	0.1521 fgh	0.2731 ef
T21 =100 mM Zn + 100 mM P	93 bcd	86 d	521 fg	0.2708 ghi	0.0757 f	0.187 gh	0.2244 g

+ Difference letters indicating significant differences at p≤0.01.

showed low EC during imbibition (Table 2). Priming techniques had significant effected on root, shoot and seedling dry weights (Table 2, Fig. 1). The greatest root dry weight was recorded in seeds primed with 50 mM P, but this was statistically similar to 10% PEG, 20% PEG, 5 mM $CaCl_2$, 10 and 100 mM P and 10 mM Zn + 10 mM P, 10 mM Zn + 50 mM P and 10 mM Zn + 100 mM P. The highest shoot dry weight was achieved from 100 mM P, which was not significantly different from 10 and 50 mM P and 10 mM Zn + 100 mM P (Table 2, Fig. 1). The largest seedling produced in response to 50 mM P that was similar to 10 and 100 mM P and 10 mM Zn + 100 mM P. Application of P solutions and 10 mM Zn + 100 mM P improved root and seedling dry weight by 25 and 12.5% over unprimed seeds, respectively (Table 2, Fig. 1). All these traits were decreased with increasing Zn concentration in priming solutions (Fig. 2). However, these qualitative traits were increased with increasing P concentration up to 50 mM, but further increase of P resulted in reducing the traits (Fig. 2).

Analysis of variance of field data showed that seedling emergence rate and percentage (seedling establishment) were significantly affected by priming methods (Table 3). Maximum seedling emergence rate belong to 10 mM Zn + 100 mM P (Table 4), but this was not statistically higher than hydropriming and 10 mM Zn. The lowest seedling emergence rate was achieved from control seeds (unprimed seeds). Seedling emergence percentage was also significantly affected by priming methods. The greatest seedling emergence percentage was obtained from 100 mM P, that improved seedling establishment by about 21% above unprimed seeds, but was not significantly different from hydropriming, 5 mM $CaCl_2$, 10 mM Zn, 50 mM P and 10 mM Zn + 50 mM P (Table 4).

Table 3. Analysis of variance of the effects of seed priming on seedling emergence rate and percentage of barley.

S.O.V	d.f	Seedling emergence rate	Seedling emergence percentage
Replication	2	0.0001	136.113
Treatment	8	0.001*	207.496*
Error	16	0.0003	81.546
C.V%	-	12.64	10.45

* Significant difference at $p \leq 0.01$.

Table 4. Means of rate and percentage of seedling emergence of barley in the field, affected by different seed priming treatment.

Seed priming treatment	Means of traits	
	Seedling emergence rate	Seedling emergence percentage
Control (no priming)	0.1213 c	79.73 b
Hydropriming	0.1691 ab	91.67 ab
%10 PEG	0.1410 bc	75 b
5 mM $CaCl_2$	0.1314 c	85.67 ab
10 mM Zn	0.1539 abc	87.67 ab
50 mM P	0.1441 bc	91.67 ab
100 mM P	0.1365 bc	100 a
10 mM Zn + 50 mM P	0.1395 bc	91 ab
10 mM Zn + 100 mM P	0.1812 a	75.67 b

+ Different letters indicating significant differences at $p \leq 0.01$.

Discussion

A pre-sowing treatment involving hydration of seeds in priming solutions improved the performance of seeds and seedlings of barley. This improvement was reflected in higher germination, root, shoot and seedling dry weights and low electrical conductivity (EC) of leachates from seeds. Several reasons have been proposed to explain the observed stimulation in early and total germination. When seeds imbibe, the water content reaches a plateau and changes little until radicle emergence [8]. Priming up to this point can have a positive effect, while extended priming duration will negatively affect germination. Heydecker *et al.*[16] noted that seed priming methods enhance the speed and uniformity of germination. Thus, up on seeding, primed seeds can rapidly imbibe and revive the seed metabolism, resulting in higher germination rate and a reduction in the inherent physiological heterogeneity in germination [26]. In short-season vegetable crops, seed hydration has resulted in earlier emergence, earlier maturity, yield increase and improved quality [13, 30].

Hydropriming, osmopriming with PEG and nutrient priming with 10 mM Zn and 10 mM Zn + 100 mM P had low EC during imbibition. The low EC for these primed seeds may be due to better membrane structure, while increased seed leachates conductivity of other seeds was probably due to the loss of ability to reorganize cellular membranes rapidly and completely [22]. Karssen *et al.*[19] and Styer and Cantliffe [29] reported that partially hydration of seeds repaired deteriorated seed parts and reduced leakage of metabolites from seeds.

High root, shoot and seedling dry weight was obtained from P solutions and 10 mM Zn + 100 mM P. Beside the direct nutritional

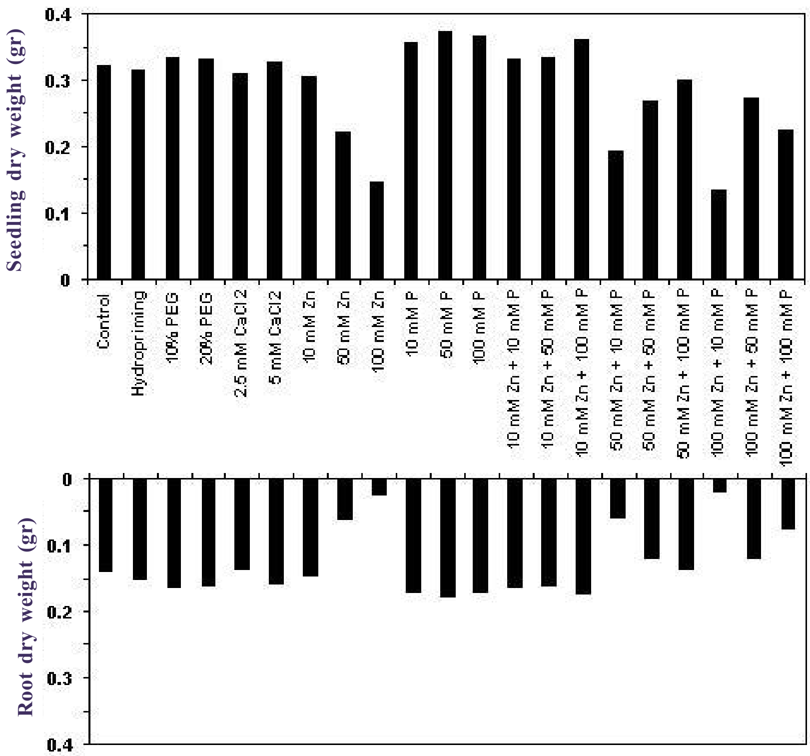

Figure 1. Effects of seed priming treatments on root and seedling dry weight of barley.

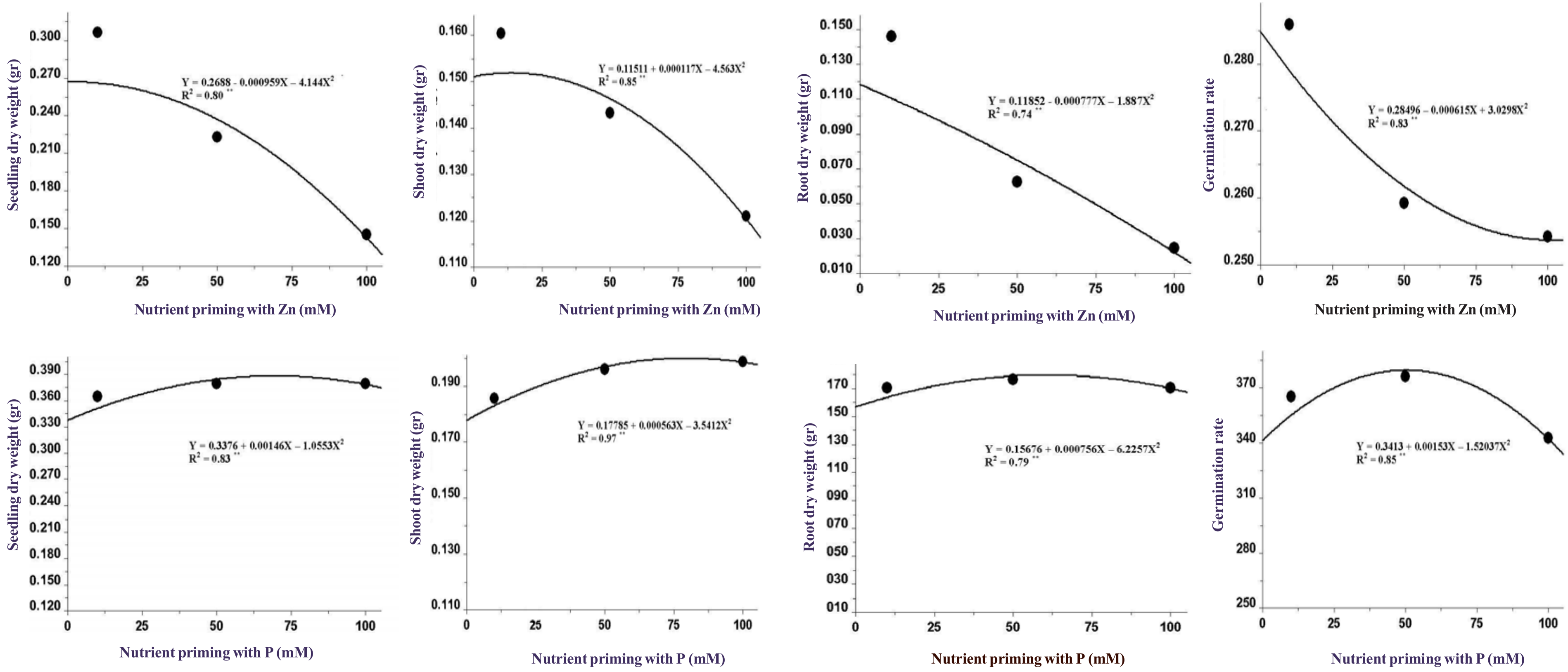

Figure 2. Effects of seed priming with 10, 50 and 100 mM of Zn and P on germination rate, root, shoot and seedling dry weight of barley.

effects of nutrient priming, it is conceivable that the observed increase in root biomass improved the access to other resources[25]. In this research, all traits were improved with increasing P concentration. Also, all traits were decreased with increasing Zn concentration in priming solutions, suggesting that Zn may have an inhibitory effect on germination. Slaton *et al*. [27] showed that radicle length tended to decrease as the Zn rate increased. Ajouri *et al*. [1] found that water priming of barley seeds for 12 hours advanced germination by up to 3 days compared to unprimed seeds, as well as addition of 10 mM Zn and 50 mM P to the priming solution increased the P and Zn content of the seeds without affecting germination. It furthermore significantly stimulated growth and P and Zn uptake by 4-week-old seedlings and improved the water use efficiency of drought-stressed plants by 44% above that of unprimed seeds. Zhang *et al*. [32] recommended soaking seeds in P solution before seeding to improve the P nutrition and germination of rice. Earlier studies on rice also suggested that seed coating with Zn or the dipping of the roots of seedlings in Zn solution is more effective than surface application of Zn in calcareous soils [10].

In the field experiment, the highest mean seedling emergence rate and percentage achieved from 10 mM Zn + 100 mM P and 100 mM P primings, respectively. Rapid seed germination and stand establishment are critical factors to crop production under drought and cold stress conditions. In many crop species, seed germination and early seedling growth are the most sensitive stages to drought stress. Drought may delay the onset, reduce the rate and increase the dispersion of germination events, leading to reductions in plant growth and final crop yield [5]. Bort *et al*. [6] stressed the importance of a rapid crop establishment and a vigorous early growth for the performance of barley in the eastern Mediterranean region with high seedling vigor being essential for the crops competitiveness for water, light and nutrients during the cold season, particularly in the rainfed environments. The widely limiting availability of water can be addressed via an improved access to soil water resources and increased water-use efficiency by an improved crop establishment and P and K nutrition [21]. The resulting improved stand establishment can reportedly increase drought tolerance, reduce pest damage and increase crop yield [14]. Kibite and Harker [20] reported that seed hydration of wheat, barley and oats improved the uniformity of seedling emergence. All of these priming technologies based on a timely availability of the nutrients which may require suitable substrates and machinery for seed coating, and necessitate an additional availability of labor at the time of seeding or transplanting.

These results suggested that seed priming, particularly with P solutions could be used to improve seedling vigor and establishment of barley in the field.

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