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Research Article

Effects of Priming with Copper, Zinc and Phosphorus on Seed and Seedling Composition in Wheat and Barley

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Abstract

Priming the seeds with plant nutrients containing solutions is economically feasible and easy way of preventing plants from future nutrient deficiency problems by enriching seed nutrition content; therefore, it has been commonly used in the last decades. Seeds of barley (*Hordeum vulgare* L., Tokak 157/57) and common wheat (*Triticum aestivum* L., Esperia) were kept in priming solutions containing phosphorus (P), copper sulphate (Cu) and zinc sulphate (Zn) in different concentrations for 10 h and washed in pure water then dried back to the initial moisture content. The chemical compositions of seeds were analysed and then the efficiency tests of priming practices were conducted in completely randomised design pot experiment with three replications. There were priming-induced increases in seeds up to 10.5, 13.4 and 1.61 fold for Cu, Zn and P respectively. The treatments resulted in an increase in the 50% emergence time. Phosphorus treatment (3%) reduced the germination rate by 33% comparing with the control. Seedling nutrient concentrations on the subject were positively responded to nutri-priming treatments. Therefore, these priming techniques could be used to improve seedling's nutrient contents to better perform in the preceding growth stages.

Key words: Barley, nutrient element priming, seedling performance, wheat.

Bakır, Çinko ve Fosfor İçerikli Priming Uygulamalarının Buğday ve Arpada Tohum ve Fide İçeriği Üzerine Etkileri

Özet

Bitki besin içerikli priming uygulamaları ile tohumun zenginleştirilmesi bitkilerin yetişme döneminde karşılaşılacak mineral madde eksikliğinin giderilmesi için ekonomik ve uygulaması kolay bir yöntemdir ve son yıllarda yoğun olarak kullanılmaktadır. Arpa (*Hordeum vulgare* L., Tokak 157/57) ve ekmeklik buğday (*Triticum aestivum* L., Esperia) tohumları farklı konsantrasyonlardaki fosfor (P), bakır (Cu) ve çinko (Zn) içerikli priming çözeltilerinde 10 saat boyunca bekletilmiş ve saf suda yıkanarak önceki nem içeriğine kadar geri kurutulmuştur. Kurutulan tohumlar analiz edilmiş ve üç tekerrürlü olarak saksılara ekim yapılmıştır. Priming işlemi tohumdaki Cu, Zn ve P içeriklerini sırasıyla 10.5, 13.4 ve 1.61 kat artırmıştır. Uygulamalar % 50 çıkış süresini artırmıştır. Fosfor uygulaması (% 3), çimlenme oranını kontrole kıyasla % 33 oranında azaltmıştır. Ön uygulama fide besin içeriklerini olumlu yönde etkilemiştir. Yöntem ilk büyüme dönemlerinde fide besin içeriklerini artırdığı için fide performansını geliştirmek için kullanılabilir.

Anahtar kelimeler: Arpa, buğday, besin elementi, priming.

Introduction

Nutrient element deficiency limits crop production in the world's arable land, where the application of fertilizers to soils is often practiced to achieve satisfactory yield and yield quality (Vance *et al.,* 2003; Fageria, 2009). Early development stages of a crop such as seed germination, seedling emergence and crop establishment are important aspects of agricultural production, and are in fact important components of seed and seedling vigour (Nadeem *et al.,* 2011; Harris *et al.,* 2007). Nutrient deficiencies related to early growth of the crop can increase susceptibility to early-season stresses (Kakhki *et al.,* 2008) and thus nutrient deficiencies result in yield loss. Common mineral nutrient deficiencies such as iron (Fe), copper (Cu), zinc (Zn), etc. are reported to be responsible for human malnutrition above 40% of the world population (White and Broadley, 2009; Cakmak *et al.,* 2010). Nutrient deficiencies in crop plants may be corrected by soil and/or foliar spraying and priming treatments. In general, soil and foliar applications of micronutrients are the most prevalent methods. The higher fertilizer requirement in soil application has disadvantages from the economic and environmental point of view due to low usage efficiency resulting from their chemical reactions with soil components and accumulation in soils, respectively. Despite spraying is a fast and efficient way of treating the micro-/macro-nutrient deficiencies (Malhi, 2009; Guo *et al*., 2016) it is not economically feasible for small stakeholders (Johnson *et al*., 2005; Farooq *et al.*, 2012) due to necessity of its repeated applications. Seed treatments were reported to be more advantageous than the soil treatments and practiced especially at early stages of the growth for micronutrients such as Mn and Zn (Moussavi-Nik *et al*., 1997; Seddigh *et al*., 2016). Priming the seeds for enriching the nutrient concentrations offers fertilizer economy and practical ease thus it has been regarded as a promising practice in the last decades. Therefore, priming practices have been used for increasing the macro and micronutrient concentrations of seeds towards enhancing the yield (Arif *et al*., 2007; Sekiya and Yano, 2010; Muhammad *et al*., 2015) and seedling quality. In addition, priming can be practiced for different purposes such as fast and homogenous germination of seeds (Rashid *et al*., 2006; Miraj *et al*., 2013), seed nutrient enrichment (Malhi *et al*., 2005; Ghassemi-Golezani and Abdurrahmani, 2012; Antonovsky and Ryant, 2015), and pesticides treatment (Khan *et al*., 2014) and microorganisms inoculation (Mahmood *et al*., 2016).

Cereals suffer from zinc and copper deficiencies (Jones, 2012; Kumar and Sharma, 2014). In general, the availability of these elements for plants are limited in different type of soils and strongly adsorbed by soil components such as iron oxides, carbonates and organic matter (Uygur and Rimmer, 2000; Flaten *et al*., 2004; Hettiarachchi *et al*., 2010, Chittamart *et al*., 2016; Udeigwe *et al.,* 2016). Copper has specific roles in nitrogen fixation, electron transport and energy absorbance, and protein and carbohydrate metabolisms (Jones, 2012; Marschner, 2012) therefore Cu deficiency results in yield and quality losses in wheat (Brennan and Bolland, 2008; Antosovsky and Ryant, 2015). The efficiency of broadcasting treatment of Cu fertilizers was reported to be limited in the first couple of years (Malhi *et al*., 2005) due to its chemical reaction with soil components. Halopriming wheat seeds in Cu solution suppressed seedling emergence, but resulted in astonishing increases in grain yield (Malhi, 2009). Priming wheat seed in Cu solution (as low as 0.04 kg Cu ha^{-1}) was an effective method to correct Cu deficiency along with significant seed yield increase. The overall effect of seed priming with Cu, as considered germination and yield, was likely to be dependent on the Cu concentration of priming solution and plant species (Malhi, 2009) with some other environmental conditions. On the other hand, Cu priming enhanced nitrogen use efficiency along with yield increase in corn cultivation (Teama, 2001).

Zinc deficiency is a worldwide incidence in calcareous soils of semi-arid regions (Gomez-Coronado *et al*., 2017; Joy *et al*., 2017; Liu *et al*,. 2017). Therefore, Zn deficiency in Turkish soils which is the subject of about 50%. Anatolian soils are reported to exist deleterious Zn deficiency due to alkaline soil reaction, high clay and very high carbonate content (Eyupoglu *et al.,* 1998), threaten crop yield of cereals, mainly wheat (Dogan, 2015) and other agronomic crops. Zinc is structural component of tens of proteins (Marschner, 2012) thus its deficiency cause significant yield loss and growth disorders in wheat (Epstein and Bloom, 2005; Zou *et al*., 2012). The usage of Zn rich seeds positively affects the yield, promotes seedling growth and corrects to some extent soil induced Zn deficiency symptoms in wheat (Farooq *et al*., 2012; Prom-u-thai *et al*., 2012) and other field crops. Despite no enhancement in grain Zn concentration, a considerable yield increase was reported due to priming with mineral Zn carriers; but some of Zn carrying amino acids improved both yield and grain Zn concentration (Seddigh *et al*., 2016). The other point is that resistance to pathogens can be often obtained by Zn priming (Graham and Rengel, 1993). It was reported that priming was superior over soil fertilizer treatments in improving the yield (Harris *et al*., 2004; Seddigh *et al*., 2016).

Phosphorus concentration of cereal grains ranks 0.2-0.5% (Marschner, 2012). Phosphorus as an essential nutrient element takes part key roles in many metabolic processes (Epstein and Bloom, 2005) and it is one of the enzyme components.

Low phosphorus availability in the soils induces root elongation (Rich and Watt, 2013), limits seedling growth performance (Nadeem *et al.*, 2011), and ultimately the yield loss (Fageria *et al.*, 2017). The most significant problem of P management in the soils is related to transformation of readily available fertilizer P into non-plant-available forms relatively in short periods depending on either alkaline or moderate to strong acid pHs and soil properties (Kacar and Katkat, 2009; Uygur, 2009; Fageria *et al.*, 2017). This nature of P in soils can be temporarily overcome by crop-based fertilization practices but the excessive P input to agricultural soils to guarantee an economical crop production lead P accumulation and pose eutrophication of water resources through runoff and soil erosion (Pantano *et al.*, 2016). Such continuous usage of P fertilisers have significantly contributed to total P losses from soil environment and reduced P use efficiency in the food chain from 35% in 1950 to 6% in 2010 (Bai *et al.*, 2016). Thus there is a need for a reasonably acceptable method of challenging of reducing P input without impairing current sustainable crop yield. Priming may be regarded as a promising method that P enriched wheat seed required lesser amounts of fertilizer P than the non-P primed counterpart (Khalil *et al.*, 2010; Sekiya *and* Yano, 2010). In a very earlier study, priming induced P enrichment in the seeds was reported to improve oat yield without any additional P fertilization (Roberts, 1948). In a recent paper majority of corn seed-P was exported towards newly growing seedlings in 2-3 weeks' time (Nadeem *et al.*, 2011) that relates the significance of seed-P content for early growth stage and seedling quality. Therefore priming may be an economically feasible and environmentally friendly way of sustainable cropping in P deficient soils.

The aim of this study was to investigate the effect of nutri-priming (Cu, Zn and P) on seed nutrient enrichments, germination and emergence properties of seeds and growth performance at seedling growth period. The results of the research may have implications on fertilizer requirement and economy with increasing yield and yield quality.

Materials and Methods

Priming procedures

Esperia bread wheat and Tokak 157/37 barley cultivars were used in the study to test the effect of water priming. Different priming solutions consisting of 0.03 (Cu1) and 0.06% (Cu2) $CuSO_4$, 0.3 (Zn1) and 0.5% (Zn2) $ZnSO_4$, and 1(P1) and 3% (P2) P_2O_5 were prepared in distilled water (Harris *et al.*, 2004; Johnson *et al.*, 2005; Abdulrahmani *et al.*, 2007; Ali *et al.*, 2008). The adequate amounts of barley and wheat seeds per treatment were soaked for 10 h in either distilled water (control) or in the required nutrient solutions (Harris *et al.*, 2001, 2004). Then seeds rinsed with plenty of distilled water and were left to air-dry until their weights reach about the initial ones.

Pot experiment

Composite surface layer (0-20 cm) of a slightly alkaline clay-loam textured soil (pH 7.9) with available P concentration 20.4 kg ha^{-1} and organic matter content 18 g kg^{-1} was used in the pot experiment. The pot experiment was set-up in completely randomised design with three replications. Soil sample was passed through 20 mm mesh sieve to homogenise and remove large fragments. 1 kg of soil sample was filled into PVC pots of 150 mm diameter. Twenty seeds were sown approximately equidistant within each pot. The moisture content was maintained about 70-80% of the field capacity by irrigating with distilled water in two days interval. Germination was observed daily according to the methods of the AOSA (Association of Official Seed Analysis) (1990). The time to obtain 50% germination (T50) was calculated from the equation given below (Farooq *et al.*, 2005):

Emergence rate = $(ni/N)*100$

Where ni the number of emerging seeds at any time and N is the total number of seeds sowed each pot.

50% emergence time, $E50 = ti + (N/2)(tj-ti)/(nj-ni)$

Germination ratio-$GP = (n_i/N)*100$

n_i number of germinated seeds, N number of sowed seeds.

Time for 50% emergence- $E_{50} = t_i + (Ng/2)(t_j-t_i) / (n_j-n_i)$

Ng total number of germinated seeds, n_i and n_j number of seeds germinating in the subsequent day (t_i and t_j), $n_i<N/2<n_j$ (adapted from Coolbear *et al.*, 1984; Farooq *et al.*, 2005).

Chemical analysis

Four weeks after sowing, the pots were soaked in a reservoir for 24 h and then the plant roots were removed from the soil by means of pressurized water and the contaminants were rinsed by tap and distilled water. Fresh weight of plants were immediately determined upon removal of excessive water and plant samples were oven-dried at 70 °C for 72 h until to a constant weight. Dry plant samples were homogenised by reducing the particle size below 0.5 mm. A scoop of plant samples was then digested with the mixture of HNO_3:$HClO_4$ (4:1 on

V/V) for mineral element analysis. Zinc and Cu concentrations of digests were determined by using atomic absorption spectroscopy (Varian AA 240FS) whereas the P concentrations were analysed by a spectrophotometer (T80 UV/VIS spectrometer, PG Instruments Ltd.) after adding vanadomolybdate colour reagent (Murphy and Riley, 1962).

Statistical analysis

The data was subjected to ANOVA using SPSS software and mean separation between the treatments was performed by means of Duncan test at $P \leq 0.05$.

Results and Discussion

Copper, Zn, and P concentration of the primed-seedlings were distinctively higher than the control groups (Table 1). Copper priming to barley seeds resulted in a drastic increase up to 6 to 10 fold for 0.03 and 0.06% treatments, respectively whereas halo-priming with Cu solution did not significantly improved Cu concentration of barley seedlings (7.60 mg kg^{-1} and 7.89 mg kg^{-1} for 0.03 and 0.06% treatments, respectively). However, wheat seedlings showed better response to priming treatments and Cu concentration increased from 4.88 to 6.91 and 9.9 mg kg^{-1} on dry weight base. Zinc priming increased barley seed concentration from 16 mg kg^{-1} to 178-210 mg kg^{-1}. A similar change was also observed for wheat seeds from 27 to 167-218 mg kg^{-1}. Both primed-barley and -wheat seedlings showed significantly higher Zn concentration than the non-primed seedlings. The effect of priming solution concentration on seedling Zn content was significant. Increasing priming concentration resulted in continuous Zn increase in barley seedlings whereas wheat seedlings responded to Zn priming differently that a negative performance was recorded for the highest Zn containing priming solution. Phosphorus priming at both solution concentrations resulted in increased seed P content for both species. Phosphorus content of control barley seeds was 2520 mg kg^{-1} and it reached up to 3100 and 4050 mg kg^{-1} by means of priming with 1 and 3% P containing solutions, respectively. Wheat seeds showed enrichment by 41.3% (3703 mg kg^{-1}) and 60.4% (4203 mg kg^{-1}) comparing to the control (2620 mg kg^{-1}). Seedlings P concentration of wheat was increased from 664 to 1013 mg kg^{-1} whereas barley seedlings did not respond to increasing priming P concentration.

Generally, a seed rate of 180-200 kg ha^{-1} is commonly practiced in Turkey. In this case, when 180 kg ha^{-1} seed rate is used, 3-7 g Cu ha^{-1}, 31-37 g Zn ha^{-1} and 104-275 g P ha^{-1} are added by primed barley seeds. The amounts given in wheat seeds are calculated as 5 g Cu, 29-38 g Zn and 198-285 g P ha^{-1}. There were no effects of priming on germination ratio, fresh and dry biomasses of barley seedlings whereas time to 50% germination of seeds became significantly longer than the one observed for the control treatment (Table 2). Seedling fresh and dry weights of wheat did not change significantly with priming practices. The maximum germination ratio (100%) was observed for 0.5% Zn priming. Phosphorus priming with 3% P solution resulted in a detrimental decrease in germination ratio to as small as 60% and increased the time to 50% germination. Mineral composition of different species has a significant degree of genetic variation (Pfeiffer and McClafferty, 2007). As reported in the earlier literature (Ajouri et al., 2004; Johnson et al., 2005) the current study showed that seed priming with different nutrient solutions was effective and practical way of improving Zn, Cu, and P contents. Seed-priming improves crop performance by inducing physiological, molecular and biochemical changes (Chen *et al*., 2012).

However, barley husk hindered the diffusion of nutrients into endosperm by absorption process. Thus the expected improvement of seedlings was not maintained by nutritional enrichment. Several reports pointed out that the potential of nutripriming by micronutrients in improving yield of wheat and other crop plants (Farooq *et al.,* 2012) wherease Johnson *et al.* (2005) and Kacar and Katkat (2009) reported that nutripriming had no-effect on either grain yield or grain nutritional status. In contrast to these findings Harris *et al*. (2004) and Arif *et al*. (2007) reported nutripriming-induced yield increases. There are relationship between P uptake and Zn and Cu uptake. Phosphorus to zinc ratio is about 200 and an increase in P uptake inversely affects Zn uptake by plants (Chang, 1999; Jones, 2012). This ratio remained well below the critical value upon nutripriming-induced enrichment of nutrient elements in seeds.

Table 1. The effect of priming practices on nutritional compositions of seeds and seedlings .

	Barley		Wheat	
	Seed (mg/kg)	**Seedling (mg/kg)**	**Seed (mg/kg)**	**Seedling (mg/kg)**
Control	3.61 c**	7.51 ns	2.70 b**	4.88 c**
Cu1	18.4 b	7.60	28.4 a	6.91 b
Cu2	37.7 a	7.89	28.0 a	9.90 a
Control	15.7 b**	31.9 c**	26.9 c**	22.6 c**
Zn1	210 a	43.6 b	167 b	57.0 a
Zn2	178 a	59.4 a	218 a	37.5 b
Control	2520 c**	990 c**	2620 c**	664 b**
P1	3100 b	2544 a	3703 b	934 a
P2	4050 a	1831 b	4203 a	1013 a

*, **: significant at P < 0.05 and P < 0.01 probability levels, respectively. ns: no significant
Means in the same columns followed by the same letters are not significantly different.

Table 2. The effects of nutripriming on some seedling properties.

Treat.	**Germination (%)**	**Time to 50% emergence (days)**	**Fresh seedling weight (g)**	**Dry seedling weight (g)**	**Treat.**	**Germination (%)**	**Time to 50% emergence (days)**	**Fresh seedling weight (g)**	**Dry seedling weight (g)**
Control	93.3 ns	10.0 c**	0.823 ns	0.120 ns	Control	93.3 ab *	11.7 b*	0.905 ns	0.129 ns
Cu1	100	11.7 b	0.760	0.102	Cu1	93.3 ab	11.7 b	0.882	0.136
Cu2	86.7	12.0 b	0.906	0.117	Cu2	86.7 ab	12.0 ab	0.721	0.113
Zn1	93.3	12.0 b	0.858	0.114	Zn1	100 a	12.0 ab	0.872	0.130
Zn2	93.3	12.0 b	0.988	0.139	Zn2	93.3 ab	12.0 ab	0.882	0.124
P1	93.3	13.0 a	0.822	0.116	P1	73.3 ab	13.7 ab	0.906	0.128
P2	93.3	12.0 b	0.884	0.125	P2	60.0 b	14.0 a	0.811	0.106

*, **: significant at P < 0.05 and P < 0.01 probability levels, respectively. ns: no significant
Means in the same columns followed by the same letters are not significantly different.

Since nutripriming practices foster earlier and stronger seedling development it leads an increase in the vegetative growth (Khalil *et al.,* 2010). Addition of Zn in priming solution further improved seedling growth in early stages depending on possible involvement of Zn in the early stages of coleoptile and radicle development (Ozturk *et al.*, 2006). The enriched amounts of Zn in seeds maintained by priming can induce vigorous seedling formation with heavier biomasses (Harris *et al*., 2007) but this doesn't always require a concentration increase in the seedlings. In accordance with this no-enrichment of Zn concentration was reported for rice seedling leaves (Johnson *et al*., 2005). Despite Zn addition in priming solution improved either fresh or dry biomasses it was not significant due possibly to shortage in growth period. This finding is in accordance with those reported by Ajouri *et al*. (2004). Concentrated nutripriming treatments may be hazardous to seeds with potential inhibition of germination (Roberts, 1948) and thus yield losses in some growth periods due to insufficient plant density (Johnson *et al*., 2005; Malhi, 2009). In this study the main inverse effect of nutripriming treatments was increased time to 50% emergence. This response may be related to nutripriming induced-toxicity or –slowdown in germination metabolisms.

Conclusions

Nutripriming practices can be used efficiently as easy and economically feasible way of increasing seed nutritional composition, but it should be careful about the concentration of the solution to eliminate its inverse effects on germination potential of seeds. The presence of seed husk can prevent the efficiency of nutripiming practice to increase seed and seedling nutrient composition. Enriching the seed nutritional status results in improvement of nutritional composition of seedlings which, in fact, may positively influence the plant developments in the subsequent growing stages.

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