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Optimizing zinc seed priming treatments for improving the germination and early seedling growth of wheat

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Key words: Germination, Micronutrient (Zn), Seed treatments, Seedling growth

Abstract

Zinc (Zn) is an essential micronutrient with various vital metabolic, enzymatic and defensive roles in crop plants. This study was conducted to optimize the seed priming treatments, with Zn in improving the germination and early seedling growth of wheat. Experiments were conducted in petri plates and sand filled pots, respectively in Allelopathy laboratory, Department of Agronomy, University of Agriculture Faisalabad, during 2012. The experiments were laid out in completely randomized design in factorial arrangement with four replications. Seeds of two wheat cultivars Lasani-2008 and Faisalabad-2008 were soaked in aerated Zn solution of various concentrations (0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 *M* Zn) for 12 h. Seeds soaked in aerated water for 12 h (hydropriming) and untreated dry seeds were taken as control. Wheat seeds primed in 0.1 to 0.01 *M* Zn solution increased the earliness, uniformity and final germination percentage in wheat. Beyond this concentration, there was adverse effect on germination and seedling growth of both wheat cultivars. Seed priming with 0.1 to 0.01 *M* Zn solution also improved the root and shoot length and seedling dry weight.

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Introduction

Zinc (Zn) is an important micronutrients for both plants and human growth. Zn is an essential micronutrient because it is involved in many metabolic processes (Rout and Das, 2003; Aravind and Prasad, 2005a, b) and it is important part (cofactor) of many enzymes (Aravind and Prasad, 2004, 2005c). Especially there are two enzymes such as Cu/Zn superoxide dismutase (SOD) and carbonic anhydrase (CA), Zn works as a cofactor of these enzymes. Carbonic anhyderase (found in cytosol and chloroplasts) catalyses the fixation of CO_2. When Zn deficiency is found Carbonic anhydrase and Cu/ZnSOD lose their activity (Rengel, 1995; Cakmak, 1997). Zinc is involved in many processes of plant such as in chlorophyll formation (Fischer, 1997) and stress (abiotic, biotic) resistance in plants (Cakmak, 2000). Biomass production is also increased in plants (Kaya and Higgs, 2002; Cakmak, 2008). Zn is also involved in the process of pollen formation and fertilization (Kaya and Higgs, 2002; Pandey *et al.*, 2006). High Zn contents in seed play a key role in many physiological processes during seed germination and early seedling growth (Cakmak, 2008).

Zinc (Zn) is involved in many vital physiological processes in early stage of radical and coleoptile development during seed germination (Ozturk *et al.,* 2006). Zn is involved in synthesis of protein, cell division and cell enlargement or cell elongation (Cakmak, 2000). It is also involved in shielding and stabilizing the structure of cell membranes in plants (Cakmak, 2008). Zinc is plays key role in metabolic process such as, synthesis and breakdown of carbohydrate, lipid, protein and nucleic acid (Auld, 2001). Zn has role in physiological process of plants, such as cell division, cell elongation and its important part for growing regions of plants especially root tips, new leaf and bud development. It is also involved in membrane function and resistance to environmental (abiotic) stresses in plants (Cakmak, 2000). Like other micronutrients, Zn fertilizer may be applied through foliar spray, fertigation, seed treatment and soil dressing. Although, each of the above methods of fertilizer application has its merits and de-merits, micronutrient application as seed treatments is gaining popularity in recent years (Farooq *et al.*, 2012). In seed priming, seeds are soaked in solutions of low water potential for a certain period of time seeds are then re-dried to permit routine handling (Farooq *et al.*, 2006). Primed seeds show uniform and early germination and sometimes greater total germination percentage over a range of environmental conditions (Farooq *et al.*, 2009). Improvement in germination followed by better stand establishment and grain yield are attributed to a buildup of germination-promoting metabolites (Farooq *et al.*, 2006), osmotic adjustment (Bradford, 1986) and metabolic repair during imbibition (Burgass & Powell, 1984).With the use of nutrient sources and commercial fertilizers as a priming, the positive effects of seed priming with an improved nutrient supply (Al-Mudaris & Jutzi, 1999; Farooq *et al.*, 2012).

However, the beneficial effects often depend on the nutrient concentration in the priming solutions. Generally, Zn seed priming treatments were applied for improving the germination and early seedling growth of rice. However, Zn has rarely been tried as priming agent in wheat. This study was therefore conducted to optimize the seed priming treatments with Zn in improving the germination and early seedling growth of wheat.

Materials and methods

Experimental site

Experiments were carried out in the Allelopathy Laboratory, Department of Agronomy, University of Agriculture, Faisalabad Pakistan, following completely randomized design with four replications.

Seed materials

Seeds of two wheat cultivars Lasani-2008 and Faisalabad- 2008, used in this study, were collected from Wheat Research program, Ayub Agriculture Research Institute, Faisalabad, Pakistan.

Seed priming treatments

Seeds, of both wheat cultivars, were soaked in solutions of Zinc sulphate (0.5, 0.1, 0.05, 0.01, 0.005 & 0.001 *M* Zn) solutions. Seeds soaked in aerated water and untreated seeds were taken as control.

In both cases, soaking was done for 12 h in aerated solution (nutripriming) or water (hydropriming) keeping seed to solution ratio 1:5 (w/v). Aeration was provided by aquarium pump.

Post Priming Operations

After removing from the respective priming solutions, seeds were thoroughly rinsed with water and re-dried with forced air under shade near to original weight (Basra *et al.,* 2002). Then seeds were sealed in polythene bags and stored in refrigerator at 4°C until used.

Experimental detail

Control and treated wheat seeds were sown in petriplates (10 each) containing moist filter paper and in sand pots (5 each) containing moist sand replicated four times and placed in incubator. Day and night lengths were kept 15 and 10 h respectively with 60% humidity at 25°C temperature. Water was applied when needed. The experiment was terminated 10 days after Sowing.

Observations

Germination Test

Time to 50% germination (T_{50}) was calculated following the formulae of Coolbear *et al.* (1984) modified by Farooq *et al.* (2005).

$$T_{50} = t_i + \frac{\left[\frac{N}{2} - n_i\right](t_j - t_i)}{n_j - n_i}$$

Where

N = final number of germination

n_i, n_j = cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$

Mean germination time (MGT) was calculated following Ellis and Robert (1981).

$$MGT = \frac{\sum Dn}{\sum n}$$

Where:

n =number of seeds, which were germinated on day D

D = number of days counted from the beginning of germination

Number of seedling emerged when constant were expressed in final emergence percentage.

Measurements

Experiment was visited daily to record germination following Association of Official Seed Analysts (AOSA, 1990) until a constant count was achieved. Time to 50% germination (T_{50}) was calculated following Farooq *et al.* (2005). Mean germination time (MGT) was calculated following Ellis and Roberts (1981) and final germination count was expressed in percentage. After the constant count, plants were thinned to maintain 3 plants per Petri plate. After 10 days seedlings were harvested and data regarding plant height, shoot length, root length, dry weights, number of leaves and secondary roots were recorded.

Statistical analyses

Experimental data were analyzed statistically using statistical software MSTAT-C. Analysis of variance was used to test the significance of variance sources, while the difference among treatment means were compared using LSD test (p=0.05) (Steel *et al.*, 1996).

Results

Pre-sowing zinc seed priming treatments significantly ($P<0.05$) affected the germination and early seedling establishment in both wheat cultivars Lasani-2008 and Faisalabad-2008 (Fig.s. 1—10). In petri-plate minimum T_{50} was noted from untreated seeds in cultivar Lasani-2008. All the seed priming treatments significantly increased the T_{50} in both wheat cultivars, while maximum T_{50} was recorded from osmopriming with 0.001 *M* Zn solution in cultivar Lasani-2008(Fig.. 1a). In sand medium minimum T_{50} was noted from seed osmoprimed with 0.01 *M* Zn solution in cultivar Faisalabad-2008, which was followed by the same treatment in cultivar Lasani-2008 and osmopriming with 0.1 *M* Zn in cultivar Lasani-2008. Maximum E_{50} was observed in control of cultivar Lasani-2008, which was followed by seed priming with 0.001*M* and 0.5 *M* Zn solution in cultivar Faisalabad-2008 (Fig.. 1 b). In petri-plates both cultivars, minimum MGT was noted from seeds osmoprimed with 0.01 *M* Zn solution while maximum MGT was recorded from control, which was followed by hydropriming in both cultivars (Fig.. 2 a). By using sand medium all Zn seed priming treatments, except hydropriming and seed osmopriming with 0.5 *M* Zn

solution in cultivar Lasani-2008 and 0.001 *M* Zn solution in cultivar Faisalabad-2008 significantly decreased mean emergence time. While maximum MET was noted in seed osmoprimed with 0.001 *M* Zn solution in cultivar Faisalabad-2008 (Fig. 2 b).

In petri-plates all the Zn seed priming treatments, except osmopriming with 0.5 *M* Zn solution in both cultivars and osmopriming with 0.005 *M* and 0.001 *M* Zn solution in cultivar Lasani-2008, significantly improved the FGP (Fig.. 3 a). In sand medium all Zn seed priming treatments except osmopriming with 0.5 *M* Zn and 0.001 *M* Zn solution in cultivar Faisalabad-2008 and osmopriming with 0.5 *M* Zn, 0.005 *M* Zn and 0.001 *M* Zn solution in cultivar Lasani-2008 significantly improved the FEP (Fig. 3 b).

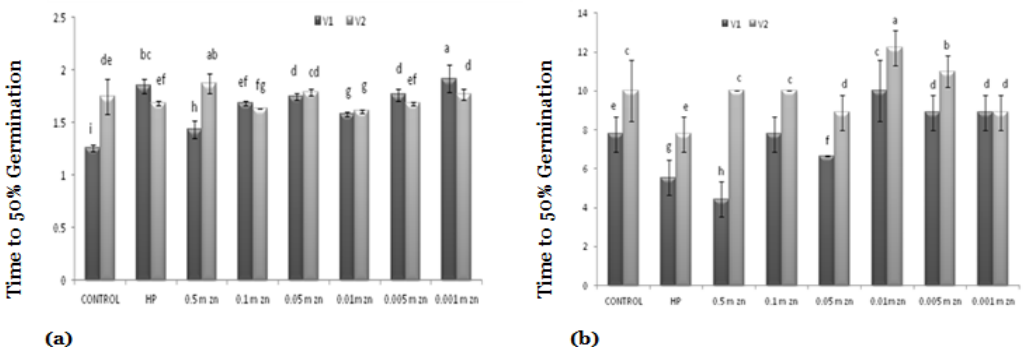

Fig. 1. Influence of zinc seed priming on shoot dry weight (mg) in wheat; V1: Lasani-2008; V2: Faisalabad-2008 ± S.E; (a) petri-plate; (b) sand filled pots. (HP = Hydropriming; 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 = Seed priming in solution of respective molar solution of zinc).

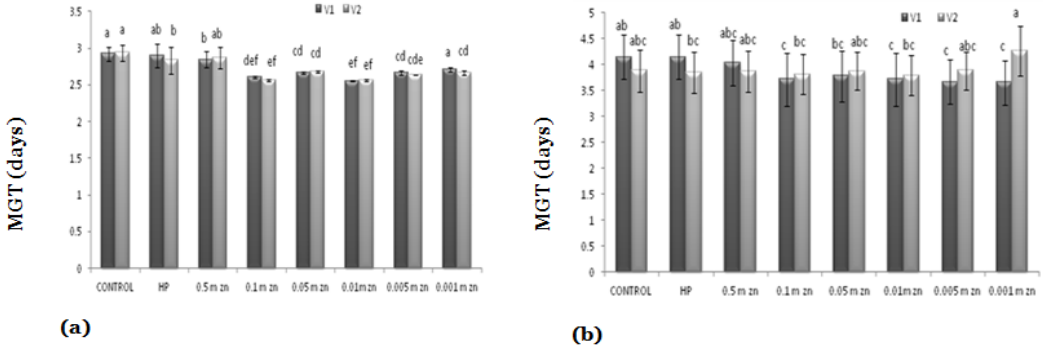

Fig. 2. Influence of zinc seed priming on mean germination time (MGT) in wheat; V1: Lasani-2008; V2: Faisalabad-2008 ± S.E; (a) petri-plate; (b) sand filled pots (HP = Hydropriming; 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 = Seed priming in solution of respective molar solution of zinc).

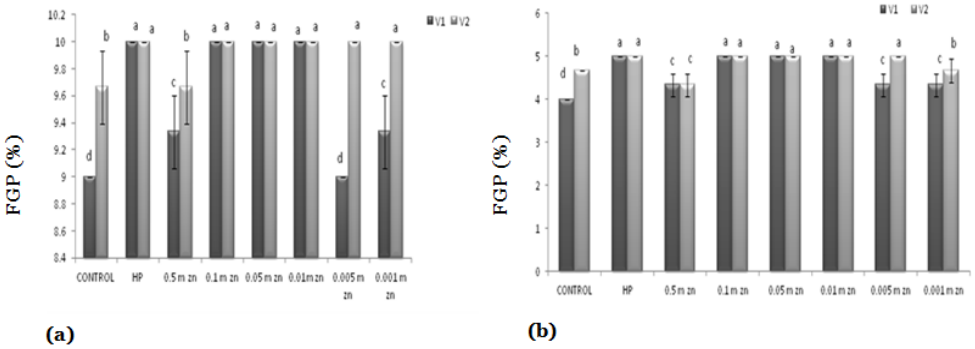

Fig. 3. Influence of zinc seed priming on final germination percentage (FGP) in wheat; V1: Lasani-2008; V2: Faisalabad-2008 ± S.E;(a) petri-plate; (b) sand filled pots. (HP = Hydropriming; 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 = Seed priming in solution of respective molar solution of zinc).

In petri-plate maximum plant height was noted in seeds primed with 0.001 *M* Zn solution in cultivar Lasani-2008, which was followed by hydropriming in the same cultivar. Minimum plant height was noted in seed osmoprimed with 0.5 *M* Zn solution in cultivar Faisalabad-2008, which was followed by seed priming with 0.001 *M* Zn solution in the same cultivar (Fig.. 4 a).

In sand medium maximum plant height was noted in hydropriming in cultivar Faisalabad-2008, which was followed by seeds osmoprimed with 0.001 *M* Zn solution in the cultivar. Minimum plant height was observed in seed priming with 0.5 *M* Zn solution in cultivar Lasani-2008. Cultivar Faisalabad-2008 had longer plants than cultivar Lasani-2008 (Fig.. 4b).

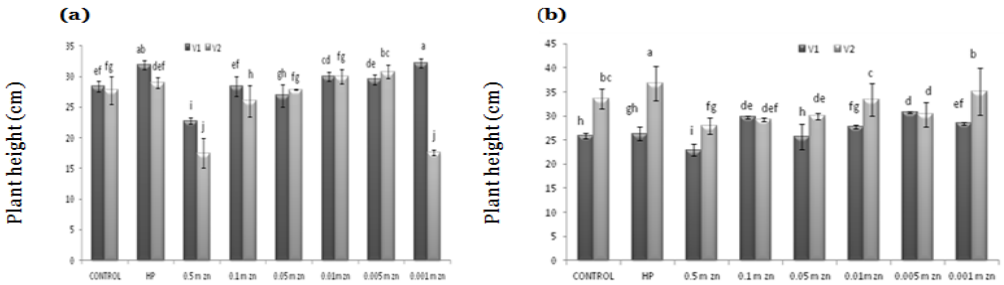

Fig. 4. Influence of zinc seed priming on plant height (cm) in wheat; V1: Lasani-2008; V2: Faisalabad-2008 ± S.E; (a) petri-plate; (b) sand filled pots. (HP = Hydropriming; 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 = Seed priming in solution of respective molar solution of zinc).

In petri-plate maximum root length was noted in hydropriming in cultivar Lasani-2008 while minimum root length was noted in seed osmoprimed with 0.5 *M* Zn solution in cultivar Faisalabad-2008, which was followed by seed osmoprimed with 0.001 *M* Zn solution in the same cultivar (Fig.. 5a). In sand pots maximum root length was noted from hydropriming in cultivar Faisalabad-2008, which was followed by the seed osmopriming with 0.001 *M* Zn solution in the same cultivar. Minimum root length was noted in untreated seed of cultivar Lasani-2008, which was followed by hydropriming and osmopriming with 0.5 *M* Zn in the same cultivar. Cultivar Faisalabad-2008 had longer roots than cultivar Lasani-2008 (Fig.. 5b).

In petri-plats maximum shoot length was observed in seed priming with 0.005 *M* Zn solution in cultivar Faisalabad-2008 while minimum shoot length was noted in seed osmoprimed with 0.5 *M* Zn solution in the same cultivar. However, shoot length of cultivar Faisalabad-2008 was more than cultivar Lasani-2008 (Fig.. 6a). By using sand filled pots maximum shoot length was note in seeds osmoprimed with 0.001 *M* Zn solution in cultivar Faisalabad-2008, which was followed by hydropriming in cultivar Lasani-2008.

Whereas minimum shoot length was noted in seed osmoprimed with 0.5 *M* Zn solution in both cultivars and seed osmoprimed with 0.1 *M* Zn solution in cultivar Lasani-2008 (Fig.. 6b).

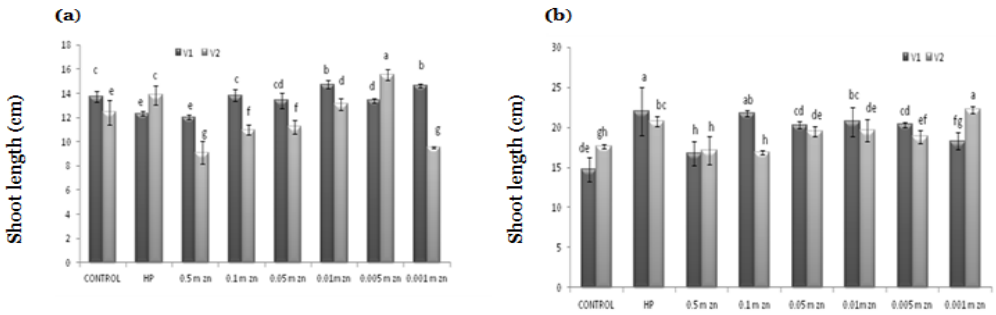

Fig. 5. Influence of zinc seed priming on shoot length (cm) in wheat; V1: Lasani-2008; V2: Faisalabad-2008 ± S.E; (a) petri-plate; (b) sand filled pots. (HP = Hydropriming; 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 = Seed priming in solution of respective molar solution of zinc).

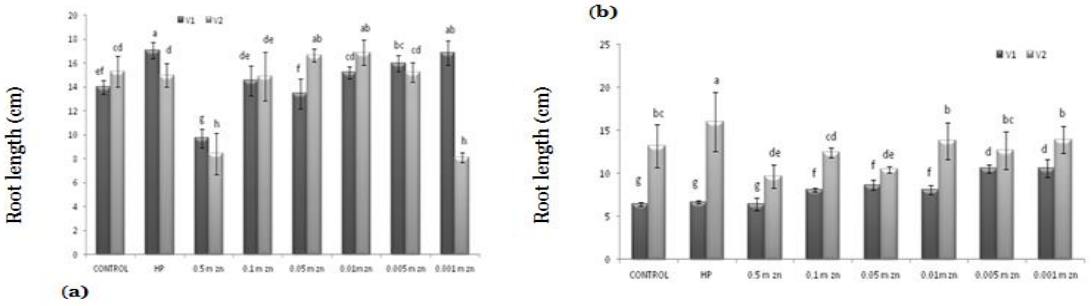

Fig. 6. Influence of zinc seed priming on root length (cm) in wheat; V1: Lasani-2008; V2: Faisalabad-2008 ± S.E; (a) petri-plate; (b) sand filled pots. (HP = Hydropriming; 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 = Seed priming in solution of respective molar solution of zinc).

In petri-plates maximum number of secondary roots per plant was noted in seed osmoprimed with 0.01 *M* Zn solution in cultivar Lasani-2008, while minimum secondary roots per plant were noted in control and osmopriming with 0.5 *M* Zn solution (Fig.. 7a). In sand filled medium maximum number of secondary roots per plant was noted in seed osmopriming with 0.005 *M* Zn solution in cultivar Faisalabad-2008, which was followed by osmopriming with 0.001 *M* Zn in cultivar Lasani-2008. Minimum number of secondary roots per plant was noted in untreated and seed priming with 0.5 *M* Zn solution in cultivar Lasani-2008. Cultivar Faisalabad-2008 had more secondary roots per plant than cultivar Lasani-2008 (Fig.. 7b). In petri-plates maximum number of leaves per plant was noted in seed osmoprimed with 0.005 and 0.001 *M* Zn solution in cultivar Lasani-2008 and osmopriming with 0.01 *M* and 0.005 *M* Zn solution in cultivar Faisalabad-2008; whereas minimum number of leaves were recorded from osmopriming with 0.5 *M* Zn solution in cultivar Lasani-2008 (Fig.. 8a). In sand medium maximum number of leaves per plant was noted in seed osmoprimed with 0.001 *M* Zn, 0.1 *M* Zn and 0.5 *M* Zn solutions in cultivar Faisalabad-2008, while minimum number of leaves per plant was noted in seed priming with 0.05 *M* Zn solution (Fig.. 8b).

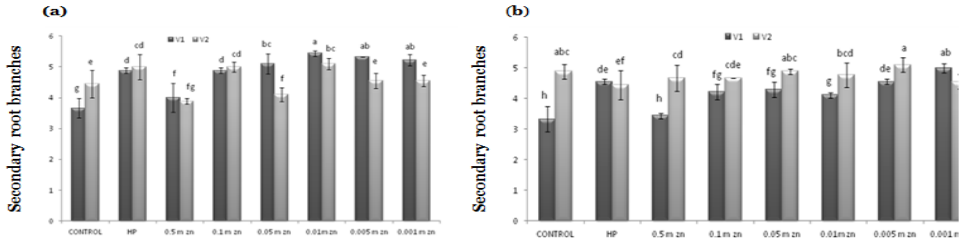

Fig. 7. Influence of Mn seed priming on secondary root branches in wheat; V1: Lasani-2008; V2: Faisalabad-2008 ± S.E; (a) petri-plate; (b) sand filled pots. (HP = Hydropriming; 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 = Seed priming in solution of respective molar solution of zinc).

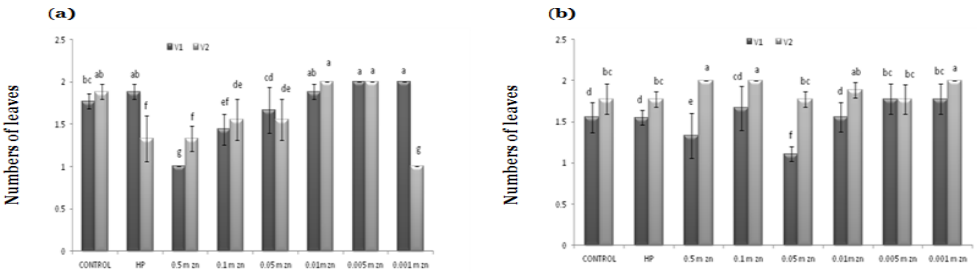

Fig. 8. Influence of zinc seed priming on no. of leaves in wheat; V1: Lasani-2008; V2: Faisalabad-2008 ± S.E; (a) petri-plate; (b) sand filled pots. (HP = Hydropriming; 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 = Seed priming in solution of respective molar solution of zinc).

In petri-plates maximum root dry weight was noted in seed priming with 0.005 *M* Zn solution in cultivar Lasani-2008 while minimum root dry weight was noted in hydropriming in the same cultivar (Fig.. 9a). By using sand filled medium mximum root dry weight was noted from seed osmoprimed with 0.005 *M* Zn solution in cultivar Faisalabad-2008, while minimum root dry weight was noted in seeds osmoprimed with 0.001 *M* Zn solution in cultivar Lasani-2008. Cultivar Faisalabad-2008 had more shoot dry weight than cultivar Lasani-2008 (Fig.. 9b). In petri-plates maximum shoot dry weight was noted in seed priming with 0.01 *M* Zn solution in cultivar Faisalabad-2008, while minimum shoot dry weight was noted in seed osmoprimed with 0.5 *M* Zn solution in cultivar Lasani-2008. Cultivar Faisalabad-2008 performed better than cultivar Lasani-2008 (Fig.. 10a). In sand medium maximum shoot dry weight was noted from seed osmoprimed with 0.005 *M* Zn solution in cultivar Lasani-2008 and in seeds primed with 0.5 *M* Zn and 0.05 *M* Zn solution in cultivar Faisalabad-2008. While minimum shoot dry weight was noted in seed priming with 0.5 *M* Zn solution in cultivar Lasani-2008. Cultivar Faisalabad-2008 had more shoot dry weight than cultivar Lasani-2008 (Fig.. 10b).

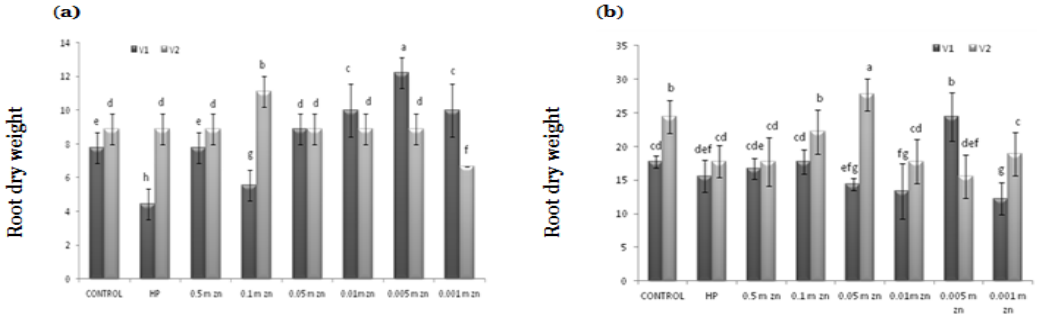

Fig. 9. Influence of zinc seed priming on root dry weight (mg) in wheat; V1: Lasani-2008; V2: Faisalabad-2008 ± S.E; (a) petri-plate; (b) sand filled pots. (HP = Hydropriming; 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 = Seed priming in solution of respective molar solution of zinc).

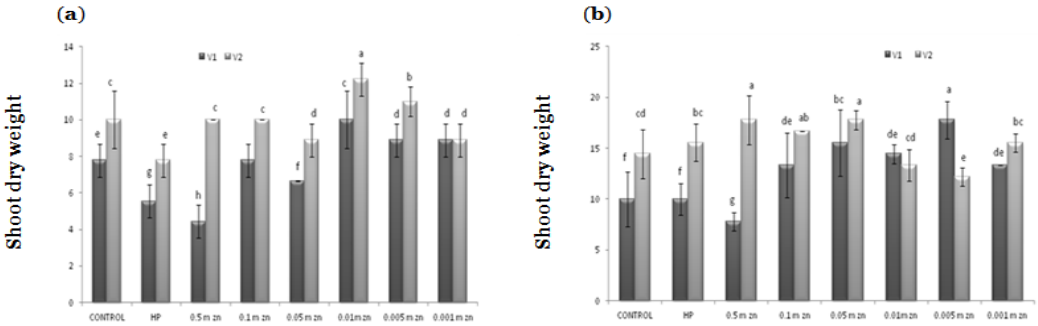

Fig. 10. Influence of zinc seed priming on shoot dry weight (mg) in wheat; V1: Lasani-2008; V2: Faisalabad-2008 ± S.E; (a) petri-plate; (b) sand filled pots. (HP = Hydropriming; 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 = Seed priming in solution of respective molar solution of zinc).

Discussion

This study reveals that Zn seed priming, particularly at lower concentrations, has significant potential to improve the stand establishment and early seedling growth of wheat. Seed priming in Zn solution of low concentration decreased the time to 50% germination (Fig. 1a, 1b), mean germination time (Fig. 2a, 2b) and final germination percentage (Fig. 3a, 3b) in wheat seedling, which indicates the possible involvement of Zn in physiological role in very low amount. Both times to 50% germination and mean germination time is important indicator of seed vigor. Seed priming triggers the hydrolytic enzymes and altered the physiology of embryos, so that germination

metabolism may take place rapidly than normal (Bam *et al.*, 2006). However, priming with higher concentration of Zn was toxic for plant growth (Fig.s. 1-10). Seed priming is a technique in which seeds are soaked in water before sowing; it reduces the time of seed germination and seedling emergence (Parera and Cantliffe, 1994). Seed germination follows three phases, imbibition, lag phase and radicle protrusion (Simon, 1984).

During seed priming, lag phase is extended and the food reserves are converted in available form (Farooq *et al.,* 2005b, 2006c). Thus primed seeds germinate in uniform and synchronized fashion upon planting (Basra *et al.*, 2002, 2004, 2005; Farooq *et al.,* 2005b, 2006c). As zinc (Zn) is involved in many vital physiological processes in early stage of radical and coleoptile development during seed germination (Ozturk *et al.,* 2006), its addition in the priming solution, in optimum range, further increased the effectiveness of seed priming. Seed priming can enhance the seed imbibitions process and revive the seed metabolism so that increases the germination rate and improved the numbers of seedling in primed seed as compared to control (Rowse, 1995).

Zinc seed priming increased the plant height (Fig.. 4a, 4b), shoot length (Fig.. 5a, 5b), root length (Fig.. 6a, 6b), root dry weight (Fig.. 9a, 9b) and shoot dry weight (Fig.. 10a, 10b) in wheat. However, optimum range of seed osmoprimed with 0.01 *M* Zn to 0.005 *M* Zn solution increased the plant height, shoot length and root length. For shoot dry weight and root dry weight, seed priming with 0.01 and 0.05 *M* Zn solution perform better than other seed treatments. Increase in root and shoot related parameters may be attributed to the fact that Zn is involved in synthesis of protein, cell division and cell enlargement or cell elongation (Cakmak, 2000). It is also involved in shielding and stabilizing the structure of cell membranes in plants (Cakmak, 2008). Zinc plays key role in metabolic process such as, synthesis and breakdown of carbohydrate, lipid, protein and nucleic acid (Auld, 2001). Enhancement of root and shoot length indicated the involvement of Zn in the meristematic growth of radical and plumule. Increases in shoot dry weight and root dry weight may be due to increases in shoot and root lengths respectively (Bohnsack and Albert, 1977).

In seed osmoprimed with 0.5 *M* Zn solution, decrease in plant height, shoot length and root length of wheat was observed (Fig.s. 4-6). It may be due to excessive amount of Zn, which causes toxicity in plants. Higher concentration of Zn decreases the growth and developments of leaves and roots in plants. Production of NADPH is decreased in chloroplast of plants when Zn concentration increased from its optimum range. Increase in the production of free radicals is also due to high concentration of Zn in plant tissues. Photosynthesis activities, ATP production and activities of RUBP carboxylase enzyme are also decreases by Zn toxicity (Prasd et al., 1999; Vitosh *et al.,* 1994; Teige *et al.,* 1990; Ruano *et al.,* 1988).

In conclusion Zn is requiring in small amount but decisive concentration, if adequate amount is not available plant will be undergoing physiological stress. Zn is an important micronutrient, which plays an important role in many physiological processed of plants. Optimum range of any nutrient is necessary for proper plant growth, because at higher concentration, micronutrients show toxic effects on plant. Seed osmoprimed with lower concentration of Zn (0.1 M Zn to 0.005 M Zn) performed better than control and osmopriming at higher concentrations.

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