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Seed priming of Zn with endophytic bacteria improves the productivity and grain biofortification of bread wheat

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ABSTRACT

Zinc (Zn) deficiency hampers the crop yield, nutritional quality and ultimately the human health. Plant growth-promoting endophyte transform unavailable Zn to available form for plant uptake which enhance the plant Zn status and growth. Therefore, a two year field study during 2013–14 and 2014–15 was conducted to evaluate the interactive effect of endophytic bacteria and Zn application methods for improving the productivity and grain biofortification of wheat. Zinc was applied to two wheat cultivars *viz*. Lasani-2008 (LS-2008) and Faisalabad-2008 (FSD-2008) as soil application (10 kg ha^{-1}), foliar application (0.025 *M*), seed priming (0.5 *M*) and seed coating (1.25 g kg^{-1} seed), while hydroprimed seeds were taken as control. Endophytic Zn solubilizing bacterial strain *viz. Pseudomonas* sp. MN12 was used in combination with different Zn application methods. Results revealed that Zn application through any method improved the grain yield and grain biofortification of bread wheat. Maximum improvement of 27.1% in grain yield was recorded when Zn was applied in combination with strain MN12 through seed priming technique against control. However, soil and foliar application of Zn with and without *Pseudomonas* sp. MN12 resulted in highest improvement in protein content, Zn concentration in whole grain, embryo, aleurone and endosperm of wheat than control. Moreover, Zn application improved the bioavailable Zn as minimum phytate concentration, phytate/Zn molar ratio and maximum bioavailable Zn was recorded for soil (43.9%) and foliar application (45.6%) of Zn + MN12, respectively. Nevertheless, highest net economic return and marginal rate of return with high benefit cost ratio (BCR) was recorded for seed priming with Zn + MN12. Furthermore, maximum grain yield, grain Zn concentration with high economic return was recorded for FSD-2008. In crux, Zn may be applied through seed priming in combination with *Pseudomonas* sp. MN12 to improve the productivity and grain biofortification of wheat.

1. Introduction

Zinc (Zn) is an important microelement essential for plants and humans. It is cofactor of many enzymes like DNA, RNA polymerases and Zn finger proteins (Coleman, 1998; Lopez-Millan et al., 2005). Zinc deficiency is receiving increasing attention due to many reports highlighting the impact of Zn dearth on crop plants and human health (Brown et al., 2004; Cakmak, 2008).

Zinc deficiency is widespread in soils with low Zn, high pH, Ca, Mg, Na, HCO_3 and phosphate contents (Alloway, 2009). Moreover, Zn deficiency is widespread in wheat producing countries (Alloway, 2008, 2009). In Pakistan, almost 70% of the agricultural land is deficient in phytoavailable Zn (Kauser et al., 2001). Wheat is inherently poor in Zn (Cakmak, 2008) and besides that Zn bioavailability to human is reduced due to presence of substances like phytic acid and polyphenols (Welch and Graham, 2004). Moreover, Zn concentration is higher in embryo and aleurone (Ozturk et al., 2006) which is lost during milling. However, Zn concentration is very less in endosperm (Li et al., 2014) which is largest fraction of wheat grain.

Zinc concentration in whole grain, grain fractions and wheat products can be augmented through supplementation and food fortification (Rehman et al., 2017). However, these approaches are being used in few countries as these are expensive and not easily approachable by the people of developing world (Bouis, 2003; Stein et al., 2007). The biofortification (genetic engineering and breeding) strategies appear to be a suitable option for increasing the micronutrient concentration in grain

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which will lead to better human health (Cakmak, 2008). The latter approaches are time consuming, costly and have some ethical issues as GM crops are not acceptable in many countries (Jaffe, 2005). Agronomic approaches involve escalating the grain Zn concentration through Zn fertilization and other management practices (Cakmak, 2008). Micronutrients (zinc) can be delivered through soil, foliar or seed treatment (Farooq et al., 2012, 2018).

To improve the plant Zn uptake, it can be supplied through soil, leaf or seed treatment (Johnson et al., 2005; Farooq et al., 2012, 2018). In this regard, soil Zn application is the principal method of Zn delivery to the plants. In wheat, $ZnSO_4$ is applied to soil before sowing to correct the Zn deficiency (Cakmak, 2008). Zinc can also be delivered through foliage application as it improves the grain Zn concentration and yield of wheat (Yilmaz et al., 1997; Johnson et al., 2005; Cakmak et al., 2010b). The alternate to soil and foliar approach is the application of Zn through seed treatment (seed coating and seed priming) which is getting popularity due to easy handling and cost effectiveness. In nutrient priming, seeds are soaked in a nutrient solution of known concentration for a specific period of time which allows the activation of germination related metabolism without radical protrusion (Farooq et al., 2009). Recently, seed priming with chelated Zn amino acids (glycine, histidine and arginine) was found to increase the Zn and protein concentration. It also reduced the phytate concentration in the grain. Seed coating is another technique of micronutrient delivery. Seed coated with Zn have better stand establishment, yield and grain Zn concentration than untreated seeds (Rehman and Farooq, 2016).

Soil microbes influence the micronutrient availability through solubility, chelation and root architecture (Prasanna et al., 2015). Plant growth promoting bacteria (PGPB) (endophytes and/or rhizosphere bacteria) enhance the essential elements availability to plants (Khalid et al., 2009). PGPE improve the plant growth through enhanced nutrient uptake, nitrogen fixation, synthesis of enzymes, production of siderophores and growth phytohormone (Mitter et al., 2013). There are many reports highlighting the role of rhizosphere microbes in improving the micronutrient availability (Gosal et al., 2010; Rana et al., 2012). However, use of endophytes in micronutrient biofortification has rarely been studied (Ren et al., 2012; Wang et al., 2014). Recently, Singh et al. (2017) found that endophytes enhanced the grain Zn concentration in wheat upto two folds than control due to higher IAA synthesis and better root growth.

Endophytes found more effective in nutrient acquisition and plant growth than rhizosphere bacteria due to better contact with plant tissues (Mitter et al., 2013). In this scenario, the use of endophytes can help to enhance the soil Zn availability to the crop plants (Singh et al., 2017). Although, Zn application improves the grain biofortification and productivity of wheat; nonetheless the Zn application in combination with endophyte bacteria have never been studied. Moreover, information about different Zn application methods on Zn biofortification and grain Zn localization is limited. For this study, it was hypothesized that Zn application with Zn solubilizing endophyte may improve the water relations, productivity, Zn bioavailability and grain Zn localization of bread wheat. The specific objective of this study was to evaluate the economics of Zn fertilization in combination with endophytic bacteria *Pseudomonas* sp. MN12 in bread wheat.

2. Materials and methods

2.1. Experimental site and treatments

This experiment was conducted for two growing seasons at Agronomic Research Area, University of Agriculture, Faisalabad (latitude 31.7°N, longitude 73.98°E) during 2013–14 and 2014–15. Seeds of two bread wheat cultivars, LS-2008 and FSD-2008 were obtained from Wheat Research Institute, Faisalabad, Pakistan. Zinc was applied as soil application (10 kg Zn ha^{-1}), foliar application (0.025 *M* Zn solution); seed priming (0.5 *M* $ZnSO_4$ for 12 h) (Rehman et al., 2015) and seed coating (1.25 g Zn kg^{-1} seed) (Rehman and Farooq, 2016). Hydroprimed seeds were taken as control. Zinc solubilizing endophytic bacteria *Pseudomonas* sp. MN12 was also used with each treatment. *Pseudomonas* sp. MN12 was previously isolated and evaluated for improving growth and yield of maize (Naveed et al., 2014) under controlled and natural soil conditions. Zinc solubilization activity of the selected strain was performed following the method of Bunt and Rovira (1955). The strain showed Zn solubilization activity as was observed the halos on the selected petri-plates.

Inoculum of strain MN12 was prepared in TSA broth in 1000 mL Erlenmeyer flasks and incubated at 28 ± 2 °C for 48 h in the orbital shaking incubator (Firstek Scientific, Tokyo, Japan) at 180 rev min^{-1}. The optical density of the broth was adjusted to 0.5 measured at 600 nm using spectrophotometer (Evolution 300 LC, Cambridge, UK) to obtain a uniform population of bacteria (10^8–10^9 colony-forming units (CFU) mL^{-1}) in the broth at the time of application. For one hectare area, 5 L microbial culture was used in each of the application method. For hydropriming and osmopriming, 5 L microbial culture was added in the solution and seeds were primed with similar to above mentioned procedure. Likewise, for seed coating, for 125 kg seeds ha^{-1}, Zn was mixed with 5 L microbial culture and Arabic gum, and the resulting slurry was coated on seeds. Moreover, in soil application for one hectare area, 5 L microbial culture was mixed with Zn and was mixed into soil before sowing. Similarly, in foliar application, 5 L microbial culture was added with spray water and was sprayed on leaves at tillering stage.

2.2. Crop husbandry

Seeds of wheat were sown on November 29, 2013 and November 26, 2014 using single row hand drill in 22.5 cm spaced rows using seed rate of 125 kg ha^{-1}. The experimental soil was sandy loam with pH 8.18, EC, 0.34, organic matter 0.92% and nitrogen 0.065% while extractable phosphorus, potassium, DTPA extractable Zn, Fe and available B and iron were 5.02, 1.68, 0.7, 6.78, and 0.55 mg kg^{-1} respectively. The extraction and analysis for soil physiochemical properties and nutrient status was done as described by ICARDA (2013).

Based on the soil analysis, 100-90-75 N, P_2O_5 and K_2O kg ha^{-1} was applied using urea (46% N), diammonium phosphate (18% N, 46% P_2O_5) and sulfate of potash (50% K_2O) as sources. Whole of the P, K and one third of the N was applied as basal dose. Remaining N was applied with 1st and 2nd irrigation in equal splits. Selected herbicide (Atlantis (iodo + mesosulfuron) at 14.4 g a.i. ha^{-1}) was applied as early post emergence on January 13, 2014 and January 15, 2015 respectively, to control weeds. In total, four irrigations (each of 3 acre inches) were applied to the crop during the growth period in addition to soaking irrigation of four acre inches. Crop was harvested on April 24, 2014 and April 26, 2015 during first and second years of the study, respectively and was threshed to record the yield and other related traits. The mean maximum temperature was 26.2, 19.5, 17.9, 21, 24.6 and 32.7 °C while mean minimum temperature was 11.6, 7.2, 6.5, 10.0, 13.6 and 19.7 °C from November to April during both years, respectively. Moreover, rain fall was 0.5, 0.0, 0.0, 14.3, 41.7 and 28.2 mm during 2013–2014, while it was 10.0, 0.0, 12.2, 20.5, 67.9 and 32.8 mm during year 2014–15 from November to April, respectively.

2.3. Data recording

2.3.1. Yield parameters

Total and productive tillers were counted from unit area (1 × 1 m) in each plot (1.8 × 6 m) at final harvest from two locations. From each plot, twenty main spikes were randomly taken and were threshed manually to separate the grain. The grains separated were counted to record number of grains per spike. Three sub-sample of 1000 grain was taken from each plot then weighted on an electric balance and average 1000-grain weight was calculated. The crop was harvested, tied into bundles and sundried for a week in respective plots. Total wheat