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Full Length Article

Zinc Biofortified Wheat Cultivar Lessens Grain Cadmium Accumulation under Cadmium Contaminated Conditions

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Abstract

Zinc (Zn) efficient wheat genotypes have been developed to enhance grain Zn concentration to combat the Zn malnutrition in humans. However, such biofortified genotypes/cultivars may also take up the toxic metals from heavy-metals contaminated soils due to similarities with Zn uptake and transport mechanisms in plants. This study was designed to explore uptake of a ubiquitous toxic metal *i.e.*, cadmium (Cd) by a Zn-efficient (Zincol-2016) and was compared with Zn-inefficient (Faisalabad-2008) wheat cultivar. Both wheat cultivars were sown in pots till maturity in Zn and Cd amended soil. Growth and yield response, and dissemination of Cd and Zn, in roots, shoot and grains were observed. Significant differences in Zn and Cd concentration in root, shoot and grain were found among both cultivars in response of Zn and Cd application. Zincol-2016 was more efficient cultivar for Zn uptake compared to the Faisalabad-2008. Cd uptake was increased by both cultivars and suppressed plant growth in Cd contaminated soil. However, Zn application in Cd contaminated soil significantly diminished the Cd uptake and vice versa. Interestingly, Cd concentration was higher in root of Zincol-2016 as compared to Faisalabad-2008; similar was the case in shoot of both cultivars, while it was lower in grain of Zincol-2016. In conclusion, Zn efficient cultivar not only produces high Zn grains but also has ability to contain Cd in root and shoot reducing its accumulation in grains. Furthermore, Zn fertilization in Cd contaminated soils can decrease Cd uptake by plants and may be used as an ameliorating strategy to grow wheat in Cd contaminated soils.

Keywords: Biofortification; Cadmium; Heavy metal; *Triticum aestivum*; Zinc

Introduction

Zinc (Zn) plays a substantial part in photosynthesis, catalytic and structural activities, protein synthesis, bio-membrane stability, energy transfer reactions and DNA replication in plants (Alloway, 2004; Hajiboland and Amirazad, 2010). It also improves the concentration of antioxidant enzymes and chlorophyll contents in plant tissues. Furthermore, it encompasses as a cofactor for the stimulation of more than 300 enzymes and plays a vital role in water uptake and transport decreasing the antagonistic effect of salt and heat stresses (McCall *et al.*, 2000; Kasim, 2007; Sbartai *et al.*, 2011). Zn is also essentially required by humans and approximately 17.3% people world-wide suffer from Zn deficiency (Wessells and Brown, 2012). Zn deficiency in soils correlates with human Zn deficiency (Joy *et al.*, 2017) and numerous factors have been reported for Zn deficiencies in soils including high clay content, high pH and soil calcareousness (Ashraf *et al.*, 2008).

Different approaches, for example Zn supplementation, food fortification and dietary diversification, are adopted to improve Zn deficiency in humans. However, these are practically and economically less feasible in the developing countries (Bouis *et al.*, 2000). Therefore, Zn biofortified cultivars of cereals were developed by HarvestPlus to fulfill the Zn scarcity in the food (Andersson *et al.*, 2017), by increasing its Zn uptake efficiency under Zn deficient soil conditions. Heavy-metals pollution in soil is a prevalent global issue (Tandy *et al.*, 2006) and has been elevated as a foremost environmental issue over the last few decades due to metals translocation in the food chain is injurious to animals and along with human health. The bioavailability of heavy metals in soils rely on their concentration in soil solution which in order is reliant on numerous soil processes

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such as specific adsorption, cation exchange complexation and precipitation as well as the uptake efficiency of crop plants (Basta *et al.*, 2005; Carrillo-González *et al.*, 2006). It is apprehended that cultivars efficient for Zn uptake may enhance the cadmium (Cd) uptake as well in contaminated soils.

Cadmium is a toxic metal pollutant and cause considerable toxicity to plants at even at low concentration. It is also deliberated as highly detrimental to human, as once it accumulates in the body remains there for a longer period of time. Compounds of Cd are comparatively more soluble than many other metals. Therefore, it can certainly become the part of food chain when existing in agricultural soils (Simmons *et al.*, 2003). Zn and Cd interact with each other in the soil, during uptake by plant from roots and its translocation to upper edible portion because of similar geochemical properties (Das *et al.*, 1997). Oxidative damage due to Cd is eased by Zn, which decreases membrane and metabolic damages by decreasing uptake translocation of Cd to edible parts (Sarwar *et al.*, 2010). Although ionic radii of Cd and Zn are dissimilar, they have equivalent electro-negativities (Abdel-Sabour *et al.*, 1988); therefore their antagonistic effect can be perceived. Zn efficient crop plants may also be efficient for Cd and can accumulate it in edible parts causing toxicity to the consumers (Adriano, 2001).

As Zn competes with Cd for plant uptake, therefore it was hypothesized that Zn efficient cultivar may also take up Cd efficiently in Cd contaminated soils. The objectives of proposed study were to explore i) Cd uptake and leading to its accumulation in grains by Zn efficient wheat cultivar in Cd contaminated soils ii) application of Zn fertilizer in Cd contaminated soils reduce the Cd uptake by plants.

Materials and Methods

Experimental Details and Treatments

This study was conducted in plastic pots (each containing 4.5 kg soil) in a green-house at Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad-38040, Pakistan in winter season. Basic analysis of soil for EC_e, pH, texture, Zn and Cd concentration is presented in Table 1. The treatments comprised of control (soil without Zn or Cd application), Zn amended soil (10 mg kg^{-1} soil), Cd amended soil (10 mg kg^{-1} soil), Zn plus Cd amended soil (10 mg kg^{-1} soil each). Source of Zn, and Cd were zinc sulfate heptahydrate ($ZnSO_4$. $7H_2O$) and cadmium chloride dihydrate ($CdCl_2$. $2H_2O$), respectively. Wheat (*Triticum aestivum* L.) cultivars Zincol-2016 (Zn efficient) and Faisalabad-2008 (Zn inefficient) were used as test material. All the treatments were arranged according to completely randomized factorial design with replicated four times. The seeds of Faisalabad-2008 were obtained from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan and Zincol-2016 from HarvestPlus Islamabad, Pakistan.

Table 1: Physicochemical characteristics of the soil used in the experiment

Parameter	Unit	Value
pH[a]	---	7.67
EC_e[a]	dS m^{-1}	1.96
Sand, Silt, Clay[b]	%	53, 24, 23
Textural class[c]	---	Sandy Loam
Zn[d]	mg kg^{-1}	0.91
Cd[d]	mg kg^{-1}	0.14

a. Measured in 1:1 soil to water
b. Hydrometer method (Page *et al.*, 1982)
c. USDA classification
d. AB-DTPA extractable (Soltanpour and Workman, 1979)

Crop Husbandry and Harvesting

Recommended dose of nutrients N, P_2O_5 and K_2O at the rate of 115, 80 and 60 kg ha^{-1} were applied as urea, triple super phosphate and sulphate of potash, respectively. Recommended dose of fertilizers were applied at the time of sowing as well as soil was contaminated at the same time. Crop was irrigated according to its water requirement at 60% of water holding capacity of soil. In each pot (containing 4.5 kg soil) primarily eight seeds were sown on 22 November 2015 and four plants were harvested from each pot after two months of sowing (before the booting stage). Remaining four plants were harvested at maturity. Plant growth attributes i.e. plant height, shoot and root fresh and dry weight were considered from first harvesting while yield parameters i.e. spike length, grain yield as well as Zn and Cd from root, shoot and grain were determined from second harvesting. Harvest index (HI) was calculated according to following formula:

$$\text{Harvest Index (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

Determination of Zn and Cd

To determine Zn and Cd concentration in the root, shoot and grain, fine ground root, shoot and grain material was digested in di-acid mixture (HNO_3:$HClO_4$ at 2:1) at 250±5°C. Dense white fumes of perchloric acid appeared in the tubes, and digestion continued for 30 min more. Then after samples were diluted with distilled water up to 25 mL volume and filtered using Whatman 42 filter paper. Zn and Cd concentrations were determined using the atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan).

Statistical Analysis

The data collected were analysed by statistical technique (Steel *et al.*, 1997) by using Statistix 8.1 (Analytical Software, Tallahassee, USA). Two way analysis of variance (ANOVA) followed by LSD test was used at $P \leq 0.05$ for the comparison of both wheat varieties as well as treatments variation.

Table 2: Effect of Zn and Cd application on growth and yield of wheat cultivars Zincol-2016 (Zn efficient) and Faisalabad-2008 (Zn inefficient). Values are mean ± standard error

Wheat cultivar	Treatments	Plant height(cm)	Total biomass(g pot^{-1})	Spike length(cm)	Grain yield(g pot^{-1})	Harvest Index(%)	Grain Zn (%)	Grain Cd (%)
Faisalabad-2008	Control	39.9 ± 0.7d	9.57±0.4cd	5.74±0.44bc	3.31±0.5bc	25.40±3.44a	45.37±4.68a	51.08±4.50a
	Zn	41.7±0.8cd	9.97±0.3bcd	6.43±0.20bc	3.00±0.2cd	23.08±0.96ab	36.65±1.47bc	49.25±3.35a
	Cd	44.4±3.0bc	8.93±0.2d	5.38±0.31c	2.23±0.5d	19.50±3.15b	34.41±4.27c	38.85±4.83bc
	Zn+Cd	44.0±0.8bcd	9.47±0.7cd	6.69±0.54bc	2.97±0.2cd	23.92±0.66ab	41.39±1.84abc	50.83±1.23a
Zincol-2016	Control	44.6±1.1bc	10.5±0.3bc	6.92±0.53ab	4.10±0.3ab	28.07±1.30a	45.68±1.56a	44.25±1.54ab
	Zn	51.7±1.2a	13.1±0.4a	8.18±0.43a	4.68±0.2a	26.32±1.21a	37.17±1.68bc	31.61±2.20c
	Cd	46.4±1.5b	10.6±0.9bcd	6.06±0.55bc	3.08±0.2cd	22.86±0.81ab	41.68±1.78abc	39.77±1.92bc
	Zn+Cd	47.1±0.9 b	11.3±0.2b	6.33±0.50bc	4.17±0.3ab	26.87±1.13a	43.59±1.47ab	40.21±1.67bc

LSD values: plant height, total biomass, spike length, grain yield, harvest index, grain Zn (%) and grain Cd (%) as 4.23, 1.5, 4.23, 0.95, 5.22, 7.56 and 8.80 respectively

Results

Plant Growth and Yield

Plant growth and yield were affected significantly in both wheat cultivars in response to various treatments. Plant height was significantly ($P \leq 0.05$) increased by application of Zn in Zincol-2016 but not in Faisalabad-2008. Total biomass (shoot + root + grain) was significantly decreased under Cd contamination in both cultivars; however application of Zn under Cd contamination improved the total biomass. Zinc fertilization in non-contaminated soil increased the total biomass production in Zincol-2016 but not in Faisalabad-2008. Spike length was not affected by Zn or Cd treatment, however Faisalabad-2008 showed shorter spike length as compared to Zincol-2016 (Table 2). Significant ($P \leq 0.05$) decrease in grain yield was observed due to Cd contamination, while Zn application improved the grain yield more in Zincol-2016 as compared to Zn-inefficient Faisalabad-2008. Similarly harvest index was significantly decreased under Cd contamination as compare to control treatment; however application of Zn under Cd contamination improved it more in Zincol-2016 as compared to control cultivar (Table 2).

Zinc Concentration in Root, Shoot and Grain

Plant samples of root, shoot and grains were analysed separately to determine the Zn distribution in different plant parts. Increased in Zn concentration was about 12-21% in root, shoot and grain of Zincol-2016 as well as Faisalabad-2008 under Zn amended soil compared to control (Fig. 1A, B and C). However, Zn concentration in root, shoot and grain of Zincol-2016 were higher compared to Faisalabad-2008 under Zn treatment. In the presence of Cd, decrease in the Zn concentration in root, shoot and grain of Zincol-2016 and Faisalabad-2008 was observed (Fig. 1A, B and C). However, Zn application under Cd contaminated soil conditions significantly ($P \leq 0.05$) improved Zn concentration in root (~26 and 25%), shoot (~30 and 28%) and grain (~28 and 26%) of Zincol-2016 and Faisalabad-2008 respectively, compared to Cd contaminated soil.

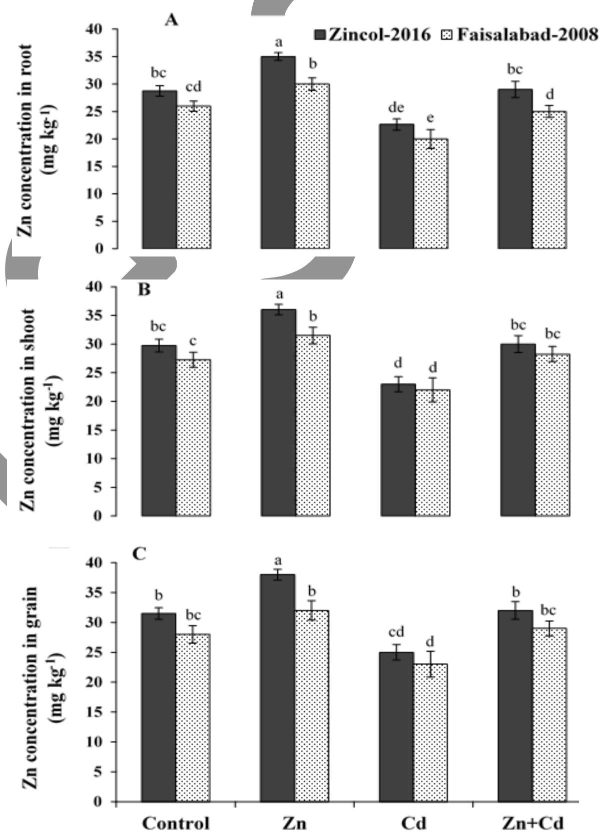

Fig. 1: Zn concentration in root (A), shoot (B) and grain (C) of wheat cultivars Zincol-2016 and Faisalabad-2008. Treatments are; control, Zn (10 mg kg^{-1} soil) application, Cd (10 mg kg^{-1} soil) amendment, and Zn plus Cd (10 mg kg^{-1} soil each) application together. Graph show the mean values and error bars indicate ± standard deviations; dissimilar letters above bars represent significant ($p \leq 0.05$) variation according to LSD (root, shoot and grain as 2.73, 3.85 and 3.86, respectively)

Cadmium Concentration in Root, Shoot and Grain

Cadmium concentration was higher ($P \leq 0.05$) in root, shoot and grain of both wheat cultivars, however its accmmulation in root was more in Zincol-2016 (~39 μg kg^{-1} dry matter), while was the highest in grains of Faisalabad-2008 (~49 μg kg^{-1} dry matter), whereas in shoot both cultivars showed similar concentration (~37 μg kg^{-1} dry matter) (Fig. 2A, B and C). Zincol-2016 exhibited significantly lower Cd concentration in wheat grain as compared to Faisalabad-2008 in Cd contaminated soils. Interesingly, application of Zn fertilizer to Cd contaminated soil sharply decreased Cd cncentration in root (~21 and 23%), shoot (~14 and 33%) and grain (~24 and 16%) of Zincol-2016 and Faisalabad-2008, respectively (Fig. 2A and B).

Discussion

Micronutrient malnutrition is a critical issue world-wide and Zn is among the most limiting micronutrients affecting a human health in various parts of world. Development of biofortified cultivars with high Zn concentration is the results of esteemed efforts of researchers (Andersson *et al.,* 2017).

Results of present study also revealed higher grain Zn accumulation in Zincol-2016 than Faisalabad-2008. Plant biomass production increased in both wheat cultivars due to Zn fertilization, however better growth of Zincol-2016 was observed in control where crop plants had to rely on native Zn concentration, which can be attributed to ability of this Zn efficient variety to acquire more Zn (Fig. 1A, B and C) from Zn deficient soils (Table 1). As many enzymatic reactions are directly related to Zn availability in the plants (Taiz and Zeiger, 2002), therefore sufficient Zn concentration is very critical for optimum crop growth. In Pakistani soils, it has already been reported that Zn applications significantly increased the plant biomass (Iqbal and Aslam, 1999), which may be due to favourable variations in root morphology and physiology (Lineman *et al.,* 1989). Furthermore, Zn fertilization significantly improves plant growth in non-contaminated calcareous soils due to lesser bioavailability of indigenous Zn content (Khattak *et al.,* 2015). Relatively better response of Zincol-2016 to Zn application may also be owing to its better Zn utilization capability.

Zincol-2016 showed higher Zn concentrations in the root (35 mg kg^{-1}), shoot (36 mg kg^{-1}) and grain (39 mg kg^{-1}) compared to Faisalabad-2008 showing more Zn accumulation in grains (Fig. 1). This suggests that Zincol-2016 has better Zn translocation mechanisms to grains which are very crucial trait for biofortified cereals. Zinc and Cd distribution index shows similar Zn partitioning percentage in both cultivars, nevertheless Cd distribution in grain is different in both cultivars. In Faisalabad-2008 Zn application enhanced the Cd partitioning in grain, while in Zincol-2016 the story was reverse. Moreover, application of Zn also decreased the Cd portioning in grain (Table 2). Not only in grain, Zn concentration in root and shoot was significantly improved with Zn application in both wheat cultivars due to better availability of Zn for plant (Joy *et al.,* 2015). Higher Zn concentrations in shoots is useful as animal fodder and a source of Zn for better animal health (Fig. 1B).

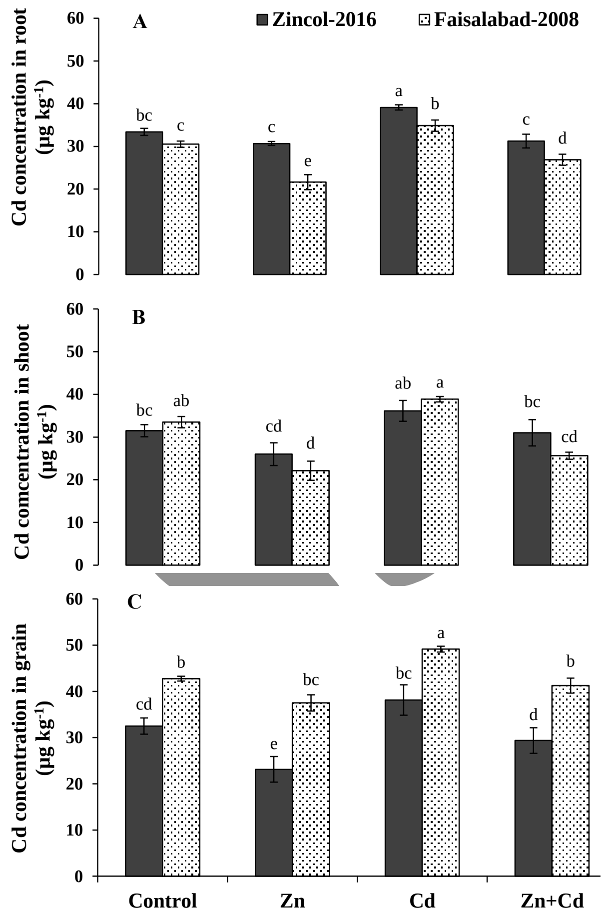

Fig. 2: Cd concentration in shoot (A), spike (B) and grain (C) of wheat cultivars Zincol-2016 and Faisalabad-2008. Treatments are; control, Zn (10 mg kg^{-1} soil) application, Cd (10 mg kg^{-1} soil) amendment, and Zn plus Cd (10 mg kg^{-1} soil each) application together. Graph show the mean values and error bars indicate ± standard deviations; dissimilar letters above bars represent significant ($p \leq 0.05$) variation according to LSD (root, shoot and grain as 3.32, 4.88 and 4.45 respectively)

Such rise in Zn concentration in wheat shoot by Zn fertilization and its beneficial effect on animal health has already been reported (Keram *et al.,* 2013). Hence, Zincol-2016 has a potential to be used as Zn biofortified cultivar for both human and animals.

In both cultivars, the growth and plant biomass were decreased when grown with Cd, while these reductions were cancelled when grown with Zn and Cd both. Reduction in plant growth and biomass production is usually deliberated as preliminary indication of Cd toxicity on Cd contaminated soil (Chen *et al.,* 2017). Cd toxicity symptom may appear at plant vegetative or reproductive growth phases. However, numerous plants may accumulate substantial concentration of Cd without displaying remarkable visual toxicity symptoms and yield loss, which can lead to Cd entry into food chain. Interestingly, Zn application significantly reduced the Cd toxicity and concentration in both cultivars, although more in Zincol-2016, might be due to the suppressive influence of Zn for Cd uptake (Dalir *et al.*, 2017; Venkatachalam *et al.*, 2017) as plant take up both Zn and Cd using same transport membrane proteins (Moustakas *et al.*, 2011).

Zincol-2016 seems more capable to reduce Cd concentration in grain in response to Zn fertilization, which was not expected as per hypothesis made for this study. Nevertheless, Zincol-2016 had more Cd concentration in roots, while similar in shoot (Fig. 2A and B). As above mentioned, it is considered that uptake and transport of Zn and Cd by plants are similar (Grant *et al.*, 1998); however, Zincol-2016 showed different pattern of transportation between Zn and Cd from root to grain. Zincol-2016 may have selective mechanism to accumulate Cd in the roots and very less translocation into the grains during accumulation of Zn into the grains. This trait is beneficial to grow in Cd contaminated soils with the apprehension of Cd entry into the food chain. Therefore, our hypothesis that Zn efficient variety also take up Cd efficiently is partially true because Cd concentration only in root was higher in Zincol-2016, while lower in grains. Genetic variation exists in many crops for uptake and accumulation of Cd (Tavarez *et al.*, 2015) and Zn (Yilmaz *et al.*, 2017). Further genetic and molecular mechanisms of Zn and Cd selection should be elucidated to design high Zn accumulator without accumulation Cd in grain, and Zincol-2016 will be a useful material to analyse this.

Conclusion

Zincol-2016 is a Zn biofortified wheat cultivar, which accumulates preferably high Zn in grains and it can also be recommended in Cd contaminated soils, because it kept very low concentration of Cd in grains. Furthermore, Zn fertilization can decrease Cd uptake more efficiently in Zincol-2016 under Cd contaminated soil conditions.

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