INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–0924/2018/20–12–2817–2822
DOI: 10.17957/IJAB/15.0840
http://www.fspublishers.org

Full Length Article

Foliage Applied Zinc Ensures Better Growth, Yield and Grain Biofortification of Mungbean

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Abstract

This study was carried out to optimize the zinc (Zn) foliar application for improving mungbean (*Vigna radiata* (L.) Wilczek) grain yield and its biofortification. The plants were either sprayed with distilled water, or with Zn solution (0.50, 1.0 and 1.5%) using $ZnSO_4.7H_2O$ as source while no spray was also taken as control. Zn foliar application improved the seedling growth, morphological and yield parameters, grain yield and grain Zn concentration in mungbean. Among the various foliar treatments, Zn foliar application at 0.5% was the most effective in improving the root system (longer roots with more roots proliferation), seedling growth, chlorophyll contents, yield related parameters, and grain yield; while for grain Zn concentration foliar applied Zn at 1% was effective. Zn application at 0.5 and 1% increased the grain yield and grain Zn concentration by 86–38% and 78–156%, respectively than no application. Thus, Zn may be foliar applied at 0.5–1% for harvesting good grain yield and Zn-enriched grains of mungbean. © 2018 Friends Science Publishers

Keywords: Zinc; Seedling growth; Mungbean; Lateral roots; Zinc grain biofortification

Introduction

Among all the food crops, pulses are important because cheaper source of proteins. Pulses are also rich in vitamin B, C and contain 2–6% fats. One hundred gram of pulse seed contains ~100–200 mg of calcium (Ca). In Asia, mungbean (*Vigna radiata* L.) is a summer pulse crop and has high contents of digestible protein than other pulses (Tabasum *et al*., 2010). It is a short duration crop (Ahamed *et al*., 2011) and can be easily grown under water limited environments. The crop residues of mungbean are used to improve the soil fertility and may also be used as fodder for the animals (Asaduzzaman *et al*., 2008). It improves the fertility status of soil through fixing atmospheric nitrogen by 63–342 kg ha^{-1} (Kaisher *et al*., 2010).

The pulse crops are usually planted on less fertile soils without application of adequate amount of fertilizer, which results in substantial reduction in grain yield due to deficiencies of different macro- and micronutrients (Srinivasarao *et al*., 2003). This problem is more in arid and semi-arid regions because of calcareous feature of soils, less organic matter, repeated drought, high pH, presence of salts and unbalance fertilizer application (Malakouti, 2008). The micronutrients play important role in the plant growth and development and in plants act as cofactor in different enzymes and take part in many redox reactions. Plants require very little amount of micronutrients for adequate growth and production (Nasiri *et al*., 2010).

Thus, it is important to apply the adequate amount of fertilizer (macro- and micronutrients) during growth period to increase the quality and yield of field crops (Sawan *et al*., 2001) including the pulses. All the micronutrients *viz*., zinc (Zn), selenium (Se), boron (B) and iron (Fe) play very crucial role in development, growth and also improve the immune system of the humans (Shenkin, 2006). Among the micronutrients, Zn is essential micronutrient necessary for the plants in low quantities. Globally, Zn is considered as crop growth and yield restrictive micronutrient because it modulates several metabolic processes including cell elongation, biosynthesis of proteins, nitrogen metabolism and photosynthesis (Cakmak, 2000; Ozturk *et al*., 2006; Potarzycki and Grzebisz, 2009; Sattar *et al*., 2017).

The uptake of nutrients by plants can be improved by appropriate application technique. Among different application methods, foliar application of micronutrients is an effective method to enhance the crop productivity (George and Schmitt, 2002). Grain yield of crop is enhanced when Zn is applied through soil but Zn concentration in grains is improved by its foliar application. Kassab (2005) demonstrated that foliar application of Zn, Mn, Fe and Mg

To cite this paper: Haider, M.U., M. Farooq, A. Nawaz and M. Hussain, 2018. Foliage applied zinc ensures better growth, yield and grain biofortification of mungbean. *Int. J. Agric. Biol*., 20: 2817–2822

considerably improved the growth and yield parameters of mungbean crop. Foliar application of Zn at flowering and grain filling period dominantly enhanced the number of pods per plant, pods weight per plant, number of seeds per pod, total pod and seed weight, 100 seed weight and biological yield as contrasted with control treatment (El-Habbasha and Magda, 2013). In another study, photosynthesis, early plant growth, nitrogen fixation, seed protein contents and total yield were improved by foliar application of Zn (Ved *et al.*, 2002).

Humans utilizing food products raised on Zn deficient soils may undergo Zn deficiency and related health issues involving stunting growth of children's, susceptibility to diseases, enhanced mortality, poor birth outcome, brain functioning and immune system (Hotz and Brown, 2004; Cakmak, 2008; Black *et al.*, 2008). The objective of Zn biofortification of grains is to enhance Zn concentration and its bioavailability in food, which is most feasible, sustainable, and economical tactic to overcome Zn shortage in the human diet (Zhao and McGrath, 2009; Salunke *et al.*, 2011; Rehman *et al.*, 2014).

Several studies have been conducted to evaluate the influence of foliage application of Zn in wheat (*Triticum aestivum* L.), chickpea (*Cicer arietinum* L.) and rice (*Oryza sativa* L.) etc., (Hoffland *et al.*, 2006; Bozoglu *et al.*, 2007; Yuan *et al.*, 2013). Improvement in growth and yield related traits of mungbean subjected to foliar application of Zn is also reported (Thalooth *et al*., 2006; Henselová and Slováková, 2010; Malik *et al*., 2015); however in all these studies Zn was applied along with other nutrients; *e.g*., with potassium and magnesium (Thalooth *et al*., 2006), molybdenum and urea (Malik *et al*., 2015) and with plant growth regulator (Henselová and Slováková, 2010). Nonetheless in all these earlier studies, effect of Zn foliar application on root system and grain Zn biofortification of mungbean is missing altogether. This study was, therefore, carried out to optimize the foliar application treatments for their effect on root and plant growth, productivity and grain biofortification of mungbean.

Materials and Methods

Site and Soil

This study was conducted in the wire house, Agronomic Experimental Area, Bahauddin Zakariya University, Multan, Pakistan (71.43° E, 30.2° N and altitude 122 meter above sea level) during the kharif season, 2016. The climate of the site is semi-arid, subtropical. The experimental soil was sandy loam having pH 8.0, electrical conductivity 2.28 dS m^{-1}, organic matter 0.45%, phosphorus 5.2 mg kg^{-1} and potassium 161 mg kg^{-1}.

Experimental Details and Crop Husbandry

Seed of mungbean cultivar NM-2006, used in the experiment, was obtained from Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan. The plants were either sprayed with distilled water or with Zn solution of 0.5, 1.0 and 1.5%, while no spray was also taken as control. Zinc was applied to foliage at 35 and 50 days after sowing (DAS) with the help of a manual sprayer. $ZnSO_4.7H_2O$ was used as source of Zn.

The pots (24 cm × 30 cm) were filled with 10 kg soil and were irrigated before mungbean sowing to attain the favorable moisture conditions, necessary for seed sowing. After reaching the moisture level at workable condition, the pots were manually cultivated with hand using a wooden stick. Seeds were sown in these pots on June 19, 2016. The experiment was laid out in completely randomized design with three replicates. Initially 10 seeds were sown in each pot, and after uniform emergence, six plants were maintained, in each pot, by thinning. Fertilizers were applied at the rate of 30 and 80 kg ha^{-1} of nitrogen and phosphorus, respectively using urea and triple super phosphate as source. The pots were irrigated regularly to avoid any moisture stress. The weeds germinated in pots were pulled out manually. The crop was harvested on September 28, 2016.

Observations and Measurements

Allometric, morphological and yield parameters: Randomly selected plants from each pot were harvested at 40, 55 and 70 DAS (days after sowing) to record the root length, number of lateral roots, leaf dry weight, shoot dry weight, root dry weight and leaf area per plant. Leaf area per plant was manually measured with the help of measuring scale. Final plant height was also measured with the measuring scale at harvest for all the plants in a pot and was averaged. The chlorophyll contents were assessed using the SPAD-502 chlorophyll meter at 40 DAS. Total numbers of vegetative and reproductive branches were counted at harvest from every plant and then averaged. The pod length of 15 pods was measured with the help of measuring scale and averaged. Pods on each plant in a pot were counted to find out the number of pods per plant. At maturity, the plants from each pot were removed and sun-dried for four days. After drying, each plant was weighed with the help of electric balance (BH-ST; Shangai Hochoice, apparatus manufacturing Co., Ltd.) to measure the biological yield. The pods were threshed manually to compute the grain yield per plant. Fifteen pods were threshed manually and number of seed was counted to estimate the number of seeds per pod. Five sub-samples of 100 seeds were taken from seed lot of each pot and weighed on an electric balance (BH-ST; Shangai Hochoice, apparatus manufacturing Co., Ltd.) to record the 100-grain weight. After calculating all the parameters, grain yield was reported at 10% moisture content. The harvest index was calculated as the ratio of grain yield to the biological yield and was expressed in percentage. To determine Zn concentration, grain samples were oven dried, ground, digested in an acid mixture ($HClO_4$+HNO_3; 3:10 ratio) on a digestion plate (Prasad, 2006), and analysed on an atomic absorption spectrophotometer (Perkin Elmer, CA, USA).

Statistical Analysis

Data were analyzed statistically with the help of Fisher's analysis of variance technique. Mean of treatments alongside diversity were compared by using the least significant test (LSD) at 1% probability (Steel *et al*., 1997). For graphical demonstration of data, the Microsoft Excel software along with ± S.E. was used.

Results

This study indicated that Zn foliar application significantly improved the root length, number of lateral roots, root dry weight, shoot dry weight, leaf dry weight and leaf area per plant. All the growth parameters (root and shoot) were improved initiating from 40 to 70 days after sowing due to Zn foliar application (Fig. 1 and 2). Among all the treatments, Zn foliar application at 0.5% was the best for improvement in root and shoot traits including leaf dry weight and leaf area per plant at each sampling date (Fig. 1 and 2).

The chlorophyll contents, morphological traits (plant height, number of vegetative and reproductive branches, pod length), yield parameters (pods per plants, seeds per pod, 100-seed weight), biological yield, grain yield, harvest index and grain Zn concentration was also significantly affected by foliar application of Zn in mungbean (Table 1 and 2). The highest plant height, pod length, number of pods per plant, 100-grain weight, grain yield per plant, biological yield per plant and harvest index were recorded with Zn foliar application at 0.5%. Foliar Zn at 0.5 and 1% was equally effective for improvement in plant height and the chlorophyll contents (Table 1). Foliar application of Zn at either concentration was equally effective for improvement in number of reproductive branches per plant and number of seeds per pod (Table 1 and 2).

The grain Zn concentration was the highest with 1% foliar application of Zn (Table 2). The percent increase in grain yield was 86.11, 37.85 and 35.33%, respectively by foliar applied Zn at 0.5, 1 and 1.5% concentration, respectively than control. The grain Zn concentration was increased by 103.67 and 141.14%, respectively by foliar application of Zn at 0.5 and 1% while it was decreased by -57.53% by foliar application of Zn at 1.5% concentration than control (Table 2).

Discussion

This study showed that mungbean grown without Zn had poor seedling growth, grain yield and grain Zn concentration. However, the mungbean plants receiving Zn as foliar spray at different levels attained the better seedling growth, grain yield and the grain Zn concentration (Table 1 and 2).

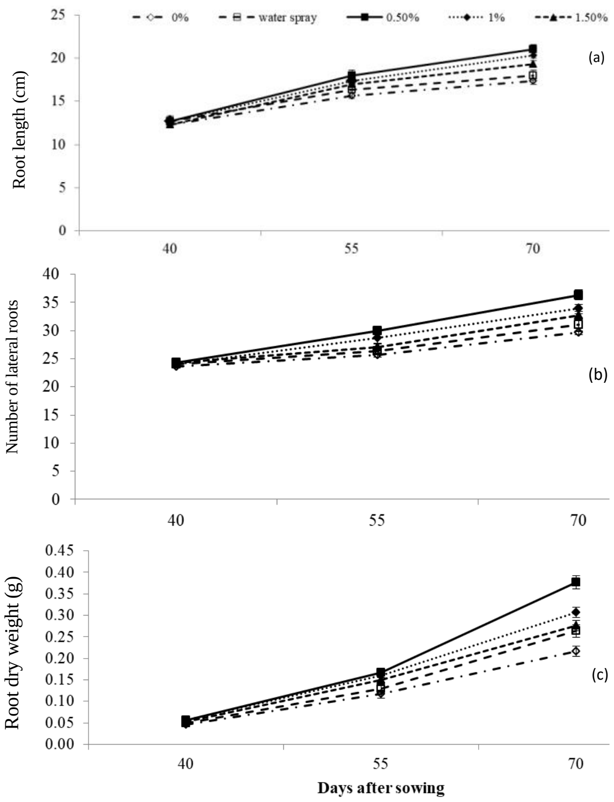

Fig. 1: Influence of foliar applied Zn on the (a) root length, (b) number of lateral roots per plant and (c) root dry weight (± S.E.) of mungbean

Indeed, Zn is one of the micronutrients required for various biochemical processes in plants like photosynthesis, respiration and chlorophyll biosynthesis (Nishizawa, 2005) and also needed for a wide range of metabolic activities including protein, lipid, carbohydrate and nucleic acid synthesis (Auld, 2001). Foliar application of Zn improved grain yield of mungbean due to improvement in number of grains per pod and grain size as was observed in this study (Malik *et al*., 2015). The application of Zn improved the root growth and shoot dry weight (Prasad *et al*., 2012) as was observed in this study as well (Fig. 1 and 2).

Zinc is necessary for the production of tryptophan, an amino acid, and the precursor of the hormone indoleacetic acid (IAA), which works as growth promoter in plants (Taiz and Zeiger, 2010). Moreover, Zn could have a positive influence on photosynthetic activity of leaves (Welch, 1995), which may enhance the shoot growth of plants, as observed in this study. This study indicated that the chlorophyll contents were improved with foliar applied Zn (Table 1). In another study, Potarzycki and Grzebisz (2009) found that Zn takes part in various plant growth cascades like photosynthesis, and synthesis of the green pigments thus enhancing the chlorophyll contents as observed in this

Table 1: Effect of zinc foliar application on plant height, chlorophyll contents, number of vegetative and reproductive branches per plant, pod length and number of pods per plant of mungbean

Zinc foliar application levels (%)	Plant height (cm)	Chlorophyll contents (SPAD value)	Number of vegetative branches per plant	Number of reproductive branches per plant	Pod length (cm)	Number of pods per plant
No application	47 E	44.3 D	5.33 C	5.00 B	7.14 E	14.7 B
Water spray	49 D	44.6 C	5.66 BC	5.66 AB	7.31 D	15.0 B
0.5	58 A	45.1 A	7.33 A	6.66 A	7.72 A	17.0 A
1.0	55 B	45.0 AB	6.66 AB	6.33 A	7.56 B	15.7 B
1.5	53 C	44.9 B	6.00 BC	6.00 AB	7.42 C	15.3 B
LSD at 1%	0.93	0.18	1.00	1.00	0.06	1.17

Means not sharing the same letters within a column differ significantly from each other at 1% level of probability

Table 2: Effect of zinc foliar application on yield related traits and grain Zn concentration of mungbean

Zinc foliar application levels (%)	Number of seeds per pod	100-seed weight (g)	Seed yield per plant (g)	Biological yield per plant (g)	Harvest index (%)	Grain Zn concentration (mg kg^{-1})
No application	6.66 B	6.02 D	3.17 E	8.79 E	36.1 CD	29.9 D
Water spray	7.00 AB	6.08 C	4.07 D	10.59 D	38.4 B	48.6 C
0.5	8.00 A	6.25 A	5.90 A	13.92 A	42.4 A	60.9 B
1.0	7.66 AB	6.16 B	4.37 B	12.33 B	35.5 D	72.1 A
1.5	7.33 AB	6.13 B	4.29 C	11.70 C	36.7 C	12.7 E
LSD at 1%	1.00	0.04	0.07	0.15	0.70	1.44

Means not sharing the same letters within a column differ significantly from each other at 1% level of probability

study. Aravind and Prasad (2003) also reported the role of Zn in chlorophyll and carotenoids synthesis in plants. The improvement in root growth due to Zn foliar application was attributed to the activation of numerous metabolic enzymes in the roots due to Zn application (Shojaei and Makarian, 2015). Broadley *et al.* (2007) concluded that plants require Zn as catalytic and structural component of enzymes and proteins for normal growth and development.

Improvement of root system *i.e.*, long roots coupled with more roots proliferation (Fig. 1) with Zn foliar application possibly enhanced the extraction of water and nutrients from the soil, which ultimately resulted in improved leaf and shoot growth in mungbean (Fig. 2). Better root system enabled the plants to explore more soil volume to extract more water and nutrients (Chassot and Richner, 2002; Luo *et al.*, 2010; Khan *et al.*, 2012). Zn application improved root growth due to its probable role in growth cascades like cell elongation, protein synthesis, carbohydrate metabolism (Cakmak, 2000; Palmer and Guerinot, 2009; Rehman *et al.*, 2018) and increase in indole acetic acid level in roots (sprouts) (Pandey *et al.*, 2010). In another study, Thalooth *et al.* (2006) found that the shoot length, number of branches, dry weight of shoot and leaves and leaf area per plant in mungbean crop was considerably enhanced with the application of Zn sulphate as foliar compared with the control plants.

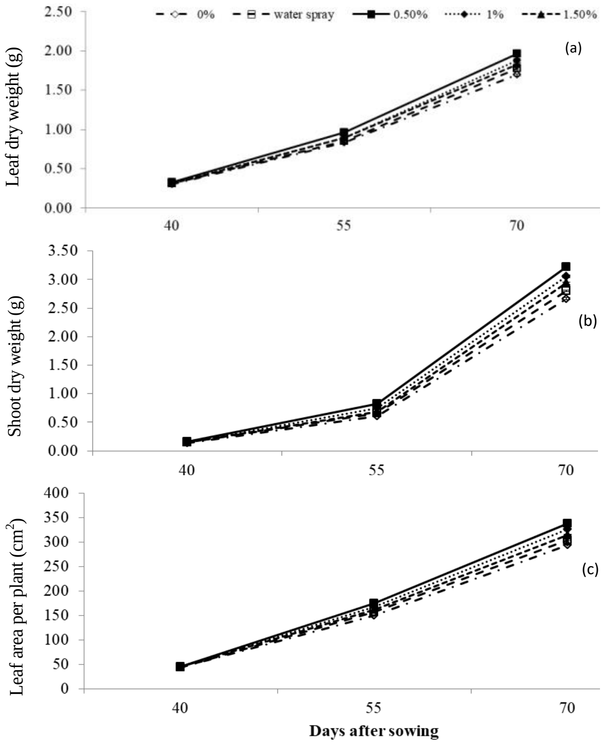

Fig. 2: Influence of foliar applied Zn on (a) leaf dry weight, (b) shoot dry weight and (c) leaf area per plant (± S.E.) of mungbean

This study indicated that the maximum grain yield was recorded with the application of 0.5% Zn as foliar spray. Improved grain yield in this treatment was the outcome of more number of seeds per pod and improvement in seed weight. Seemingly, Zn application improved the root system (Fig. 1), leaf area (Fig. 2) and chlorophyll contents (Table 1) which might have resulted in better grain partitioning thus enhancing seed weight and seed number, and finally the grain yield (Henselová and Slováková, 2010; Malik *et al.*, 2015; Table 1 and 2).

Zn application at higher level (*i.e.*, 1.5%) proved toxic, which hampered the mungbean growth and yield and reduced grain Zn concentration. Reduction in growth and grain Zn concentration might be due to Zn toxicity. Several reports highlighted that excess of Zn above the required level inhibited growth and development of plants and behaved heavy metals as lead and cadmium (Ali *et al*., 1999, 2000). In another study, Zn application at 300 mg L^{-1} reduced dry weight as well as roots and shoot growth of wheat (Glińska *et al*., 2016). Rout and Das (2009) concluded that in root tip cells, chromatin material was condensed, cytoplasm became structure less, breakdown of cell organelles and vacuole was also observed due to excess of Zn. Zn toxicity also exerts disturbed nutrient balance and oxidative stress in plants (Wang *et al*., 2009). Indeed, the margin of deficiency, sufficiency and toxicity of the micronutrients including Zn is very low which should be critically considered when optimizing their use for crop production.

Conclusion

Zn foliar application at 0.5–1% improved the seedling growth, root system, grain yield and grain Zn concentration than control. However, Zn foliar application at 0.5% was the most effective for improvement in yield parameters and grain yield. The grain Zn concentration was higher when Zn was foliar applied at 1%. Thus, Zn foliar application at 0.5–1% might be a pragmatic option to improve the grain yield and grain Zn concentration in mungbean.

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(Received 02 July 2018; Accepted 08 August 2018)