



Optimizing zinc seed coating treatments for improving growth, productivity and grain biofortification of mungbean

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Abstract

Zinc (Zn) is a crucial micronutrient required by plants and human beings. In this study, it was hypothesized that Zn seed coating may improve the stand establishment, growth, grain Zn biofortification and seed yield of mungbean (*Vigna radiata* (L.) Wilczek). The experiment consisted of four seed coating treatments viz. 0.50, 1.0, 1.5 and 2 g of Zn kg⁻¹ seed; non-coated seeds being taken as control. The findings of this study disclosed that Zn seed coating was beneficial for enhancing the seedling growth, morphological and yield parameters, grain yield and grain Zn concentrations of mungbean. Among the Zn coating treatments, 2 g Zn kg⁻¹ was the most superior for stand establishment, seedling growth, morphological/yield parameters, grain yield and grain Zn biofortification. Zn seed coating increased the seed yield of mungbean by 6.8-40.6% and grain Zn concentration by 12.0-34.4%, respectively, than the non-coated seeds. Thus, the mungbean seeds should be coated with 2 g Zn kg⁻¹ to achieve better stand establishment, seedling growth, morphological and yield parameters, seed yield and grain Zn biofortification.

Keywords: Zinc, seed coating, mungbean, malnutrition, biofortification

Introduction

Pulses are consumed by billions of people across the globe. Indeed, the protein available through pulses is easily accessible by the poor people (Usman *et al.*, 2007). Among all pulses, mungbean (*Vigna radiata* L.) Wilczek) is an ancient and valuable pulse crop due to its nutritional worth (Shanmugasundaram, 2004). It is a short period crop which can be grown in spring as well as summer season in Pakistan. It is a marvelous crop having excellent quality protein which is easily digestible. Mungbean crop may have protein contents of 18 to 25%. Besides its nutritional value, mungbean is also beneficial for improvement of soil fertility due to its natural ability to fix the atmospheric nitrogen (Ashraf *et al.*, 2003).

However, the average yield of mungbean in Pakistan is lower than most of the countries of the world. The reasons for this low yield include the poor stand establishment, biotic and abiotic stresses and little use of micronutrients at farmer field. Among these micronutrients, the Zn deficiency is regarded as an important factor for decreasing the mungbean yield. Indeed, Zn is necessary for protein synthesis (Cakmak, 2008), DNA synthesis, cell division

(Nadergoli *et al.*, 2011), proper functioning of pollen, fertilization, germination and chlorophyll production (Cakmak, 2008). Thus, the Zn deficiency may hamper the growth of legumes including mungbean.

In plants, Zn can be applied through various methods (Zafar *et al.*, 2016; Haider *et al.*, 2018a, b; Ullah *et al.*, 2019). For instance, Zn foliage application enhanced the growth, seed yield and grain Zn concentration in mungbean (Haider *et al.*, 2018a). The soil applied micronutrients are pragmatic solution for reducing the micronutrient deficiencies in crop plants like mungbean (Haider *et al.*, 2018b). However, both soil and foliar application have some limitation. For example, soil application required high quantities of micronutrients and the uptake of the micronutrients may be affected due to soil properties (Koca, 2016). The environmental conditions may affect the efficacy of foliar applied micronutrients (Fageria *et al.*, 2009) thus decreasing the wide scale adoption of foliar application at farmers' field.

In this scenario, seed treatments viz. seed coating, seed pelleting, might be a useful option for micronutrient delivery to seed in order to improve the performance of

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crops (Farooq *et al.*, 2012). Among the seed treatments, seed coating is most beneficial than other seed treatments owing to very low use of micronutrient and direct delivery of the required micronutrient on seed surface. Many studies have reported that seed coating of micronutrients improved the yield of field crops (Wiatrak, 2013; Korishettar *et al.*, 2016; Ullah *et al.*, 2019).

Besides plants, in developing world, the micronutrient deficiencies are exacerbating in humans where most of the people are suffering from poverty (Bhandari and Banjara, 2015). They are unable to purchase the micronutrient fortified food. According to World Health Organization, ~2 billion peoples in the world (especially developing countries) are suffering from minerals and vitamin deficiencies. As mungbean is staple of many people in world, improving Zn concentration in seeds might be a pragmatic option to improve the micronutrient delivery to humans through grains, which will ultimately reduce the malnutrition. Thus, it was hypothesized that Zn seed coating may improve the stand establishment, seedling growth, morphological and yield parameters, grain yield and grain Zn concentration in mungbean.

Materials and Methods

Site and soil

This study was conducted at Bahauddin Zakariya University, Multan, Pakistan during the summer season of 2016. The climatic conditions of the study site are semi-arid, subtropical. The experimental soil was sandy, having pH of 8.1, electrical conductivity of 2.39 dS m⁻¹, organic matter of 0.47%, phosphorus of 5.4 mg kg⁻¹, potassium of 160 mg kg⁻¹ and Zn of 0.61 mg kg⁻¹.

Experimental details

Seeds of mungbean cultivar NM-2006 were obtained from NIAB, Faisalabad, Pakistan. The experimental treatments comprised of four levels of Zn seed coating viz. 0.50, 1, 1.5 and 2 g Zn kg⁻¹ seed using ZnSO₄·7H₂O as source of Zn while non-coated seeds being taken as control. For seed coating, arabic gum was used as sticky material to adhere the Zn on seeds. After coating, the seeds were dried under shade near to its original weight. The coated seeds were kept in refrigerator (5°C) in polythene bags, till sowing. The experiment was laid out following completely randomized design and each treatment was replicated four times.

Crop husbandry

Each pot (24 cm × 30 cm) was filled with 10 kg soil. Before mungbean sowing, the pots were irrigated to achieve the favorable moisture conditions for sowing of seeds.

When the moisture level of soil reached at workable level, the soil of pots was manually prepared with hand using a wooden stick. The coated and non-coated seeds were planted in pots on June 19, 2016. Firstly, ten seeds were manually sown in each pot, which were later on thinned to six plants per pot after uniform emergence. Pots were irrigated weekly to avoid any moisture stress. Pots were fertilized at the rate of 60 and 30 kg ha⁻¹ of phosphorus and nitrogen, respectively, using triple super phosphate and urea as source. The weeds in pots were pulled out manually. The crop was harvested on September 27, 2016.

Data recording

The pots were visited daily to record the emergence of seedlings each day. Mean emergence time and emergence index were calculated as detailed by Ellis and Roberts (1981) and Association of Official Seed Analysts (1983), respectively. The final emergence count was estimated as the total number of seedlings emerged to the total number of seeds sown in a pot and was expressed in percentage. Energy of emergence was taken as the percentage of emerged seeds from sowing to 4th day of emergence.

Plant sampling was done at 40, 55 and 70 days after sowing (DAS) of crop to record the root length, leaf area per plant, number of lateral roots, and root/stem/leaf dry weights. The final plant height was recorded at harvest with the help of measuring scale for all the plants in a pot and was averaged. The chlorophyll contents were estimated using the SPAD-502 chlorophyll meter at 40 DAS. Total numbers of vegetative and reproductive branches were calculated from every plant and then averaged. The pod length of 10 pods was measured with a measuring scale and was averaged. The numbers of pods per plant in a pot were counted to work 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 digital balance to measure the biological yield. The pods were threshed manually to compute the grain yield per plant. Ten pods were threshed manually, and the number of seeds was calculated to estimate the seed number per pod. Five sub-sample of 100 seeds were taken from seed lot of each pot and were weighed to record the 100-grain weight. The harvest index was calculated as the ratio of grain yield to the biological yield and was expressed in percentage.

Grain Zn concentrations

For determining grain Zn concentrations, the ground seed samples (0.5 g) were digested in a mixture of 70% HClO₄ (2:1 v/v) and HNO₃ (5 mL) in Pyrex digestion flasks for overnight. This was followed by heating this mixture at temperature of 150 °C on hot plate to the point



Table 1: Influence of zinc seed coating on early stand establishment of mungbean

Zinc seed coating level (g kg ⁻¹ of seed)	Energy of emergence (%)	Mean emergence time (days)	Emergence index	Final emergence percentage (%)
0.0	60 b	4.2	3.9 b	67 c
0.5	63 b	4.8	5.4 ab	77 bc
1.0	63 b	5.0	5.9 ab	83 ab
1.5	57 b	4.2	3.9 b	70 c
2.0	80 a	4.4	6.1 a	93 a
LSD ($p \leq 0.01$)	10	NS	2.1	10

Table 2: Influence of zinc seed coating on plant height, chlorophyll contents, number of vegetative and reproductive branches, pod length and number of pods per plant of mungbean

Zinc seed coating level (g kg ⁻¹ of seed)	Plant height (cm)	Chlorophyll contents (spade value)	Vegetative branches per plant	Reproductive branches per plant	Pod length (cm)	Pods per plant
0.0	37.7 e	38.4 e	4.0 d	3.0 c	7.1 d	5.0 d
0.5	41.0 d	39.5 d	4.7 cd	3.7 bc	7.4 c	6.0 d
1.0	43.7 c	41.6 c	5.3 bc	4.0 bc	7.9 b	8.0 c
1.5	48.3 b	44.6 b	6.0 b	4.7 ab	8.1 b	10.0 b
2.0	51.3 a	46.3 a	7.0 a	5.3 a	8.4 a	11.7 a
LSD ($p \leq 0.01$)	1.5	0.3	0.9	1.0	0.2	1.4

when the red fumes production was ceased. Then the temperature of hot plate was moved to 250 °C until the samples of mixture became the transparent substance. These digested samples were diluted to 25 mL with distilled water followed by filtration. Grain Zn contents (mg kg⁻¹) were determined using atomic absorption spectrophotometer (Perkin Elmer, CA, USA) following the procedure of Prasad *et al.* (2006).

Statistical analysis

Data were analyzed statistically with the assistance of Fisher's ANOVA method and treatments mean along with diversity were assessed by utilizing least significant test (LSD) at 1% probability level (Steel *et al.*, 1997). For graphical representation of data Microsoft Excel software along with \pm S.E. was used.

Results

Seed coating significantly affected the final emergence count, emergence index and energy of emergence; results being non-significant for mean emergence time (Table 1). The highest final emergence count, energy of emergence and emergence index were noted when the seeds were coated with 2 g Zn kg⁻¹ seed and that was followed by seed coating with 0.5 or 1 g Zn kg⁻¹ seed for emergence index and with 1 g Zn kg⁻¹ seed for final emergence count (Table 1). The mungbean grown through non-coated seeds had poor seedling growth and seed yield.

Periodic data (Figure 1a-f) indicated that root length, number of lateral roots, root/stem/leaf dry weight and leaf area per plant was gradually enhanced starting from 40 to 70 days after sowing. Among all the seed coating treatments, seed coating at 2 g Zn kg⁻¹ seed was most beneficial for improvement in root length, number of lateral roots, leaf area per plant, and root/stem/leaf dry weight at all sampling dates; while the plants grown in control pots performed poor in this regard (Figure 1a-f).

The chlorophyll contents, morphological traits (vegetative/reproductive branches, plant height, pod length), yield parameters (100-seed weight, pods per plant, seeds per pod), biological yield, grain yield and grain Zn concentrations were significantly affected by various Zn seed coating treatments (Tables 2 and 3). The highest number of vegetative and reproductive branches, plant height, pod length, chlorophyll contents, pods per plants, 100-grain weight, biological and grain yield were noted with Zn seed coating at 2 g Zn kg⁻¹ seed and that was statistically similar with 1.5 g Zn kg⁻¹ seed for the number of reproductive branches per plant (Tables 2 and 3). Different Zn seed coating treatments increased the yield of mungbean by 6.8-40.6%; Zn seed coating at 2 g Zn kg⁻¹ seed was the best treatment with 40.6% improvement in grain yield. Nonetheless, different Zn seed coating treatments enhanced the grain Zn concentration by 12.0-34.4%, respectively, than the non-coated seeds. However, the maximum improvement in grain Zn concentration (34.4%) was resulted by Zn seed coating at 2 g Zn kg⁻¹ seed (Table 3).



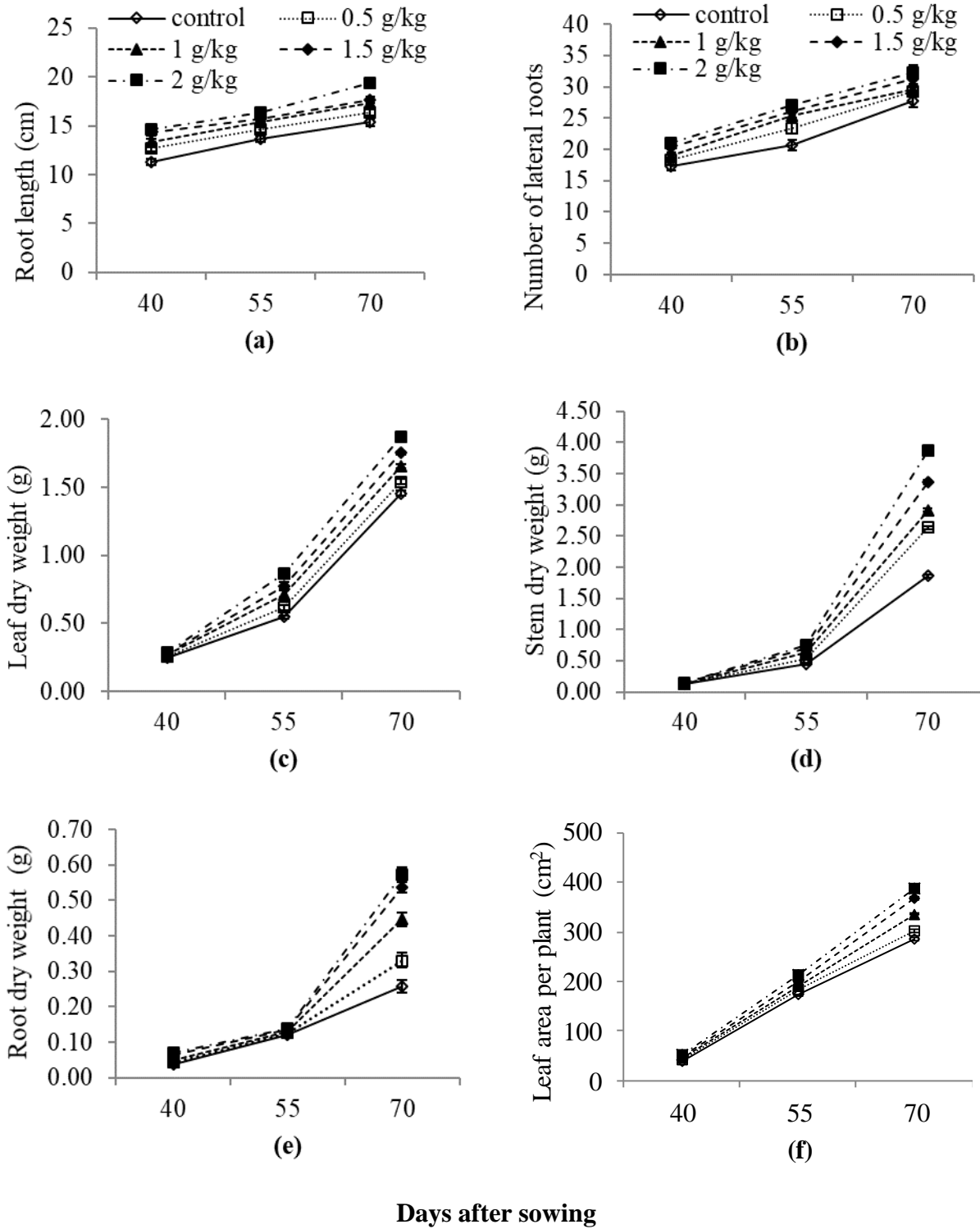


Figure 1: Influence of zinc seed coating on root length (a), number of lateral roots (b), leaf dry weight (c), stem dry weight (d), root dry weight (e) and leaf area per plant (f) (\pm SE) of mungbean



Table 3: Influence of zinc seed coating on yield parameters and grain Zn contents of mungbean

Zinc seed coating levels (g kg ⁻¹ of seed)	Number of seeds per pod	100-grain weight (g)	Grain yield per plant (g)	Biological yield per plant (g)	Grain Zn concentrations (mg kg ⁻¹)
0.0	6.72 e	5.55 e	3.08 e	6.77 e	35.33 c
0.5	7.18 d	5.62 d	3.29 d	7.28 d	41.33 b
1.0	7.64 c	5.68 c	3.67 c	8.18 c	39.58 b
1.5	8.19 b	5.83 b	3.95 b	10.01 b	40.25 b
2.0	8.70 a	6.11 a	4.33 a	11.19 a	47.50 a
LSD ($p \leq 0.01$)	0.45	0.05	0.16	0.31	1.92

Discussion

Application of Zn through seed coating significantly enhanced plant growth, grain yield and grain Zn concentrations of mungbean (Tables 1-3). The better stand establishment, seedling growth and grain yield in Zn coated seeds might be attributed to involvement of Zn in lipid/protein/carbohydrates metabolism, acid nucleic/tryptophan synthesis and its role in various plant growth cascades including germination, photosynthesis, germination and tissue turgor (Rout and Das, 2003; Tsonev and Lidon, 2012). In a recent study Zaman *et al.* (2018) and Ullah *et al.* (2019) reported higher rice (*Oryza sativa* L.) and chickpea (*Cicer arietinum* L.) productivity in consequence of Zn seed coating.

In this study, increase in chlorophyll contents was due to involvement of Zn in chlorophyll formation which ultimately enhanced the synthesis of chlorophyll and carotenoids which are finally involved in the plant photosynthetic mechanism (Aravind and Prasad, 2003). Better root growth and increase in number of lateral roots due to Zn seed coating in this study (Figure 1 a-f) might be attributed to the role of Zn in root cell elongation by reducing the free radical injury to cell (Cakmak, 2000). Early stand establishment and well-developed root system due to seed coating (Table 1; Figure 1) might improve nutrients uptake which resulted in more leaf area. Being units of plant assimilatory system, higher leaf area along with higher chlorophyll contents maybe resulted in more assimilates production which leads to significant improvement in yield related traits (Table 3). This improvement in grain yield due to Zn seed coating might be attributed to enhanced growth and improvement in yield related parameters (e.g. grain weight, grain number). Indeed, 100-seed weight, pods per plant and seeds per pod are important yield contributing traits in legumes and any improvement in these traits will improve the seed yield as was observed in this study. Masuthi *et al.* (2009) reported that Zn seed coating improved the grain weight of a legume crop. In an earlier study, Nadergoli *et al.* (2011) found that Zn application improved the number of seeds pod⁻¹ in green gram.

Yield improvement with Zn seed coating was possibly due to more Zn availability as Zn was available in the vicinity of emerging seedling which resulted in better stand establishment leading to improvement in yield contributing traits and ultimately grain yield (Table 3). Zinc application increases the chlorophyll synthesis, photosynthesis, which result in more photo assimilate formation and better yield (Rehman and Farooq, 2016; Rehman *et al.*, 2018). Moreover, substantial improvement in pod formation and grain weight contributed to higher grain yield. Zinc supply in adequate amount helps in better seed setting owing to better pollen germination and fertilization (Pandey *et al.*, 2006). Zinc seed coating also improved the grain Zn concentration (Table 3). The increased Zn accumulation in grains was possibly due to better root system (Figure 1), which might have helped in better soil exploration and uptake of Zn by mungbean roots. Moreover, presence of Zn in close vicinity of roots due to seed coating might have resulted in more Zn uptake (Rehman *et al.*, 2018).

Conclusion

Mungbean seed coating with Zn improved the stand establishment, seedling growth, grain yield and grain Zn content than non-coated seed. Thus, the mungbean seeds should be coated with 2 g Zn kg⁻¹ to achieve better stand establishment, grain yield and grain biofortification.

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