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Basal Application of Zinc to Improve Mung Bean Yield and Zinc-Grains-Biofortfication

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Abstract: Worldwide, the dietary deficiency of zinc (Zn) is prevailing in almost all arid and semi-arid regions. Zinc deficiency is not only the major constraint of lower yield, but also dietary Zn deficiency in cereals grains may cause increasing malnutrition and chronic health problems in human. Exogenous application of Zn through basal soil nutrition might be a useful option to recover Zn deficiency in mung bean. Therefore, field study was conducted to optimize the optimum level and method of Zn nutrition to enhance crop yield and Zn biofortification of mung bean through basal application. Zinc was applied at 0, 5, 10 and 15 kg/ha as basal application and side dressing, and in combination (50% basal application + 50% side dressing). The results highlighted that Zn nutrition prominently improved the mung bean yield as compared with control (no Zn applied). The maximum grains yield and Zn concentration in grains were obtained where Zn was applied at 15 kg/ha as basal application as compared with all other combinations. Better improvement in grain yield was due to significant increase in more number of pods and grain size owing to welldeveloped root system, improved leaf area index and high chlorophyll contents in mung beans leaves. Amongst all applied Zn nutrition's the basal application of Zn (15 kg/ha) was a viable option to get higher yield and Zn biofortification of mung bean.

Keywords: Mung bean; zinc; biofortification; basal application; soil dressing

1 Introduction

Micronutrients deficiency in arable soils is main issue for poor crop growth and yield. Recently, the degree and extent of nutrients deficiency in arable soils have serious consequences due to intensive agricultural practices, unwise use of mineral nutrition, breeding of high yielding and advanced varieties, and removal of huge quantities of nutrients at every crop harvest with lower nutrients returns to soils, thus contributing lower micro nutrients including zinc (Zn) in harvested grains [1,2].

Among the legumes, mung bean (*Vigna radiata* L.) is the main conventional pulses crop with relatively higher protein contents [3] and considered as "poor man's meat" due to three times more protein contents in comparison to cereals. Moreover, it has ~50% more carbohydrates contents along with substantial amount of phosphorus (367 mg/100 g of seed) and calcium (132 mg 100 g of seeds) as well [4]. Owing to its short duration period [5], it can be grown two times in a year i.e., Kharif (July to October) and summer (March to June) season. It flourishes better in wet environment, however can also be seeded



under water limited condition [6]. Therefore, mung bean is a better choice since its productivity will be achieved as seed return and the soil fertility status will be recovered by fertilization of mineral Zn nutrition, incorporating the crop residues which contain atmospherically fixed nitrogen [7].

Worldwide, the food security is the most important issue that can only be resolved by improving the crop productivity and yield. Zinc is utmost widespread deficient micronutrient [8] and nearly 50% soils are Zn deficient, and dietary Zn deficiency is a main cause for existence of Zn deficiency in human's beings. Zinc is very important micronutrient for healthy life [9]. The daily Zn dietary deficiency may cause acute health problems in humans including inhibition of children's growth, more vulnerability to diseases, reduced birth outcome, damage brain working, and immune system [10,11]. Zinc plays exclusively large key functions in human health and considered "metal of life" [12].

Photosynthetic activity of the plant is seriously affected due to deficiency of Zn by a change in chloroplast pigments [13]. Therefore, it is essential to supply sufficient quantity of mineral fertilization during growth period to improve the quality and yield of field crops [14] including the pulses. Zinc plays role in stabilization of ribosomal portions, auxin formation and establishment of dehydrogenase enzymes [15] and improves the crop productivity [16].

Biofortification of food crops by Zn either through fertilization or by breeding for greater uptake efficacy can be a feasible option to overcome emerging extensive dietary deficiency of Zn in human being [17]. Thus, mung bean cultivation with Zn-enriched grains might be a feasible strategy to address Zn malnutrition in human population. Various scientists worked to improve the Zn contents in a number of crops including rice (*Oryza sativa* L.) [18], wheat (*Triticum aestivum* L.) [19], and chickpea (*Cicer arietinum* L.) [20].

The uptake of nutrients by plants can be enhanced by adopting suitable application method (e.g., foliar application, basal addition and seed treatment) [21]. Biofortification is a way to mitigate the effect of malnutrition, improved the yield and quality of mung bean crop. There are various methods to enhance the Zn contents in mung bean through agronomic practices and plant breeding. Among these, soil application is the easiest and cheapest way to minimize the malnutrition problem in Pakistan. This technique was used to increase the Zn concentration in mung bean by increasing crop growth. The field study was undertaken to evaluate efficacy of Zn biofortification for improving performance and grain yield of mung bean through soil basal Zn addition.

2 Materials and Methods

2.1 Site Description

This field study was conducted at Agronomy Farm, Bahauddin Zakariya University (BZU), Multan, (71.43° E, 30.2° N and altitude 122 m) during spring 2017. The climate of the site is semi-arid. The experimental soil was silty clay loam in texture, having pH 8.2, electrical conductivity (EC) 3.42 dS/m, organic matter 0.83%, available phosphorus 6.30 mg/kg and potassium 127 mg/kg.

Months	Monthly mean temperature (°C)	Monthly mean relative humidity (%)	Total rainfall (mm)
March	21.75	68.35	0.00
April	30.00	53.50	5.30
May	34.00	63.05	0.10
June	33.10	74.90	45.60

 Table 1: Weather data during the growth cycle of crop

Source: Pakistan central cotton committee (PCCC) Multan

2.2 Experimental Details

Seeds of mung bean cultivar (Azri-2006) were obtained from NIAB (Nuclear Institute of Agriculture and Biology, Faisalabad). Zn was applied (0, 5, 10 and 15 kg/ha using ZnSO₄ (21% Zn) as source) as basal application, side dressing and in combination (50% basal application + 50% side dressing). The experiment was laid out according to randomized complete block design (RCBD) under factorial arrangement and each treatment was replicated thrice. For basal application, Zn was band placed at the time of sowing, while Zn was side dressed at 30 days after sowing following the 1st irrigation.

2.3 Crop Husbandry

A pre-soaking irrigation of 10 cm was applied to the field. When the soil reached a workable moisture level, seedbeds were prepared by cultivating the field two times with the help of tractor-mounted cultivator followed by planking. Sowing was done with hand drill in 30 cm spaced rows using seed rate of 20 kg/ha. After 1st irrigation thinning was done to maintain plant to plant distance of 10 cm. Weeds were controlled manually. Overall, three irrigations were applied i.e., 3 weeks after germination, at flowering and at pod formation stage to avoid moisture stress. The fertilizers were applied at the rate of N, P, K 20-60-0 kg/ha by using urea, di-ammonium phosphate (DAP), sulphate of potash (SOP) as source, respectively. Crop was harvested (after 80 days of sowing) when 90% pods were matured and threshed after drying in sunlight.

2.4 Parameters and Calculations

Randomly selected three plants from each plot were harvested at 30, 45 and 60 DAS (days after sowing) to record the leaf area index, number of roots and chlorophyll contents. Leaf area per plant was manually measured with the help of measuring scale. The chlorophyll contents were calculated using the SPAD-502 plus chlorophyll meter. Number of roots was counted manually. Final plant height was measured with the measuring scale at harvest for ten plants in a plot and was averaged. Number of monopodial and sympodial branches were counted at harvest from randomly selected ten plants of each plot and then averaged. The pod length of ten pods from every plot was measured with the help of scale and then valued were averaged. Number of pods on ten plants in each plot were counted, and then averaged. At maturity, the plants from each plot were removed and sun-dried for four days. After drying the plants were weighed to measure the biological yield. The pods were threshed manually to compute the grain yield per plot. Ten pods were threshed manually and number of grains was noted to estimate the number of grains per pod. Three samples of 1000 seeds were taken from every plot and weighed to record the 1000-grain weight. Grain yield was calculated at 10% plant moisture contents. The harvest index was calculated as the ratio of grain yield to the biological yield. The Zn content in mung beans was analyzed after acid digestion ((HClO₄ + HNO₃; 3:10 ratio) on digestion plate, and Zn concentration was determined on an atomic absorption spectrophotometer (Perkin Elmer, CA, USA).

2.5 Statistical Analysis

The collected data was checked statistically by Statistix 8.1 at 5% probability level by using Fisher's analysis of variance technique and least significant difference (LSD) was applied to compare the treatments' means by using the computer statistical program [22].

3 Results

The interactive effect of applied Zn levels and application methods had significant effect on entire yield related traits of mung bean. Growth related attributes such as leaf area index, chlorophyll contents, plant height, number of roots, grains yield, and biological yield were regularly increased with life cycle of crop; Zn application levels improved all these parameters as compared with control. Obtained data highlighted that Zn applied methods, doses and their interaction prominently influenced the total leaf area index (LAI) of mung bean after 15, 30 and 45 days interval (Fig. 1(A)).

The maximum LAI 1.49, 1.70, and 2.46 was observed where Zn 15 kg/ha was applied as compared to control after 15, 30 and 45 days interval, respectively (Fig. 1(A)). Regarding interaction the highest LAI was recorded when 15 kg/ha of Zn was applied with basal application method after 15, 30 and 45 days of intervals, whereas lowest LAI was observed under no Zn (control).

Statistical data regarding chlorophyll content expressed prominent behavior when mung bean plants were subjected to Zn exposure as compared to control. The maximum values of chlorophyll contents were 47, 50, and 54 with respect to basal application (BA), side dressing (SD), and basal + side dressing (BA + SD), respectively as compared to control when Zn 15 kg/ha was applied. However the minimum chlorophyll content was measured in control (Fig. 1(B)).

Roots of mung beans expressed significant behavior when subjected to Zn, and higher increase in number of roots (BA 15.33, SA 15.67, and BA + SA 18) were observed when Zn 15 kg/ha was applied as compared to the control (12.11) (Fig. 1(D)). Therefore, maximum plant roots (15.42) were observed when Zn was applied through basal application method, whereas minimum number were recorded under controlled soil (Fig. 1(D). The prominent impact of Zn nutrition on plant height was observed under various Zn applied methods and levels as compared to no applied Zn (Fig. 1(C)). The maximum plant height was observed under interactive effect (BA + SA) where 15 kg/ha was applied and shorter plants was recorded in controlled soil (Fig. 1 (C)).



Figure 1: Interactive effects of zinc doses (kg/ha) and application methods on (A) leaf area index (LAI) (B) chlorophyll contents, (C) plant height and (D) number of roots per plant of mung bean ± SE Error bars are the SD of the means (n = 3) and (p < 0.05)
Here BA = Basal application; SD = Soil dressing and BA + SD = 50% BA + 50% SD

Number of monopodial expresses the significant behavior under all Zn levels and methods, its application and their interaction. Amongst all applied Zn application methods, the more increment in monopodial numbers was observed under basal application of Zn and the greater number of monopodial (10.24) were recorded when 15 kg/ha of Zn was applied as compared to control (7.16) (Fig. 2(A)). Whereas, the minimum number of monopodial branches were observed under the side dressing (SD) methods as compared with control soil (Fig. 2(A)). Similarly, the number of sympodial branches were significantly increased under Zn 15 kg/ha with basal nutrition, while minimum number of sympodial branches of mung beans were noted under controlled soil (no Zn) (Fig. 2(B)).



Figure 2: Interactive effects of Zinc doses (kg/ha) and application methods on (A) Monopodial branches (B) Sympodial branches (C) Number of pods per plant and (D) Pod length of mung bean \pm SE Error bars are the SD of the means (n = 3) and (p < 0.05)

Here BA = Basal application; SD = Soil dressing and BA + SD = 50% BA + 50% SD

Different Zn levels, application methods and their interaction prominently effected number of pods per plant (Fig. 2(C)). The highest number of pods per plant (21.97) was noted under Zn 15 kg/ha, whereas the lower number was observed where no Zn was added (control). Moreover, basal application method resulted in the highest number of pods per plant as compared with other application methods.

The prominent impact of Zn nutrition on pods length of mung beans plants was observed and pods length continuously increased with increasing Zn nutrition under all application methods. Basal application of Zn at 15 kg/ha observed the highest pods length (8.27 cm) compared with other treatments (Fig. 2(D)).



Figure 3: Interactive effects of Zinc doses (kg/ha) and application methods on (A) Number of grains per pod (B) 1000-grain weight (C) Biological yield and (D) Grain yield of mung bean ± SE

Error bars are the SD of the means (n = 3) and (p < 0.05)

Here BA = Basal application; SD = Soil dressing and BA + SD = 50% BA + 50% SD

Numbers of grains per pod and grains weight was prominently increased with exposure of Zn nutrition under all applications methods (basal, side dressing and interactive of basal and side dressing) (Figs. 3(A)-3(B)). The significant increase in numbers of grains per pods and grains weight was measured under Zn basal addition, and this increment was boosted with raising Zn nutrition. The highest increases in numbers of grains per pods (11.33) and grains weight (52.27 g) was recorded when Zn BA 15 kg/ha was added to soil, as compared to their respective control 7.33 g and 44.53 g (Figs. 3(A)-3(B)). While, the lowest grains per pods and grains weight was observed where no Zn was applied as compared to all applied Zn basal, side-dressing, and basal + side-dressing application to mung beans.

Our results demonstrated that grains yield and biological yield and harvest index of mung beans were influenced with Zn application. Amongst all Zn application methods, the basal application of Zn nutrition attracts more attention and significantly increased harvest index (Fig. 4(A)), biological and grains yield (Figs. 3(C)-3(D)) of mung beans. Grains yield and harvest index of mung beans were increased with increasing Zn nutrition under all application methods while highest increased in grains yield (26%), biological yield (8%) and harvest index (19%) was observed where Zn 15 kg/ha was applied as compared to no Zn applied soil.

The Zn concentration in grains was increased with increasing Zn nutrition through all application methods (BA, SA, BA + SA). The highest Zn percentage 56% in grains was increased with basal application BA 15 kg/ha, as compared to control (Fig. 4(B)).



Figure 4: Interactive effects of Zinc doses (kg/ha) and application methods on (A) Harvest Index and (B) Grain Zn concentration of mung bean \pm SE

Error bars are the SD of the means (n = 3) and (p < 0.05)

Here BA = Basal application; SD = Soil dressing and BA + SD = 50% BA + 50% SD

Table 2: Statistical summary of growth, yield related traits and grain Zn of mung bean grown with different Zn doses and methods of application

Parameters	Zn application methods	Zn doses	Zn application methods × Zn doses
Leaf area index	212.48**	1029.71**	150.14**
Chlorophyll contents	32.66**	267.87**	2.77*
Plant height (cm)	7.91*	56.81**	1.22^{NS}
Number of roots	2.95 ^{NS}	23.97**	3.76*
Monopodial branches	10.58**	124.43**	4.45*
Sympodial branches	3.42*	77.01**	4.93*
Number of pods per plant	19.04**	77.64**	3.12*
Pod length (cm)	27.68**	110.97**	41.68**
Number of grains per pod	25.23**	148.04**	6.31**
1000-grains weight (g)	93.26**	1021.30**	28.69**
Biological yield (t/ha)	80.63**	248.02**	21.47**
Grain yield (t/ha)	117.59**	633.64**	11.05**
Harvest index (%)	43.69**	320.04**	5.21**
Grain Zn concentration (mg/kg)	305.84**	4835.25**	60.51**



4 Discussion

The results of this study disclosed that the positive effects of Zn nutrition on mung bean growth and Zn-biofortification in grains were observed. The enhanced mung bean yield and Zn biofortification in grains was possibly attained due to improved chlorophyll contents, leaf area index, plant height and better root system of mung bean. Results are in accordance with Usman et al. [23] who observed that plant

height of green gram was greatly increased with application of Zn at 15 kg/ha. Zn nutrition considerably improved the chlorophyll contents, enhanced uptake of the root absorption and ultimately increased the photosynthetic rate in mung bean, resultantly plant growth was increased. It is also responsible for extending the leaf area index of mung bean [24].

Dashadi et al. [25] stated that Zn play prominent role in the production of auxin that maximizes cell volume which improved the plant height. Better plant roots system responsible to uptake more available Zn to flourish plants more. Zn contributes more to activated enzymatic activity, and root cell elongation, and minimizing the free radical injury to the cell [26,27]. Thalooth et al. [28] found that the number of branches and leaf area per plant in mung bean crop were significantly improved with the application of ZnSO₄ as compared to control. Zinc involved in formation of stamens and pollens that may helpful for production of more sympodial branches [26].

Zn biofortification greatly influenced the number of pods per plant and pods length particularly at 15 kg/ha as basal application that improved the number of pods per plant and pods length. Nadergoli et al. [26] reported that the highest number of seeds per pod and pods bearing branches were recorded when ZnSO₄ 20 kg/ha was applied and that gave about equal beneficial results as compared where ZnSO₄ 15 and 25 kg/ha was applied in soil.

Zn nutrition also improved the number of grains/pods, and 1000-grain weight of mung bean when Zn was applied before the flowering stage. In a study, grains weight and number of grains per pods prominently increased in chickpea with exposure of Zn application [20].

Results disclosed that all Zn levels and application methods, especially 15 kg/ha of Zn as basal application improved the biological yield and grain yield in mung bean. Shahab et al. [29] stated that soil Zn application to maize crop resulted in greater biological yield. Khorgamy et al. [20] reported that Zn application affected the harvest index significantly as was observed in this study. Zn concentration in mung beans grain was also improved [30], these results are in line with our current results.

Rathi et al. [31] demonstrated that chickpea witnessed an increase in its seed weight as a result of optimized Zn application. Verma et al. [32] found that biological yield and weight of grains in mung bean crop increased by combined application of boron and zinc.

Zn nutrition through basal application improved the Zn biofortification to mung beans and increased the harvesting index, and these results are in agreement with the Valenciano et al. [33] who reported that among applied zinc, molybdenum and boron fertilization, the highest harvest index of chickpeas was observed under Zn nutrition.

5 Conclusion

Overall, mung bean positively responded to Zn nutrition. Among all applied application methods and Zn doses, the optimized basal application of Zn 15 kg/ha was found to be more effective, viable and applicable to enhanced mung bean growth and yield. Moreover, BA 15 kg/ha could be more effective to enhance Zn biofortification in mung bean grains by minimize Zn deficiency cereal in daily foods.

Conflicts of Interest: None of the authors have any conflict of interest.

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