Research Article | Open Access

Dev et al., 2023. 25 (1&2): 1-17. J. Environ. Agric. Sci. (ISSN: 2313-8629)

Zinc Biofortification of Mungbean (*Vigna radiata* L.) as Influenced by Varieties and Zinc Fertilization

Pushkar Dev [1], Ummed Singh [1,*], L. Netajit Singh [2], Y. S. Shivay [3], Manoj Kumar [4], P. R. Raiger [5]

Edited by:
Muhammad Imtiaz,
National Institute of Biotechnology and Genetic Engineering, Faisalabad, Pakistan

Reviewed by:

Hamid Nawaz,
Islamia University, Bahawalpur, Pakistan

Gurdeep Singh Malhi,
CCS Haryana Agricultural University, Rohtak, India

Received
January 2, 2023
Accepted
February 20, 2023
Published Online
March 10, 2023

Abstract: A field experiment was conducted during kharif season of 2019 under a factorial randomized block design replicated thrice in Zn-deficient loamy sand soil of arid region to study Zn biofortification of mungbean in four different varieties including “GM 4,” “GAM 5”, “GM 6”, and “IPM 02-3” under seven different Zn levels i.e., 0, 1, 2, 3, 4, 5, and 6 kg Zn ha^{-1}. Mungbean genotype ‘GM 4’ substantially fetched higher Zn concentration by grain (46.6 mg kg^{-1}) and stover (36.0 mg kg^{-1}) and Zn uptake by grain (52.1 g ha^{-1}) and stover (99.6 g ha^{-1}). Application of 6 kg ha^{-1} Zn resulted in significantly higher Zn concentration in the grain (39.6 mg kg^{-1}) and stover (29.2 mg kg^{-1}). Among the mungbean varieties, ‘GM 6’ fetched substantially higher ZUE (454.6 kg grain increased kg-1 Zn applied), IZUE (11.9 kg grain kg^{-1} Zn uptake), and PZUE (28.1 kg grain increment g^{-1} Zn uptake). Application of Zn to mungbean significantly influenced the Zn use indices. Increasing levels of Zn recorded decreasing ZUE (1035.8–187.9 kg grain increased kg^{-1} Zn applied), IZUE (9.89–9.15 kg grain kg^{-1} Zn uptake), AZUE (48.6–21.9 kg grain increased kg^{-1} Zn applied), and PZUE (37.4–14.1 kg grain increment g^{-1} Zn uptake). Efficient genotype selection and appropriate Zn application are potential approaches for Zn biofortification of mungbean under Zn-deficient soil conditions.

Keywords: Biofortification, mungbean, nutrient use efficiency indices, zinc concentration

***Corresponding author:** Ummed Singh, email: singhummed@yahoo.co.in

Cite this article as: Dev, P., U. Singh, L.N. Singh, Y.S. Shivay, M. Kumar and P.R. Raiger. 2023. **Zinc biofortification of mungbean (*Vigna radiata* L.) as influenced by varieties and zinc fertilization**. Journal of Environmental & Agricultural Sciences. 25 (1&2): 1-17.

Journal of Environmental & Agricultural Sciences (JEAS). Volume 24, Issue 3 & 4

1. Introduction

Globally, zinc (Zn) deficiencies in soils have been documented as a nutritional constraint (Hui et al., 2019; Khan et al., 2022) and are the most widespread deficiencies in crops including pulses (de Valença et al., 2017; Singh et al. 2016a). Moreover, Zn is one of the most important micronutrients essentially required and plays a vital role not only in plants nutrition but equivocally in animals and human nutrition (Bevis et al., 2023; Prasad 2012; Prasad et al. 2014). In plants, prolonged Zn deficiency reduces vegetative growth, sexual development, and lowered grain Zn concentration, shortening of internodes, epinasty, and reduction of leaf size (Natasha et al., 2022).

India has one of the highest rates of Zn deficiencies in soils and people. Presently, ~50 % of Indian soils are Zn deficient and will be rising to 63% by 2025, if management precautions not advocated (IZA, 2017). Deficiency of Zn mainly depends on nature of parent material, processes of soil formation, climatic

---

[1] Department of Agronomy, College of Agriculture, Agriculture University, Jodhpur-342304, Rajasthan, India
[2] Department of Agricultural Statistics, College of Agriculture, Agriculture University, Jodhpur-342304, Rajasthan, India
[3] Division of Agronomy, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India
[4] ICAR-All India Coordinated Research Project on Pearl Millet, ARS, Mandor, Jodhpur-342304, India
[5] Department of Soil Science and Agricultural Chemistry, College of Agriculture, Agriculture University, Jodhpur-342304, Rajasthan, India

conditions, and management practices. In Indian soils, Zn deficiency is widely spready, however, approximately half of the Indian soils are Zn deficient (Suganya et al., 2020).

Deficiency of Zn appears to be a limiting factor in several parts of the country including mungbean growing areas of India (Shukla et al. 2012). Henceforth, Zn fertilization is recommended in several crops and parts of the country (Rattan et al. 2008) mainly ascribed to two reasons (a) to enhance productivity and profitability and (b) to enrich Zn concentration in grains (Prasad et al. 2014) and thereby provide biofortified food to the consumers (Singh et al. 2016b). Recommendation of Zn for the first reason is outcome of response of plant in terms of enhanced grain yield after Zn fertilization (Prasad et al. 2013); whereas the second reason is prevailing disquiet of Zn deficiency in humans and animals leading to micronutrient malnutrition not only in India but also throughout the world (Ali et al. 2021; Singh et al. 2016c).

Response of Zn fertilization is also recognized from its widespread deficiency in Indian soils (Shukla and Behera 2012), heavy removal of soil Zn through crop harvest (Shukla et al. 2012) and providing resilience against abiotic stresses (Akram et al. 2022). Further, the problem of Zn deficiency is more aggravated in the arid and semi-arid regions, wherein the soils are not only thirsty but also these are hungry and sick.

On health front, there is a high degree of correlation between Zn deficiency in soils and that in human beings. Zn is an essential nutrient for human health (Sangeetha et al., 2022). Life on earth can rarely survive without Zn. Deficiency of Zn is the 5th leading cause of deaths, disorders and diseases in developing countries, however, it ranked 11th globally (Lagoriya et al., 2023). About one-third of the world's population suffers from Zn deficiency (Das and Green, 2016). Zn deficiency is causing 45% of child deaths. According to the World Health Organization (WHO), death of approximately 800,000 humans is linked with the Zn deficiency, including 450,000 children under the age of five (De Benoist et al., 2007; WHO, 2000).

To address micronutrient deficiencies comprehensively, several approaches are needed simultaneously (Bhatt et al., 2020; Gomes et al., 2023; Gupta et al., 2021). Despite past progress in mitigating micronutrient deficiencies through supplementation and food fortification, new approaches are required for expanded provision of food-based interventions (Ali et al., 2021). Biofortification, a promising approach that relies on agronomic interventions especially fertilization, along with conventional plant breeding and modern biotechnology to increase the micronutrient density of staple crops (Praharaj et al., 2021). Biofortification potentially can improve the nutritional status and health of poor populations in both rural and urban areas of the developing world (Kiran et al., 2022).

Pulses are major source of plant-based proteins, particularly for vegetarians. Pulses provide about 14% of the total protein of average Indian diet (Singh and Pratap, 2016). In India production of pulses (52 g day^{-1} per capita) is inadequate even to meet minimum consumption requirement i.e., 80 g day^{-1} per capita. Moreover, this availability has been continuously decreasing. (DAC, 2017). In India, currently, mungbean is cultivated in an area of 5.13 million hectares and contributes 3.08 million tonnes of production with average crop yield of 601 kg ha-1 in the country (DES, 2021). Mungbean plays an important role not only in enriching the human diet, but also in improving soil health (Nath et al. 2023).

Therefore, it important to search cultivars with higher Zn efficiency indices and accumulation in the edible parts (grains) and assess the suitable levels of Zn application to soil supplementation and their relation with Zn uptake, plant growth, grain yield and yield attributes.

Therefore, present study was designed with a group of promising mungbean varieties under graded levels of Zn to find out genotypic variations in terms of Zn concentration, Zn use efficiency indices, and identify efficient varieties for Zn concentrations. Results of the present study would contribute to provide suitable mungbean genotype and level for Zn enrichment in mungbean grains to mitigate Zn deficiency and hidden hunger.

2. Materials and Methods

2.1. Experimental site and treatment details

A field experiment was conducted during rainy season of 2019 at Instructional Farm, College of Agriculture, Jodhpur, India. Laboratory analysis was performed during winter season 2020. Geographically, experimental site was located between 26^{o} 15' N to 26o 45' North latitude and 73^{o} 00' E to 73^{o} 29' East longitude at an altitude of 231 meter above mean sea level. This region falls under agroclimatic zone IA (Arid Western Plains Zone) of Rajasthan, India.

Table 1. **Physico-chemical characteristics of soil of the experimental soil (0-30 cm)**

Particulars	Values	Method of analysis	Reference
Organic carbon (%)	0.13	Walkley and Black's method	Walkley and Black, 1934
Available N (kg ha^{-1})	174.0	Alkaline $KMnO_4$ method	Subbiah and Asija, 1956
Available P (kg ha^{-1})	22.2	Olsen's method	Olsen et al. 1954
Available K (kg ha^{-1})	325.0	Flame photometric Method	Jackson, 1973
Available S (mg kg^{-1})	10.6	$CaCl_2$-extratable S	Williams and Steinbergs, 1959
Available Zn (mg kg^{-1})	0.48	DTPA extractable Zn using Atomic Absorption Spectrophotometer	Lindsay and Norvell, 1978
Available Fe (mg kg^{-1})	3.21	DTPA extractable Fe using Atomic Absorption Spectrophotometer	Lindsay and Norvell, 1978
EC (dSm^{-1}) (1:2 soil water suspension at 25°C)	0.12	Method No. 4 USDA Handbook No.60	Richards, 1954
pH (1:2 soil water suspension)	7.8	Method No. 21 b, USDA Handbook No. 60	Richards, 1954

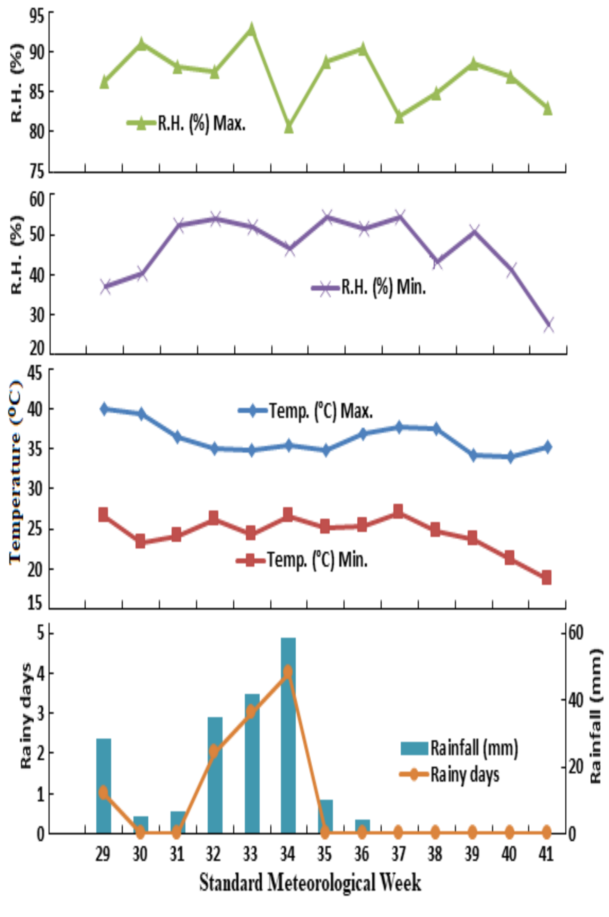

Fig. 1. Weather variables during rainy crop season (kharif), 2019.

The average annual rainfall and evaporation of the zone is 367 mm and 1843 mm, respectively (Sihag et al., 2020). The details of weather variables recorded during the crop season are presented in Fig. 1. Soil of the experimental field was loamy sand in texture, non-saline, slightly alkaline with a mechanical composition of 7.8% coarse sand, 68.4% fine sand, 10.7% silt and 13% clay. Experimental field had low organic carbon, low available nitrogen, low available phosphorus, high available potassium, low DTPA–extractable Zn and iron (Fe). Details of physico-chemical characteristics of the experimental soil along with methodologies executed are given in Table 1.

The experiment having twenty-eight treatment combinations and three replications with eighty-four plots in total was laid out in Factorial Randomized Block Design. The treatments were allocated randomly to different plots by using the random number table (Fisher and Yates, 1963). Treatments comprised of four varieties of mungbean (GM 4, GAM 5, GM 6 and IPM 02-3) and seven Zn levels (Control, 1, 2, 3, 4, 5 and 6 kg Zn ha^{-1}) were undertaken in the experimentation.

2.2. Crop Establishment and Management

In the well-prepared seedbeds, quality seeds of the mungbean varieties (GM 4, GAM 5, GM 6 and IPM 02-3) were sown in 3rd week of July using the seed rate of 15 kg ha^{-1}. Before sowing, the seeds were treated with thiram at 2 g kg^{-1} seed to prevent seed-borne diseases. The mungbean seeds were sown manually (using 'kera' method), wherein, one man drops the seeds behind the furrow. Seeds were placed 5-6 below the soil surface, and plant spacing was maintained (row × row 30 cm; plant × plant 10 cm). A basal dose of fertilizer (12 kg N ha^{-1}; 30 kg P_2O_5 ha^{-1}) was supplied to the crop using urea and diammonium phosphate (DAP), at the depth of 7 to 8 cm. In Zn treatment plots, in addition to N and P, Zn was manually applied in the furrows, as Zn oxide (80% Zn) considering treatments, as described earlier. Except treatment, all other recommended management practices were performed uniformly, and suitable protection measures were adopted to keep crop stress free. Hoeing was done (31 days after sowing, DAS) of crop, to remove weeds. Due to prevailing dry spell, a life-saving irrigation was given to the crop at the pod-development stage for achieving proper pod development.

2.3. Chemical Analysis of Plant Samples

Plant samples (grain, stover, root, leaf, stem and pod wall) were collected from the respective plots and grounded using mortar and pestle. For determination of micronutrients (Zn and Fe), finely ground plant material (0.5g) was transferred in 100 ml volumetric flask and added 10 ml of diacid acid mixture (nine parts of HNO3 and four parts of $HClO_4$) for digestion. On completion of digestion, 30 ml of deionized water was added and filtered it through Whatman No. 1 filter paper into a 100 ml volumetric flask. After 3-4 washings of 10-15 ml portions of distilled water the volume was made to 100 ml. A blank digestion was also carried out following all steps, excluding the plant material to avoid any impurity in the reagents. Mineral concentrations of the filtrates (Zn and Fe) were measured using Atomic Absorption Spectrophotometer for zinc (213.7 nm) and iron (248.7 nm) (Singh and Praharaj, 2017). Concentrations of Fe and Zn in the sample was calculated using formulae:

Zn/Fe in plant sample = AAS reading (µg/ml) × Dilution factor.

Dilution factor= [(Final volume of digested sample (ml) ÷ Weight of plant tissue (g)].

Here, final volume was made 100 ml and plant sample weight was 0.5 g.

Zn/Fe concentration in the plant sample = Concentration read from the AAS × total dilution factor =Zn/Fe conc. (mg kg^{-1}).

Total nitrogen content in plant materials (grain and stover) was estimated through Kjeldahl method following digestion, distillation, and titration. Finely ground grain (0.2 g) and stover (0.5 g) samples were taken in digestion tube and added10 ml of concentrated H_2SO_4 for better digestion, 3g catalyst mixture (K_2SO_4+$CuSO_4$; 5:1 ratio) was also added prior to H_2SO_4. The digestion tubes were placed on digestion block and set the digestion system to attain a temperature of about 410 °C and then attach the digestion tube to the heating unit as per the instructions given in the operation manual.

Subsequently, content was allowed the digestions continuously till completion of aliquot (no black or brown colour). Water was added (10 ml distilled water) in the digestion tubes before distillation. Distillation was done through Automatic Nitrogen Analyser (Kelplus Classic-DX VA) using 4% boric acid and 40% NaOH. After completion of distillation, the boric acid containing mixed indicator was titrated against 0.1 N HCl. Blank was also run to the same end point as that of sample (Singh and Praharaj, 2017).

2.4. Zn use efficiency indices

In crop production system, nutrient use efficiency is a critically important, Zn use efficiency indices were measured by following the formulae (Yoshida, 1981; Singh and Ahlawat 2007; Fixen et al. 2015).

2.4.1. Zinc Use Efficiency (ZUE)

Zn use efficiency (ZUE) was calculated in terms of grain yield kg^{-1} of Zn fertilizer applied (Eq 1).

$$ZUE = \frac{GY}{Zn\ Applied} \qquad [1]$$

where, grain yield kg ha^{-1}); Zn Applied, amount of Zn applied, kg ha^{-1}.

2.4.2. Agronomic Zinc Use Efficiency (AZUE)

Agronomic Zn use efficiency (AZUE) was calculated in terms of yield increased per unit of nutrient applied (Eq 2).

$$AZUE = \frac{GY(Tzn) - GY(ck)}{Zn\ Applied} \qquad [2]$$

Where AZUE, agronomic Zn use efficiency; GY(Tzn), grain yield of Zn fertilizer treated plot (kg ha^{-1}); GY(ck) grain yield of CK (without Zn application) plot (kg ha^{-1}); Zn Applied, amount of Zn applied, (kg ha^{-1}). AZUE) indicates yield increased per unit of nutrient applied.

2.4.3. Internal Zinc Utilization Efficiency (IZUE)

Internal Zn utilization efficiency of Zn (IZUE) was calculated as kg grain kg^{-1} Zn uptake (Eq 3)

$$IZUE = \frac{GY}{TUzn} \qquad [3]$$

IZUE, internal Zn use efficiency; GY, grain yield (kg ha^{-1}); TUzn, total Zn uptake (kg ha^{-1}); IZUE) indicates kg grain kg^{-1} Zn uptake.

2.4.4. Physiological Zinc Use Efficiency (PZUE)

Physiological Zn use efficiency (PZUE) was measured as the grain yield obtained per unit of Zn adsorbed. It was computed according to Eq 4.

$$PZUE = \frac{GY(Tzn) - GY(ck)}{Uzn\,(Tzn) - Uzn(ck)} \qquad [4]$$

Where PZUE, physiological Zn use efficiency; GY, grain yield (kg ha^{-1}); TUzn, total Zn uptake (kg ha^{-1}); IZUE) indicates kg grain kg^{-1} Zn uptake.

2.4.5. Apparent Zinc Recovery Efficiency (AZRE)

Apparent Zn recovery efficiency (AZRE) measured as the quantity of Zn absorbed per unit of Zn applied and is expressed as per cent.

$$AZRE = \frac{\mathrm{Yt} - \mathrm{Yo}}{\mathrm{Znt}} \times 100 \qquad [5]$$

Where Yt, Uptake of Zn in particular treatment (kg ha^{-1}); Yo, Uptake of Zn in unfertilized plot (kg ha^{-1}); Znt = Quantity of Zn applied for the treatment (kg ha^{-1})

2.4.6. Zinc Harvest Index (ZHI)

Zn harvest index: Zn harvest index (ZHI) is the ratio between nutrient uptake in grain and nutrient uptake in grain plus straw or shoot and is expressed as per cent.

$$ZHI\ (\%) = \frac{UGZn}{UGZn + USZn} \times 100 \qquad [6]$$

Where ZHI, Zn harvest index (%); UGZn, Zn uptake by grain (g ha^{-1}); USZn, Zn uptake by stover (g ha^{-1}).

2.5. Statistical analysis

Analyses of variance (ANOVA) of experimental data, correlation and regression analysis were performed using linear model procedures of the Statistical Analysis System 9.2 (SAS Institute, Cary, NC) for RBD (Gomez and Gomez, 1984). Means of two treatments pairs were compared by using the least significant test (LSD) at 0.05 level of significance (Steel et al. 1997). Principal component analysis for different variables was performed by XLSTAT version 2020.3.

3. Results

3.1. Crop Nutrient Content

An overview of Zn content in different parts (root, stem, leaf, and pod wall) of the mungbean plant at different growth stages [25 days after sowing (DAS), 52 DAS, and at harvest or maturity] varied substantially (Table 2). Mungbean varieties differed substantially in terms of Zn content in roots, stem and leave at 25 DAS, 50 DAS and harvest stages. At early growth stages (25 DAS), Zn content in roots (28.7 to 39.8 mg kg^{-1}) and stem (17.7 to 41.8 mg kg^{-1}) parts were observed highest compared with later growth stages at 50 DAS and harvest or maturity stages.

Table 2. Effect of varieties and zinc levels on zinc content (mg kg^{-1}) in different plant parts of mungbean

Mungbean Varieties	**Zn in Roots**			**Zn in Stem**			**Zn in Leaf**			**Pod wall**
	25 DAS	**50 DAS**	**At harvest**	**25 DAS**	**50 DAS**	**At harvest**	**25 DAS**	**50 DAS**	**At harvest**	**At harvest**
GM 4	39.9	13.1	23.8	38.0	26.8	11.2	49.2	57.4	26.9	21.7
GAM 5	32.2	19.7	17.5	41.8	14.7	36.97	46.4	53.2	35.3	22.4
GM 6	28.7	15.0	23.2	17.7	23.3	21.9	49.2	53.9	32.9	19.1
IPM 02-3	34.9	16.3	34.3	36.6	23.3	34.5	44.9	51.4	38.4	18.2
SEM±	0.27	0.17	0.21	0.23	0.23	0.28	0.33	0.62	0.33	0.25
CD (P=0.05)	0.76	0.49	0.60	0.66	0.65	0.78	0.92	1.77	0.94	0.71
Zinc levels (kg ha^{-1})										
Zn_0	33.0	15.6	23.6	32.3	21.2	25.3	45.9	52.3	32.0	19.8
Zn_1	33.0	15.6	23.9	32.4	21.5	25.4	46.0	52.3	32.2	19.9
Zn_2	33.1	15.7	24.1	32.6	21.6	25.5	46.1	52.3	32.8	20.1
Zn_3	33.4	15.9	24.3	33.0	21.9	25.8	46.8	53.5	33.1	20.2
Zn_4	34.0	16.2	24.8	33.5	22.0	26.2	47.6	54.7	33.4	20.5
Zn_5	35.0	16.6	25.5	34.9	22.8	27.0	48.9	55.8	34.6	20.9
Zn_6	36.0	16.9	26.5	35.9	23.2	27.7	50.7	56.9	35.5	21.2
SEm±	0.36	0.23	0.28	0.31	0.30	0.37	0.43	0.83	0.44	0.33
CD (P=0.05)	1.01	0.64	0.80	0.87	0.85	1.04	1.22	2.34	1.25	0.94
Zn × Cultivar Interaction										
SEM±	0.71	0.45	0.56	0.61	0.60	0.73	0.86	1.65	0.88	0.66
CD (P=0.05)	NS	NS	NS	1.74	NS	NS	NS	4.68	2.50	NS

*Significant at 5% level of significance. Zn_0 (control, without Zn); Zn_1: 1 kg Zn ha^{-1}; Zn_2: 2 kg Zn ha^{-1}; Zn_3: 3 kg Zn ha^{-1}; Zn_4: 4 kg Zn ha^{-1}; Zn_5: 5 kg Zn ha^{-1}; Zn_6: 6 kg Zn ha^{-1}.

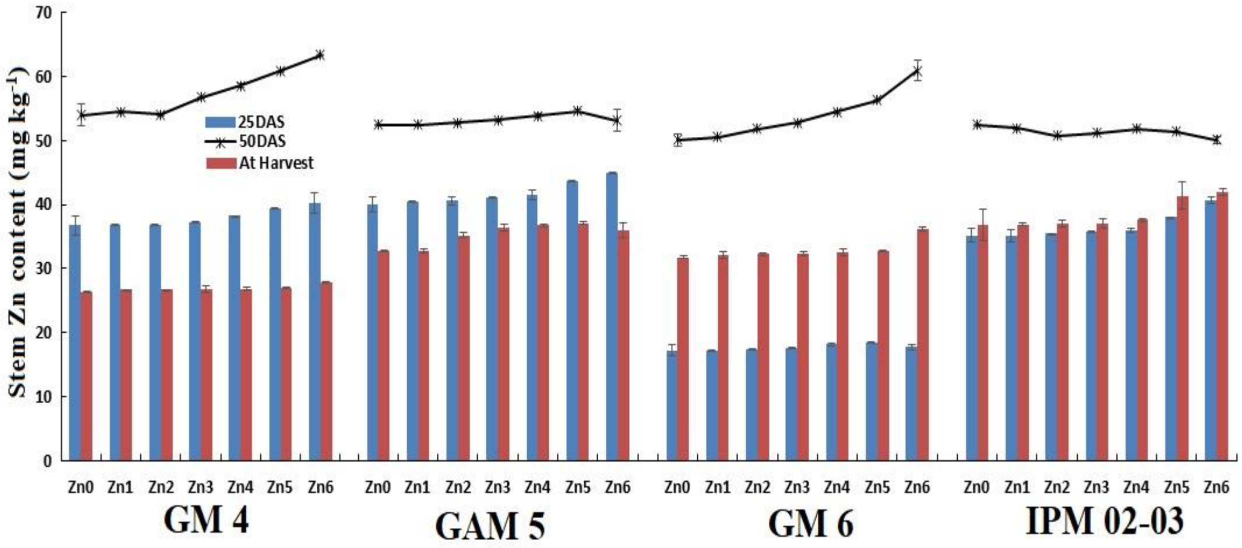

Fig. 2. Interaction effects of varieties and zinc level on zinc Stem Zn content (mg kg^{-1}) in mungbean stem at 25DAS, leaves at 50DAS and at harvest. Bar show standard error of mean with LSD value 1.72 (25 DAS), 4.64 (50 DAS) and 2.50 (At harvest) at P=0.05 to determine the significant differences among the treatment mean. Zn_0 (control, without Zn); Zn_1: 1 kg Zn ha^{-1}; Zn_2: 2 kg Zn ha^{-1}; Zn_3: 3 kg Zn ha^{-1}; Zn_4: 4 kg Zn ha^{-1}; Zn_5: 5 kg Zn ha^{-1}; Zn_6: 6 kg Zn ha^{-1}.

However, the trend was different in case of mungbean leaves, wherein, highest Zn content in leaves was observed at 50 DAS (51.4 to 57.4 mg kg^{-1}) compared with early growth stage (25 DAS) and later stage at maturity (at harvest). Further, it has also been observed that the genotype 'GM 4' had recorded the highest content of Zn in roots and leaves at early growth stage (25 DAS). Albeit the trend was not similar at later stages of the growth (50 DAS and at maturity). Among mungbean varieties, the highest Zn content in pod wall (22.4 mg kg^{-1}) was observed with the mungbean variety 'GAM 5'.

Zn content analysis done in different plant parts (roots, stem, leaves and pod wall) at different growth stages (25 DAS, 50 DAS and at maturity) of mungbean crop provided somewhat contrasting results among treatments. Maximum Zn content in roots, stem and leaves was observed with the application of highest level of Zn i.e., 6 kg Zn ha^{-1} at all the growth stages (25, 50 DAS and at maturity) and remained significantly superior over control or no application of Zn. Among different plant parts, higher Zn content in roots and stem was noticed at early stage (25 DAS) followed by harvest stage and 50 DAS. However, in case of leaves, the trend of Zn content was in the order: 50 DAS>25 DAS>at harvest. Furthermore, application of 6 kg Zn ha^{-1} substantially improved Zn content in all the tested plant parts viz: roots, stem, and leaves at all the growth stages (25, 50 DAS and at harvest) over control or no application of Zn. Likewise, application of 6 kg Zn ha^{-1} also enhanced Zn content in pod wall (21.2 mg kg^{-1}).

Statistical analysis of data visualizes interaction effect of mungbean varieties and Zn levels significantly on Zn content in mungbean stem at 25 DAS, leaves at 50 DAS and at harvest stage and crude protein content in grain (Fig. 2). Application of 6 kg Zn ha^{-1}to mungbean genotype 'GAM 5' substantially improved Zn content in stem at 25 DAS (45.0mg kg^{-1}). However, highest Zn content in mungbean leaves at 50 DAS was observed in 'GAM 5' mungbean with the same level of Zn application i.e., 6 kg Zn ha^{-1}. Further, at harvest stage, application of 5 kg Zn ha^{-1} to mungbean genotype 'IPM 02-3' recorded the highest Zn content (42.0 mg kg^{-1}).

Mungbean varieties and zinc levels considerably influenced Zn, Fe, and nitrogen content in grain and stover of mungbean and crude protein content in grain (Table 3). Among varieties, the highest Zn content in grain (46.6 mg kg^{-1}) and stover (36.0 mg kg^{-1}) was recorded with 'GM 4' mungbean variety which was significantly superior over the rest of varieties. Whereas the highest Fe content in grain (80.7 mg kg^{-1}) and stover (392.1 mg kg^{-1}) was recorded with 'IPM 02-3' genotype.

Table 3. Effect of varieties and zinc levels on zinc, iron, nitrogen, and protein content in mungbean

Treatments	Zn content (mg kg^{-1})		Fe content (mg kg^{-1})		N content (%)		Crude protein content (%)
	Grain	Stover	Grain	Stover	Grain	Stover	Grain
Mungbean Varieties							
GM 4	46.6	36.0	73.2	175.0	3.59	2.44	22.5
GAM 5	37.7	25.2	56.5	341.4	4.05	2.40	25.3
GM 6	30.5	23.2	75.5	234.9	3.47	2.61	21.7
IPM 02-3	33.5	26.1	80.7	392.1	3.73	2.04	23.3
SEm±	0.24	0.30	0.90	2.62	0.04	0.04	0.27
CD (P=0.05)	0.69	0.85	2.54	7.43	0.12	0.10	0.75
Zinc levels (kg ha^{-1})							
Zn$_0$	35.8	26.8	74.6	294.9	3.54	2.28	22.2
Zn$_1$	35.9	26.9	74.3	293.7	3.57	2.29	22.3
Zn$_2$	36.0	27.0	73.7	291.0	3.62	2.32	22.6
Zn$_3$	36.5	27.2	72.2	287.5	3.67	2.35	22.9
Zn$_4$	37.3	27.7	69.7	282.7	3.77	2.40	23.6
Zn$_5$	38.5	28.5	68.8	277.4	3.88	2.46	24.2
Zn$_6$	39.6	29.2	67.0	273.6	3.93	2.52	24.6
SEm±	0.32	0.40	1.19	3.46	0.06	0.05	0.35
CD (P=0.05)	0.92	1.13	3.36	9.82	0.16	0.13	1.00
Zn × Cultivar Interaction							
SEm±	0.65	0.80	2.37	6.93	0.11	0.09	0.70
CD (P=0.05)	1.83	NS	NS	NS	0.32	NS	1.99

*Significant at 5% level of significance. Zn$_0$ (control, without Zn); Zn$_1$: 1 kg Zn ha^{-1}; Zn$_2$: 2 kg Zn ha^{-1}; Zn$_3$: 3 kg Zn ha^{-1}; Zn$_4$: 4 kg Zn ha^{-1}; Zn$_5$: 5 kg Zn ha^{-1}; Zn$_6$: 6 kg Zn ha^{-1}.

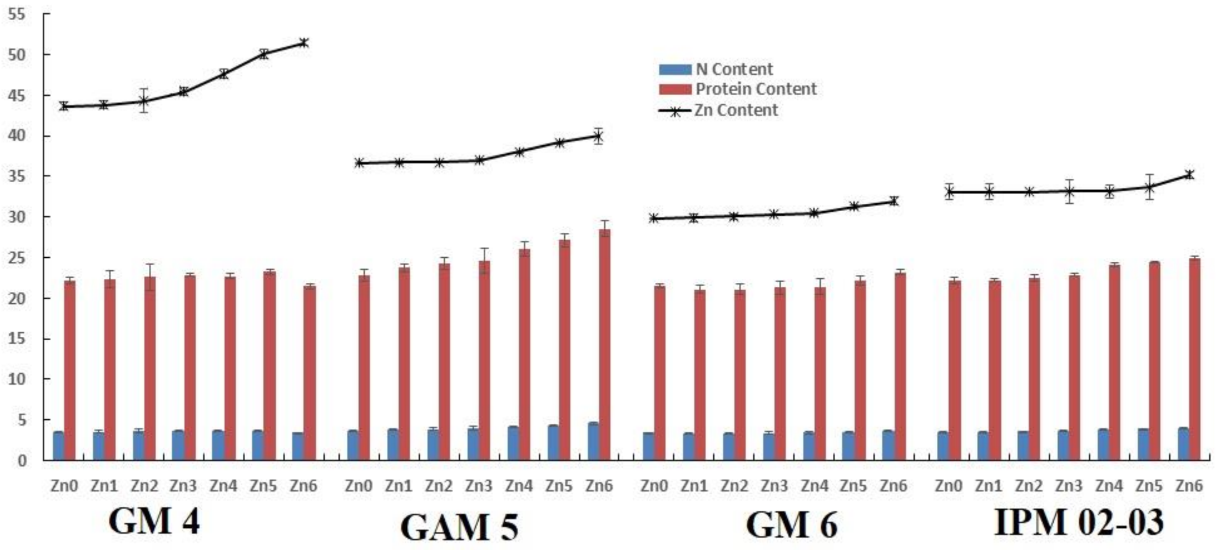

Fig. 3. Interaction effects of varieties and zinc level on zinc content (mg kg^{-1}), protein content (%) and nitrogen content in grain of mungbean. Bar show standard error of mean with LSD value 1.99 (protein content), 2.49 (Zn content) and 0.32 (N content) at P=0.05 to determine the significance differences among the treatment mean. Zn$_0$ (control, without Zn); Zn$_1$: 1 kg Zn ha^{-1}; Zn$_2$: 2 kg Zn ha^{-1}; Zn$_3$: 3 kg Zn ha^{-1}; Zn$_4$: 4 kg Zn ha^{-1}; Zn$_5$: 5 kg Zn ha^{-1}; Zn$_6$: 6 kg Zn ha^{-1}.

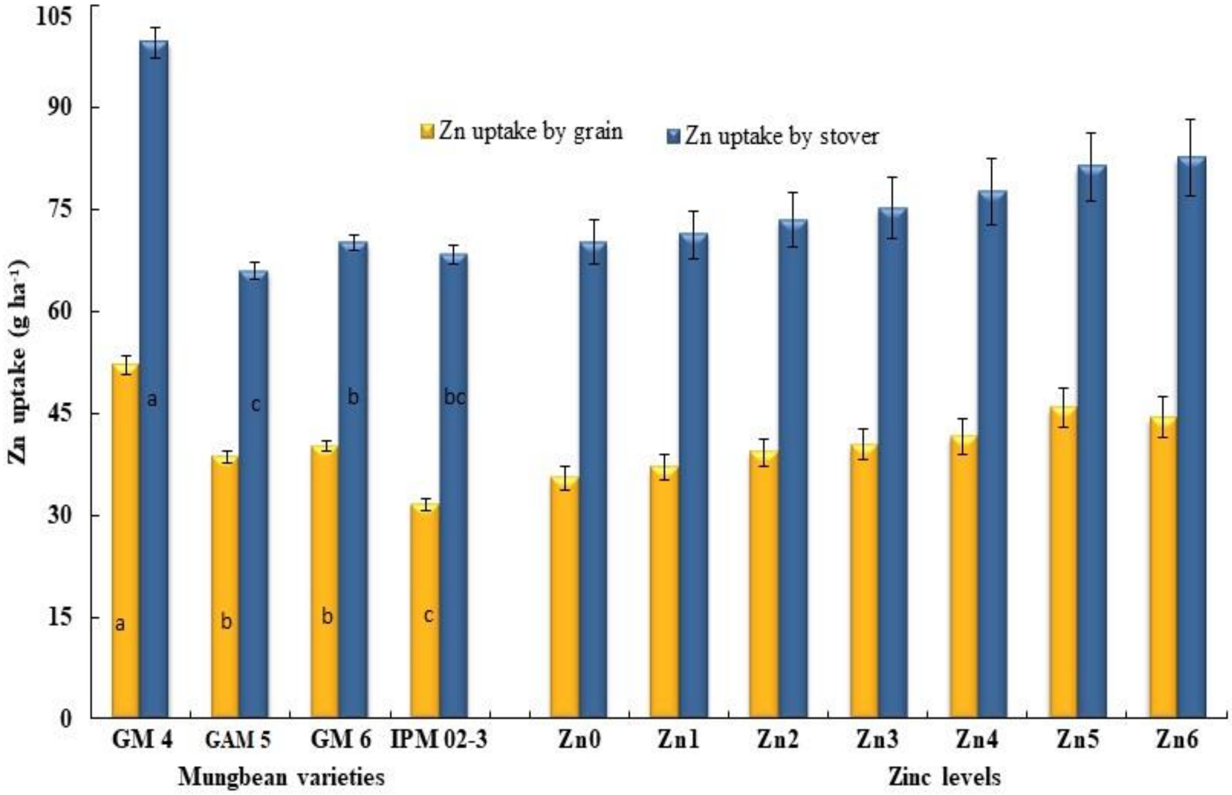

Fig. 4. Effect of varieties and zinc levels on zinc uptake by grain and stover (g ha^{-1}) of mungbean. Bars show LSD values at P = 0.05 to determine the significance differences among treatment means. Zn_0 (control, without Zn); Zn_1: 1 kg Zn ha^{-1}; Zn_2: 2 kg Zn ha^{-1}; Zn_3: 3 kg Zn ha^{-1}; Zn_4: 4 kg Zn ha^{-1}; Zn_5: 5 kg Zn ha^{-1}; Zn_6: 6 kg Zn ha^{-1}.

Although, the trend in terms of nitrogen content in grain and stover was dissimilar. Statistically, the highest nitrogen (4.05%) and crude protein (25.3%) content in grain was fetched by the genotype 'GAM-5' and nitrogen content in stover (2.61%) by 'GM 6'. Furthermore, application of 6 kg Zn ha^{-1}to mungbean enhanced Zn (39.6 and 29.2 mg kg^{-1}) and nitrogen content (3.93 and 2.52%) in grain and stover both at the harvest stage over control and preceded levels of Zn applied. Albeit, increasing levels of Zn to mungbean from 0 to 6 kg ha^{-1} decreased the Fe content both in grain and Stover. However, increasing levels of Zn enhanced crude protein content in grain.

In mungbean grain, statistical differences as positive interaction was found in respect to Zn, nitrogen, and crude protein content (Figure 3). The highest Zn content in mungbean grain was observed with mungbean genotype 'GM 4' (51.5 mg kg^{-1}) with the application of 6 kg Zn ha^{-1}. Although, 'IPM 02-3' had the highest nitrogen content in grain (4.0%) when fertilized with same level of Zn i.e., 6 kg Zn ha^{-1}. Contrary to this, the highest crude protein content in grain (28.6%) was attained by the genotype 'GAM 5' with the application of 6 kg Zn ha^{-1}.

3.2. Crop Nutrient Uptake

Nutrient uptake is one of the important indicators of nutrient acquisition, enrichment, and mobilization in the plant parts. Mungbean varieties and Zn levels noticeably affected Zn (Figure 4), Fe (Figure 5) and nitrogen (Figure 6) uptake by the grain and stover. Among mungbean varieties, substantially higher uptake of Zn by the grains (52.1 g ha^{-1}) and stover (99.6 g ha^{-1}) was attained by 'GM 4' over 'GM 6', 'GAM 5' and 'IPM 02-3'. On the other hand, greatest Fe uptake by grain (99.2 g ha^{-1}) was recorded with 'GM 6' and by stover (1027.5 g ha^{-1}) was attained with 'IPM 02-3'. Further, significantly higher uptake of nitrogen both in grain (45.6 kg ha^{-1}) and in stover (78.7 kg ha^{-1}) was recorded by the genotype 'GM 6'.

Table 4. Effect of varieties and zinc levels on zinc use efficiency indices

Treatments	ZUE (kg grain kg^{-1} Zn applied)	IZUE (kg grain g^{-1} Zn uptake)	AZUE (kg grain increased kg^{-1} Zn applied)	PZUE (kg grain increment g^{-1} Zn uptake)	AZR (%)	Zinc harvest index (%)
Mungbean Varieties						
GM 4	386.4	7.39	31.1	13.1	2.14	34.3
GAM 5	353.0	9.77	31.6	18.3	1.41	36.8
GM 6	454.6	11.94	29.4	28.1	1.11	36.4
IPM 02-3	326.7	9.46	34.5	26.6	1.19	31.7
SEm±	4.62	0.17	1.01	1.21	0.21	0.60
CD (P=0.05)	13.10	0.49	2.87	3.38	0.53	1.70
Zinc levels (kg ha^{-1})						
Zn_0	—	9.55	—	—	—	33.5
Zn_1	1035.8	9.72	39.9	37.4	1.53	34.2
Zn_2	546.5	9.89	48.6	30.9	1.89	34.9
Zn_3	371.1	9.85	39.1	27.8	1.66	35.1
Zn_4	280.6	9.65	31.6	20.8	1.56	34.9
Zn_5	239.4	9.67	40.2	19.6	2.08	36.0
Zn_6	187.9	9.15	21.9	14.1	1.50	35.1
SEm±	6.11	0.23	1.35	2.03	0.32	0.79
CD (P=0.05)	17.32	0.65	3.78	5.81	0.71	2.23
Zn × Cultivar Interaction						
SEm±	12.22	0.45	12.90	10.94	0.05	1.59
CD (P=0.05)	34.65	NS	NS	NS	NS	NS

*Significant at 5% level of significance. Zn_0 (control, without Zn); Zn_1: 1 kg Zn ha^{-1}; Zn_2: 2 kg Zn ha^{-1}; Zn_3: 3 kg Zn ha^{-1}; Zn_4: 4 kg Zn ha^{-1}; Zn_5: 5 kg Zn ha^{-1}; Zn_6: 6 kg Zn ha^{-1}. AZR, Apparent Zn recovery efficiency; IZUE, internal Zn use efficiency; PZUE, Physiological Zn use efficiency; ZUE, Zinc use efficiency.

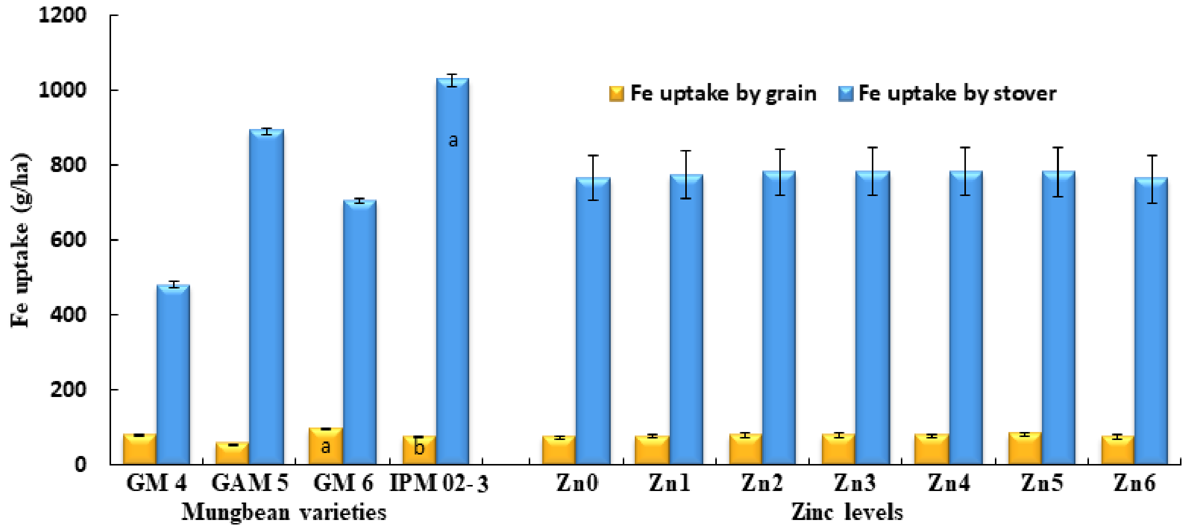

Fig. 5. Effect of varieties and zinc levels on Fe uptake by grain and stover (g ha^{-1}) of mungbean. Bars show LSD values at P = 0.05 to determine the significance differences among treatment means. Zn_0 (control, without Zn); Zn_1: 1 kg Zn ha^{-1}; Zn_2: 2 kg Zn ha^{-1}; Zn_3: 3 kg Zn ha^{-1}; Zn_4: 4 kg Zn ha^{-1}; Zn_5: 5 kg Zn ha^{-1}; Zn_6: 6 kg Zn ha^{-1}.

Interestingly, application of Zn up to highest level i.e., 6 kg Zn ha^{-1}enhanced Zn uptake by stover (82.7 g ha^{-1}), albeit, in case of grain (45.9 g ha^{-1}) it was noticed highest up to application of 5 kg Zn ha^{-1} only. Contrarily, the analysis did not show any differences in Fe uptake by grain and stover in response to Zn application. Conversely, marked improvement in nitrogen uptake by both grain and stover was noticed with the application of Zn.

3.3. Zn use efficiency indices

Indigenous nutrient supply and nutrient use efficiency indices are important parameters affecting crop growth and grain yield. Interestingly, mungbean varieties and varying levels of Zn had significant influence on Zn use efficiency, internal Zn utilization efficiency, agronomic Zn use efficiency, physiological Zn use efficiency, apparent Zn recovery and Zn harvest index (Table 4). Among the mungbean varieties, 'GM-6' fetched substantially higher ZUE (454.6 kg grain kg^{-1} Zn applied), IZUE (11.9 kg grain kg^{-1} Zn uptake) and PZUE (28.1 kg grain increment g^{-1} Zn uptake). However, markedly higher apparent Zn recovery (2.14%) and Zn harvest index (36.8%) was fetched by the mungbean genotype 'GM 4' and 'GAM 5', respectively. Unexpectedly, highest AZUE (34.5 kg grain increased kg^{-1} Zn applied) was noticed with 'IPM 02-3'.

Application of Zn to mungbean influenced substantially Zn use indices. Increasing levels of Zn recorded decreasing trend of ZUE (1035.8–187.9kg grain kg^{-1} Zn applied), IZUE (9.89–9.15 kg grain kg^{-1} Zn uptake), AZUE (48.6–21.9kg grain increment kg^{-1} Zn applied) and PZUE (37.4–14.1 kg grain increment g^{-1} Zn uptake). Contrarily, application of 5 kg Zn ha^{-1} fetched significant improvement in AZR (2.1%) and ZHI (36.0%).

3.4. Regression

Simple linear regression coefficients (b) of different characters on Zn content in grain varied due to plant characters (Table 5). The regression coefficients of all the characters were found highly significant with positive effects (values). The positive values of regression coefficients suggested that the rate of increases of Zn content in grain due to one unit increase in the independent variable (plant characters). The regression coefficient value of number of secondary branches (23.1) signifies that the Zn content in the mungbean grains which might have increased by increasing number of secondary branches. The highest regression coefficient was found in number of secondary branches (23.1) followed by LAI (13.8) and pod length (12.0). Similarly, Zn content in mungbean grain may also be enhanced by increasing the others plant characters.

The coefficients of determination (R^2) values of all the mungbean characters for grain yield under study were found in the range from 0.337 to 0.937. The highest value of R^2 (0.937) was recorded in number of primary branches and number of grains pod^{-1}, it suggests that 93.7% of the variation in grain yield could be explained by the number of primary branches and number of grains pod^{-1}when others variables are kept constant. Hence, the traits like number of branches and number of grains pod^{-1} are the most important component of grain yield in mungbean followed by stover yield (R^2=0.930), dry matter accumulation (R^2=0.892), number of pods (R^2=0.850) and so on.

Table 5. Regression coefficients (b values) and intercept (a) of different component traits on grain yield in mungbean along with their coefficient of determination (R^2)

Plant characters	Correlation coefficient		a (Intercept)		b (Regression coefficients)		R^2	
	Grain yield	Grain Zn content	Grain yield	Grain Zn content	Grain yield	Grain Zn content	Grain yield	Grain Zn content
Number of nodules plant^{-1}	0.737**	0.423*	-525.07	-21.67	64.39**	5.48*	0.544**	0.179*
Nodules fresh weight (mg)	0.603**	0.084	-204.75	89.77	2.87**	0.59	0.364**	0.007
Nodules dry weight (mg)	0.580**	0.170	-323.03	54.69	20.38**	0.88	0.337**	0.029
Crop Growth Rate (CGR)	0.718**	0.255	175.47	68.15	164.37**	8.625	0.517**	0.065
Plant height (cm)	0.880**	0.512**	-126.55	10.98	27.09**	2.33**	0.774**	0.262**
Dry matter accumulation (g)	0.944**	0.544**	48.75	26.94	60.08**	5.13**	0.892**	0.296**
SCMR	0.736**	0.450*	-73.58	41.49	34.28**	1.21*	0.542**	0.202*
Leaf area index (LAI)	0.726**	0.517**	-157.38	-15.61	416.23**	13.82**	0.525**	0.267**
Number of primary branches	0.968**	0.441*	172.07	53.98	146.59**	9.91*	0.937**	0.159*
Number of secondary branches	0.912**	0.631**	367.41	41.46	224.81**	23.05**	0.832**	0.339**
Number of pods plant^{-1}	0.922**	0.568**	128.02	27.91	34.60**	3.16**	0.850**	0.323**
Pod length (cm)	0.881**	0.533**	-168.99	2.97	134.05**	12.02*	0.777**	0.284*
Number of grains pod^{-1}	0.968**	0.457*	29.00	41.78	109.85**	7.68*	0.937**	0.209*
Test weight (g)	0.695**	0.181	-80.71	70.79	24.99**	0.97	0.483**	0.033
Stover yield (kg ha^{-1})	0.964**	0.319	-1105.22	8.69	0.80**	0.04	0.930**	0.101

* and **Significant at 5% and 1% level of significance respectively. Zn_0 (control, without Zn); Zn_1: 1 kg Zn ha^{-1}; Zn_2: 2 kg Zn ha^{-1}; Zn_3: 3 kg Zn ha^{-1}; Zn_4: 4 kg Zn ha^{-1}; Zn_5: 5 kg Zn ha^{-1}; Zn_6: 6 kg Zn ha^{-1}. SCMR=SPAD Chlorophyll Meter Reading.

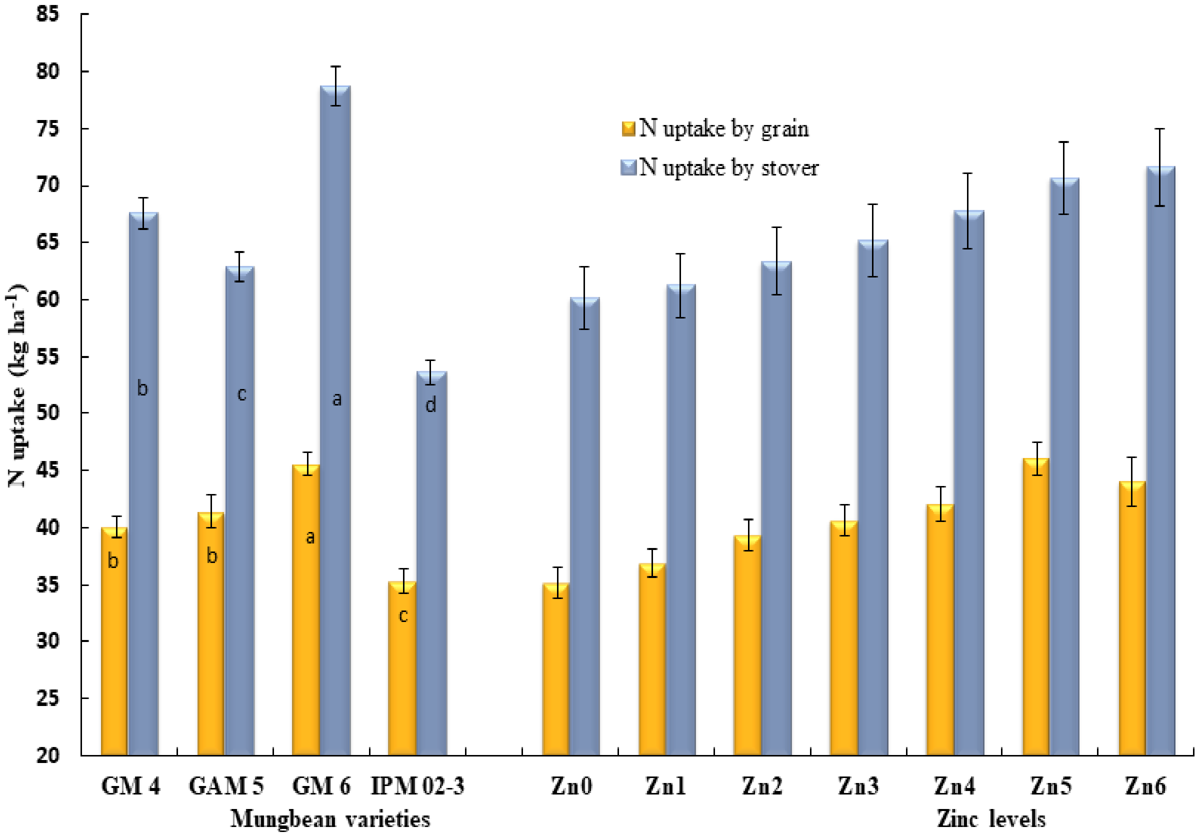

Fig. 6. Effect of varieties and zinc levels on N uptake by grain and stover (kg ha^{-1}) of mungbean. Bars show LSD values at P = 0.05 to determine the significance differences among treatment means. Zn$_0$ (CK, without Zn); Zn$_1$: 1 kg Zn ha^{-1}; Zn$_2$: 2 kg Zn ha^{-1}; Zn$_3$: 3 kg Zn ha^{-1}; Zn$_4$: 4 kg Zn ha^{-1}; Zn$_5$: 5 kg Zn ha^{-1}; Zn$_6$: 6 kg Zn ha^{-1}.

The R^2 values of nodules dry weight (0.337), nodules fresh weight (0.364), 1,000-grains weight (0.483), CGR (0.517), LAI (0.525) and SCMR (0.542) were comparatively lower than the others plant characters. Thus, these variables had comparatively lesser influence on grain yield compared with other remaining variables. Further, the coefficients of determination (R^2) values of all the mungbean characters for grain Zn content were recorded in the range from 0.007 to 0.339. The highest value of R^2 (0.339) for Zn content in grain was recorded in number of secondary branches followed by number of pods plant^{-1} (0.323), dry matter accumulation (0.296) and pod length (0.284).

3.5. Principal Component Analysis

The first two principles components were found to be sufficiently to explain the variation of plant characters *i.e.*, 83.8% (Fig. 7). The first PC 1 contributes 74.4% of total variation and PC 2 contributes 9.3% of total variation. From the analysis it is evident that all the plant characters are found to be sufficiently associated to PC 1. Dry matter accumulation contributed maximum associated to the PC 1 followed by number of primary branches, number of grains pod^{-1}, total nitrogen uptake and grain yield. Angles between the errors of plant character are found to be narrow, it justified that all the variables are associated with each other's.

4. Discussion

Zn content and its uptake are interactively influenced by varying rates of Zn and different mungbean varieties (Table 2-3; Figure 2-4). In the present study, accumulation of Zn in plant parts *viz.*, root, stem, leaf, pod wall, grain and stover at different developmental stages of mungbean showed a significant positive correlation with varieties and Zn levels. Zn contents in mungbean plant parts elevated with increasing soil Zn status. Roots had greater Zn accumulation than stems. The reason for greater sensitivity of roots to Zn than stems might be related to the fact that roots are the first organs to be in contact with Zn and consequently accumulate Zn at much higher amounts than stems, which agrees with the previous studies (Hassan et al., 2005).

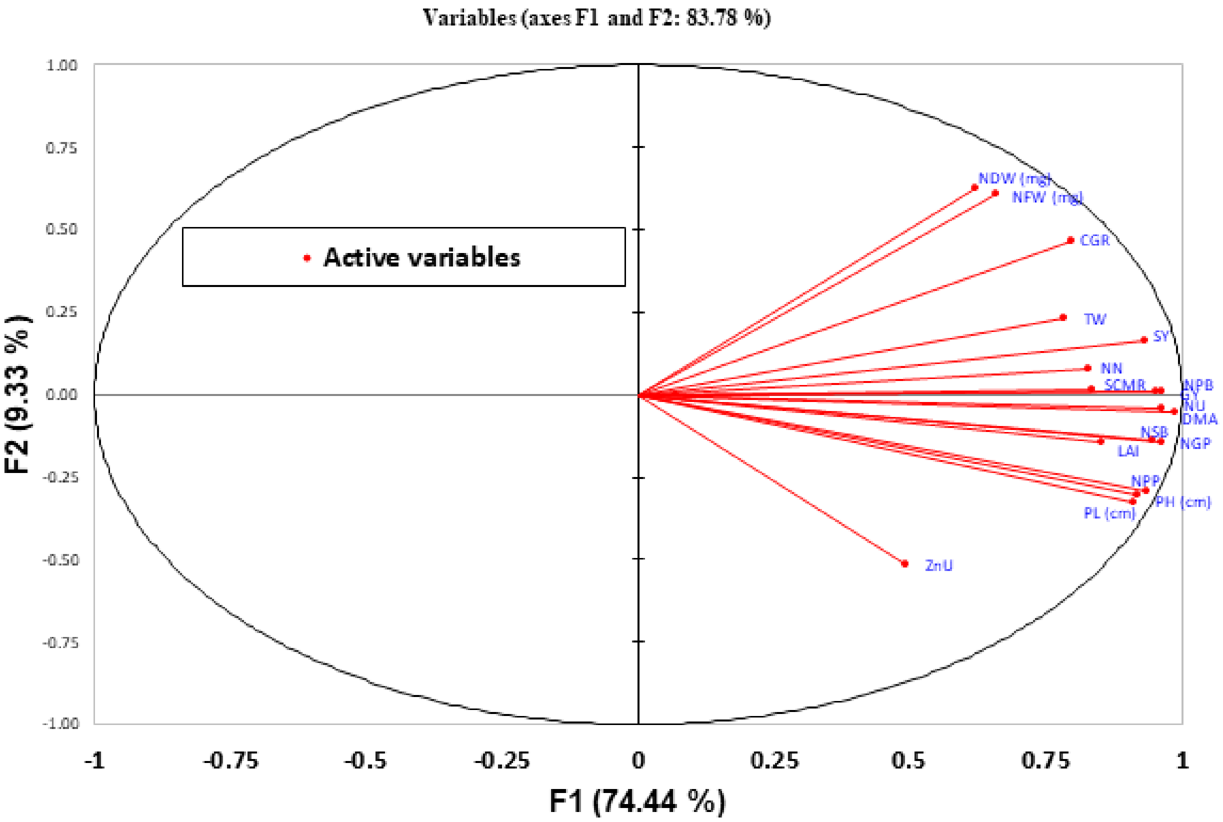

Fig. 7. Relationship between plants characters and components (PCA). NN = Number of nodules plant^{-1}; NDW = Nodules dry weight (mg plant^{-1}); NFW = Nodules fresh weight (mg plant^{-1}); CGR = Crop growth rate; LAI = Leaf area index; SCMR = SPAD chlorophyll meter reading; NPB = Number of primary branches plant^{-1}; NSB = Number of secondary branches plant^{-1}; PL = Pod length (cm); TW = Test weight (g); GY = Grain yield (kg ha^{-1}); SY = Stover yield (kg ha^{-1}); ZnU = Zinc uptake (g ha^{-1}); NU = Nitrogen uptake (kg ha^{-1}).

Root tissues can have higher Zn accumulation of Zn as compared to stem tissues (Fan et al., 2021). A critical Zn concentration of 35.9 mg kg^{-1} in whole stems at early growth stage (25 DAS) of mungbean was determined in the present study. Roots had higher Zn content than the stem when no Zn was applied, but the concentration in the root did not increase until Zn was applied (Khan et al. 1998). The concentration of Zn in the plant varies considerably during different plant growth stages, which generally bears positive correlation with the dry matter yield. Further, leaves have been the most appropriate part of the plant to sample for nutrient status for Zn (Venkatesh et al. 2014). In the present study, highest Zn content in leaf was observed with 'GM 4' variety followed by 'GM 6' variety at 25 and 50 DAS growth stage of mungbean crop while, minimum Zn content was observed in leaf at harvest stage. This can be attributed to significant genotypic variation, Zn levels and environmental effect (Saeed et al., 2021; Nawaz et al. 2015; Venkatesh et al. 2014;). Mungbean varieties significantly influenced tissue Zn concentration at different stages. Plant tissue Zn concentration was moderate to highly correlated with dry matter production throughout the mungbean crop growth stages. Based on the quadratic relationship with dry matter production, the critical concentration of tissue Zn was higher at early stage (25 DAS) which was reduced at 50 DAS where the concentration generally declines during the growing season. Considerable variation was also observed in critical Zn concentration with leaf position. Based on the variability of Zn concentration in plant tissues and relation with dry matter production, young fully expanded leaf at 50 DAS was found to be the efficient sampling plant part for the plant diagnostics of Zn (Venkatesh et al. 2014). Gradual increase in dose of Zn to mungbean through soil application help to attain higher availability of Zn in the rhizosphere coupled with better root development and thereby led to greater absorption of nutrients from the soil. The higher uptake of nutrients was also a consequence of increased seed and stover yield and their concentration in seed and stover under better treatments. Soil application of Zn can increase grain and stover Zn content and uptake in plants with adequate Zn mobility in the phloem (Fan et al., 2021).

Soil application of Zn significantly enhanced Zn content in root, stem and leaf at 25, 50 DAS and at harvest stage of mungbean crop. At early growth stage, high Zn content in seed has important physiological roles during seed germination and early seedling growth. It is well documented that, during seed germination, Zn concentration of newly developed radical and coleoptiles is extremely high (up to 200 mg kg^{-1}), indicating critical physiological roles of Zn during early seedling development (Ozturk et al. 2006). In such highly metabolically active root and coleoptiles tissues, Zn is most probably used for protein synthesis, membrane function, especially membrane stability, cell elongation and tolerance to environmental stresses (Cakmak, 2000). Roots had higher Zn concentrations than the shoot, when no Zn was applied, but the concentration in the root did not increase until Zn was applied (Khan et al. 1998). Applying Zn fertilizers to crop grown in in Central Anatolia under field conditions improved not only productivity but also grain Zn concentration (Yilmaz et al. 1997).

Soil application of Zn as Zn oxide significantly increased the content and uptake of Zn in grain and stover over control (0 kg Zn ha^{-1}). The role of Zn in increasing the cation exchange capacity of roots might have led to more absorption of nutrients from soil and thereby more translocation to different vegetative and reproductive parts which ultimately led to higher content in the grain and stover of mungbean. Earlier, Karmaker et al. (2015) and Haider et al. (2018) obtained higher Zn content and uptake of mungbean due to Zn application. In present study, such increment in content of Zn in grains and stover with the application of Zn might be due to more availability and absorption of Zn in rhizosphere resulting from Zn application to deficient soil (0.48 mg Zn kg^{-1} soil).

Content and uptake of Fe by mungbean grain and stover decreased with increasing levels of Zn (Table 3, Fig. 5). There is a complex interaction of Zn with other nutrients (Fan et al., 2021). Decreased Fe content and uptake with increased Zn application doses led to profuse plant vegetative growth and negative effect on Fe transportation under Zn deficient soils (Singh and Prasanna, 2020; Yaseen and Hussain, 2021). Furthermore, Zn fertilizer decreases Fe content in plant because of competition for the same carrier site in the casparian bands or plasmalemma. The antagonistic effect of Zn is especially prevalent with Fe and Cu (Tisdale et al. 2010). Genetic makeup of the plants further influences their ability to take up Fe from the soil.

Nitrogen plays a critical role in uptake and translocation of Zn in plants. Zn-N crosstalk have confirmed synergistic interaction between both nutrients, and application of Zn results in increases accumulation of N and Zn and higher crude protein in grain (Fan et al., 2021; Samreen et al., 2017). Nitrogen content and its uptake in grain and stover and crude protein content in grain of mungbean influenced significantly by varieties and levels of Zn application (Table 3; Figure 3, 6). Deficiency of Zn affects nitrogen metabolism, and ultimately leading to reduced protein contents and amino acids in Zn-deficient plant tissues (Khan et al., 2022). Plant tolerance to Zn deficiency varies widely among varieties (Venkatesh et al., 2014). Gradual increase in crude protein content in all the varieties due to increasing dose of Zn and higher crude protein content in grain under treatments might be the outcome of increased concentration of nitrogen in grain of mungbean by Zn application which promotes protein synthesis (Shivay et al. 2014).

Application of Zn up to 6 kg ha^{-1} exhibited significant increment in nitrogen content and its uptake by grain and stover and crude protein content in grains. The increased crude protein content might be due to increased nitrogen content, as Zn takes part in nitrate conversion to ammonia in plants (Boorboori et al. 2012). The role of Zn in indole acetic acid synthesis results in amino acids which in turn makes protein (Moussavi-Nik and Kiani 2012). The increase in protein content may also be due to increased photosynthetic rates and leaf chlorophyll content. The role of Zn in increasing the cation exchange capacity of roots might have led to absorption of more nutrients from soil and in turn led to more translocation to different vegetative and reproductive parts which ultimately resulted into higher content in the grain and stover of mungbean (Srivastava et al. 2006; Habibullah et al. 2014; Tak et al. 2014; Karmaker et al. 2015). Moreover, Zn is required as structural and catalytic components of protein and enzymes for normal growth and development (Natasha et al., 2022).

It is not clear at present whether there are common factors or mechanism influences Zn use indices viz; ZUE, IZUE, AZUE, PZUE, AZR and ZHI. Under our study, varietal differences were noticed for Zn use indices. The variety ‘GM 6’ fetched higher ZUE, IZUE and PZUE. However, variety ‘GAM 5’ recorded higher AZUE and ZHI and the variety ‘GM 4’ had higher AZR. Further, varying Zn levels also influenced the Zn use indices and the trend was not uniform for all the indices. It is Astonishing that the influences of varieties and varying Zn levels is not uniform in terms of Zn use indices recorded. In our study the increase in AZR and ZHI in mungbean with increasing doses of Zn could be attributed to relatively higher grain yield and Zn uptake by the crop. The reduction in ZUE, IZUE and PZUE with increasing levels of Zn ascribed to relatively lower Zn uptake and higher dry matter and grain yield (Shivay et al. 2015).

There are significant genotypic variations for nutrient acquisition and translocation efficiencies, these processes are vital for the growth and development of plants and play a critical role in determining the nutritional content and potential for biomass production. Promising cultivars can be used for breeding programs aimed at development of nutrient enrichment (Fan et al., 2021; Ma et al., 2022; Rehman et al., 2018; Saudy et al., 2022; Sultana et al., 2020).

5. Conclusion

Application of Zn to mungbean up to 6 kg ha^{-1} enhanced concentration and its uptake and also nitrogen in edible plant parts i.e. grain and stover (animal fodder). Increasing levels of Zn to soil negatively influenced Fe concentration and its acquisition in mungbean under Zn deficient soil. Further, Zn use efficiency indices viz; ZUE, IZUE, AZUE, PZUE, AZR and ZHI were also markedly influenced with the mungbean varieties and levels of Zn applied. Thus, a pragmatic strategy for Zn biofortification of mungbean, selection of suitable varieties and Zn application could be an option for grain Zn biofortification in mungbean.

Competing Interest Statement: The authors have declared that they have no competing interests and there is no conflict of interest exists.

Author's Contribution: P.D: Field observations, micronutrient analysis, Writing - review & editing. **U.S.:** Conceptualization, Supervision, Methodology, Writing - original draft, Writing - review & editing. **L.N.S.:** Statistical analysis of data, graphical representation of data. **Y.S.S.:** Micronutrient analysis. **M.K.:** Field layout plan, Editing. **P.R.R.:** Soil sampling.

Acknowledgment: The authors would like to acknowledge the College of Agriculture, Agriculture University, Jodhpur, India for facilitating and financing the study.

References

Akram, R., Jabeen, T., Bukari, M.A., Wajid, S.A., Mubeen, M., Rasul, F., Hussain, S., Aurangzaib, M., Bukhari, M.A., Hammad, H.M. and Zamin, M., 2022. Research on Climate Change Issues. In: Building Climate Resilience in Agriculture. Springer, Cham. P.255-268.

Ali, M. F., S. B. Ali Ammar, U. Ali, N. Huma and M. Adnan. 2021. Mitigating zinc deficiency in plants and soils through agronomic techniques: A review. J. Environ. Agric. Sci. 23 (1&2): 1-10.

Bevis, L., K. Kim and D. Guerena. 2023. Soil zinc deficiency and child stunting: Evidence from Nepal. J. Health Econ. 87: 102691.

Bhatt, R., A. Hossain and P. Sharma. 2020. Zinc biofortification as an innovative technology to alleviate the zinc deficiency in human health: A review. Open Agric. 5: 176-187.

Boorboori, M.R., D.R. Asli, and M. Tehrani. 2012. The effect of dose and different methods of iron, zinc, manganese and copper application on yield components, morphological traits and grain protein percentage of barley plants in green house conditions. Adv. Environ. Biol. 6:740-746.

Cakmak, I. 2000. Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. New Phytol. 146(2):185-205.

DAC. 2017. Pulses in India: Retrospect and Prospects. Department of Agriculture, Cooperation and Farmers Welfare, Government of India. URL: https://farmer.gov.in/imagedefault/prospects_2017.pdf. Accessed 13 August 2020.

Das, S. and A. Green. 2016. Zinc in crops and human health. In: Biofortification of Food Crops (Eds., U. Singh et al. Springer (India) Pvt. Ltd., New Delhi). p. 31-40.

De Benoist, B., I. Darnton-Hill, L. Davidsson, O. Fontaine and C. Hotz. 2007. Conclusions of the joint WHO/UNICEF/IAEA/IZiNCG interagency meeting on zinc status indicators. Food Nutr. Bull. 28: S480-S484.

de Valença, A. W., A. Bake, I. D. Brouwer and K. E. Giller, 2017: Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. Glob. Food Secur. 12: 8-14.

DES. 2021. Department of Agriculture Cooperation & Farmers Welfare, Directorate of Economics and Statistics, 2020-21, Government of India. URL: https://eands.dacnet.nic.in/APY_96_To_06.htm (Accessed on 02-02-2022).

Fan, X., X. Zhou, H. Chen, M. Tang and X. Xi. 2021. Cross-Talks Between Macro- and Micronutrient

Uptake and Signaling in Plants. Front. Plant Sci. 12: 663477.

Fisher, R.A. and F. Yates. 1963. Statistical tables, Oliver and Boyd, Edinburgh, Tweeddate Court, London (U.K).

Fixen, P., F. Brentrup, T. Bruulsema, F. Garcia, R. Norton and S. Zingore. 2015. Nutrient/fertilizer use efficiency: measurement, current situation and trends. In: Managing Water and Fertilizer for Sustainable Agricultural Intensification (Eds. Drechsel, P., Heffer, P., Magen, H., Mikkelsen, R. and Wichelns, D., IFA, IWMI, IPNI and IPI). p. 1-30.

Gomes, M. J. C., H. S. D. Martino and E. Tako. 2023. Zinc-biofortified staple food crops to improve zinc status in humans: a systematic review. Crit. Rev. Food Sci. Nutrit. https://doi.org/10.1080/10408398.2021.2010032

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research. John Wiley & sons.

Gupta, P. K., H. S. Balyan, S. Sharma and R. Kumar. 2021. Biofortification and bioavailability of Zn, Fe and Se in wheat: present status and future prospects. Theor. Appl. Genet. 134: 1-35.

Habibullah, M., T.A. Noor, M.Z.K. Roni, A.T.M. Shamsuddoha and J.A.F.M. Uddin. 2014. Influence of Different Doses of Zn on Growth and Yield of Mungbean (*Vigna radiata L.*). Int. J. Bus. Soc. Sci Res. 2(2):169-172.

Haider, M.U., M. Hussain, M. Farooq and A. Nawaz. 2018. Soil application of zinc improves the growth yield and grain zinc biofortification of mungbean. Soil Environ. 37:123-128.

Hassan, M.J., G.P. Zhang, F.B. Wu, K. Wei and Z. Chen. 2005. Zinc alleviates growth inhibition and oxidative stress caused by cadmium in rice. J. Plant Nutr. Soil Sci. 168(2):255-261.

Hui, X., L. Luo, S. Wang, H. Cao, M. Huang, M. Shi, S.S. Malhi and Z. Wang. 2019. Critical concentration of available soil phosphorus for grain yield and zinc nutrition of winter wheat in a zinc-deficient calcareous soil. Plant Soil. 444: 315-330.

IZA, 2017. Fertilizing crops to improve human health. URL: https://crops.zinc.org/wp-content/uploads/sites/11/2017/01/IFA_Infographic_ZINC_V10_final.pdf.

Jackson, M.L. 1973. Soil Chemical Analysis (II Edition). Prentice Hall of India Private Limited. New Delhi, India.

Karmakar, P.C., A.H.M. Abdullah, M. Asrafuzzaman, K.K. Poddar and S. Sarker. 2015. Effects of Zinc on the Concentrations of N, P, K, S and Zn in Mungbean (Bari Mung 6) Stover and Seed. Int. J. Res. Rev. 2(6):307-310.

Khan, H.R., G.K. McDonald and Z. Rengel. 1998. Assessment of the Zn status of chickpea by plant analysis. Plant Soil. 198(1):1-9.

Khan, S. T., A. Malik, A. Alwarthan and M. R. Shaik. 2022. The enormity of the zinc deficiency problem and available solutions; an overview. Arabian J. Chem. 15: 103668.

Kiran, A., A. Wakeel, K. Mahmood, R. Mubaraka, Hafsa and S. M. Haefele. 2022. Biofortification of staple crops to alleviate human malnutrition: contributions and potential in developing countries. Agronomy. 12: 452.

Lagoriya, D. S., S. J. Harishma and S. K. Singh. 2023. Agronomical Approaches for Biofortification of Cereal Crops. In: R. Deshmukh, A. Nadaf, W. A. Ansari, K. Singh and H. Sonah eds. *Biofortification in Cereals: Progress and Prospects*. Springer Nature Singapore, Singapore. *p.* 1-19.

Lindsay, W.L. and W.A. Norvell. 1978. Development of DTPA soil test for zinc, iron, manganese and copper. Soil Sci Soc Am. J. 42: 421-428.

Ma, Q., R. W. Bell and E. M. Mattiello. 2022. Nutrient Acquisition with Particular Reference to Subsoil Constraints. In: T. S. d. Oliveira and R. W. Bell eds. *Subsoil Constraints for Crop Production*. Springer International Publishing, Cham. *p.* 289-321.

Moussavi-Nik, M.S. and Z. Kiani. 2012.Considering interaction effects of micro nutrition’s elements (Fe, Zn and Mn) on qualitative yield of wheat varieties. Ann. Biol. Res. 2:1467-1472.

Natasha, N., M. Shahid, I. Bibi, J. Iqbal, S. Khalid, B. Murtaza, H. F. Bakhat, A. B. U. Farooq, M. Amjad, H. M. Hammad, N. K. Niazi and M. Arshad. 2022. Zinc in soil-plant-human system: A data-analysis review. Sci. Total Environ. 808: 152024.

Nath, C. P., N. Kumar, A. Dutta, K. K. Hazra, C. S. Praharaj, S. S. Singh and K. Das. 2023. Pulse crop and organic amendments in cropping system improve soil quality in rice ecology: Evidence from a long–term experiment of 16 years. Geoderma. 430: 116334.

Nawaz, H., N. Hussain, A. Yasmeen, M. Arif, M. Hussain, M.I.A. Rehmani, M.B. Chattha and A. Ahmad. 2015. Soil applied zinc ensures high production and net returns of divergent wheat cultivars. J. Environ. Agric. Sci. 2(1):1-7.

Olsen, S., C. Cole, F. Watanabe and L. Dean.1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture Circular, no. 939, Government Print Office, Washington, D.C.

Ozturk, Levent., M.A. Yazici, C. Yucel, A. Torun, C. Cekic, A. Bagci, H. Ozkan, H.J. Braun, Z. Sayers and I. Cakmak. 2006. Concentration and localization of zinc during seed development and germination in wheat. Physiol. Plant. 128(1):144-152.

Praharaj, S., M. Skalicky, S. Maitra, P. Bhadra, T. Shankar, M. Brestic, V. Hejnak, P. Vachova and A. Hossain. 2021. Zinc Biofortification in Food Crops Could Alleviate the Zinc Malnutrition in Human Health. Molecules. 26: 3509.

Prasad, R. 2012. Micro mineral nutrient deficiencies in humans, animals and plants and their amelioration. Proc. Nat. Acad. Sci. India Section B: Biol. Sci. 82 (2):225–33.

Prasad, R., Y.S. Shivay and D. Kumar. 2013. Zinc fertilization of cereals for increased production and alleviation of zinc malnutrition in India. Agric. Res. 2(2):111–8.

Prasad, R., Y.S. Shivay and D. Kumar. 2014. Agronomic bio-fortification of cereal grains with zinc and iron. Adv. Agron. 125:55–91.

Rattan, R.K., S.P. Datta and J.C. Katyal. 2008. Micronutrient management-research achievements and future challenges. Indian J. Fert. 4(12):93–118

Rehman, A., M. Farooq, L. Ozturk, M. Asif and K. H. M. Siddique. 2018. Zinc nutrition in wheat-based cropping systems. Plant Soil. 422: 283-315.

Richards, L.A. 1954. Diagnosis and improvement of saline and alkali soil. USDA Hand Book No. 60 Oxford & IBH Publication Company, New Delhi.

Saeed, B., A. Nawab and S. Rani. 2020. Genotypic variation in micronutrient composition of potato (*Solanum tuberosum*). J. Environ. Agric. Sci. 22(1): 64-70.

Samreen, T., Humaira, H. U. Shah, S. Ullah and M. Javid. 2017. Zinc effect on growth rate, chlorophyll, protein and mineral contents of hydroponically grown mungbeans plant (*Vigna radiata*). Arabian J. Chem. 10: S1802-S1807.

Sangeetha, V. J., S. Dutta, J. A. Moses and C. Anandharamakrishnan. 2022. Zinc nutrition and human health: Overview and implications. eFood 3: e17.

Saudy, H. S., G. A. A. El–Samad, M. E. El–Temsah and Y. A. E. G. El–Gabry. 2022. Effect of Iron, Zinc, and Manganese Nano-Form Mixture on the Micronutrient Recovery Efficiency and Seed Yield Response Index of Sesame Genotypes. J. Soil Sci. Plant Nutrit. 22: 732-742.

Shivay, Y.S., R. Prasad and M. Pal. 2014. Effect of variety and zinc application on yield, profitability, protein content and zinc and nitrogen uptake by chickpea (*Cicer arietinum*). Indian J. Agron. 59(2):317–321.

Shivay, Y.S., R. Prasad and M. Pal. 2015. Effect of source and method of zinc application on yield, zinc biofortification of grain and Zn uptake and use efficiency in chickpea (*Cicer arietinum* L.). Commun. Soil Sci. Plant Anal. 46(17):2191–2200.

Shukla, A.K. and S.K. Behera. 2012. Micronutrient fertilizer and higher productivity. Indian J. Fert. 8(4):100–117.

Shukla, A.K., S.K. Behera, Y.S. Shivay, P. Singh and A.K. Sing. 2012. Micronutrient and field crop production in India: A review. Indian J. Agron. 57:123–30.

Sihag, J., D. Prakash and P. Yadav. 2020. Evaluation of Soil Physical, Chemical Parameter and Enzyme Activities as Indicator of Soil Fertility with SFM Model in IA–AW Zone of Rajasthan. In: Soft Computing: Theories and Applications, Singapore.

Singh, D. and R. Prasanna, 2020. Potential of microbes in the biofortification of Zn and Fe in dietary food grains. A review. Agron. Sustain. Develop. 40: 15.

Singh, N.P. and A. Pratap. 2016. Food Legumes for Nutritional Security and Health Benefits In: Biofortification of Food Crops (Eds., U. Singh et al. Springer (India) Pvt. Ltd., New Delhi). p. 479-492.

Singh, S.S., K.K. Hazra, C.S. Praharaj and U. Singh. 2016c. Biofortification: Pathway Ahead and Future Challenges In: Biofortification of Food Crops (Eds., U. Singh et al. Springer (India) Pvt. Ltd., New Delhi). p. 479-492.

Singh, U. and C.S. Praharaj. 2017. Practical Manual-Chemical Analysis of Soil and Plant Samples, ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh- 208 024, India. p. 58.

Singh, U. and I.P.S. Ahlawat. 2007. Phosphorus management in pigeon pea – wheat cropping system. Indian J. Agron. 52(1):21-26.

Singh, U., C.S. Praharaj, S.K. Chaturvedi and A. Bohra. 2016b. Biofortification: Introduction, Approaches, Limitations and Challenges In: Biofortification of Food Crops (Eds., Ummed Singh et al. Springer (India) Pvt. Ltd., New Delhi). p. 3-18.

Singh, U., C.S. Praharaj, S.S. Singh and N.P. Singh. 2016a. Biofortification of Food Crops. Springer (India) Pvt. Ltd. https://doi.org/10.1007/978-81-322-2716-8

Srivastava, A.K., P.N. Tripathi, A.K. Singh and R. Singh. 2006. Effect of rhizobium inoculation, sulphur and zinc levels on growth, yield, nutrient uptake and quality summer of greengram (*Phaselous radiatus* L). Int. J. Agric.Sci. 2(1):190-192.

Steel, R.G.D., J.H. Torrie and D.A. Dicky. 1997.

Principles and procedures of statistics: A biometrical approach. 3rd eds. New York: McGraw Hill Book Co. Inc. p. 400–28.

Subbiah, B.V. and G.L. Asija. 1965. A rapid procedure for the estimation of available nitrogen in soils. Curr. Sci. 25:259-260.

Suganya, A., A. Saravanan and N. Manivannan, 2020: Role of zinc nutrition for increasing zinc availability, uptake, yield, and quality of maize (*Zea mays* L.) grains: An overview. Commun. Soil Sci. Plant Anal. 51: 2001-2021.

Sultana, S., R. W. Bell and W. H. Vance. 2020. Genotypic variation among chickpea and wild Cicer spp. in nutrient uptake with increasing concentration of solution Al at low pH. Plant Physiol. Biochem. 157: 390-401.

Tak, S., S.K. Sharma, and M.L. Reager. 2014. Effect of vermicompost and zinc on yield attributes, yield and quality of green gram [*Vigna radiata* var. *aureus* (L.) Wilczek] in arid western Rajasthan. Int. J. Agric. Sci. 10(1):138-141.

Tisdale, S.L., W.L. Nelson, J.D. Beaton and J.L. Havlin. 2010. Soil fertility and fertilizer: An introduction to nutrient management. PHI Learning Pvt. Ltd., New Delhi, pp. 184.

Venkatesh, M.S., K.K. Hazra and P.K. Ghosh. 2014. Critical tissue concentration of zinc in short duration mungbean (*Vigna radiata*). Indian J. Agric. Sci. 84(7):892-895.

Walkley, A.J. and I.A. Black. 1934. Estimation of soil organic carbon by the chromic acid titration method. Soil Sci. 37(1):29-38.

WHO, 2000; World Health Organization. The world health report, health systems: improving performance; 2000, p. 1–10.

Williams, C.H., A. Steinbergs. 1959. Soil sulphur fractions as chemical indices of available sulphur in some Australian soils. Aust. J. Agric. Res. 10:340-352.

Yaseen, M. K. and S. Hussain. 2021. Zinc-biofortified wheat required only a medium rate of soil zinc application to attain the targets of zinc biofortification. Archiv. Agron. Soil Sci. 67: 551-562.

Yilmaz, A., H. Ekiz, B. Torun, I. Gultekin, S. Karanlik, S.A. Bagci and I. Cakmak.1997. Effect of different zinc application methods on grain yield and zinc concentration in wheat grown on zinc-deficient calcareous soils in Central Anatolia. J. Plant Nut. 20(4-5):461-471.

Yoshida, S. 1981. Fundamental of Rice Crop Science. International Rice Research Institute, Los Baños, Laguna, Philippines, p.269.