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ANATOMICAL VARIATIONS IN THE STEM AND LEAF EPIDERMIS OF MUNG BEAN (*VIGNA RADIATA* L.) WITH FOLIAR APPLICATION OF MANGANESE AND ZINC

M.S.B. AL–HAYANI[1*], O.I.M. AL-DULAIMI[1], and M.A.H. AL-HDEETHI[2]

[1]Department of Field Crops, College of Agriculture, University of Anbar, Anbar, Iraq
[2]Department of Biology, College of Education for Pure Sciences, University of Baghdad, Iraq
*Corresponding author's email: muazaz.a.h@ihcoedu.uobaghdad.edu.iq
Email addresses of co-authors: mah20g3001@uoanbar.edu.iq, muazaz75@gmail.com

SUMMARY

A field experiment determined the effects of foliar application of zinc (0, 25, and 50 mg Zn L^{-1}) and manganese (0, 30, and 60 mg Mn L^{-1}) concentrations on growth variables of three mung bean cultivars (Indian Green VC6089A10, Indian Green VC6173B1319, and Indian Black Gold Star). The study commenced in the crop season 2022 at the College of Agriculture, District Ramadi, Anbar Governorate, Iraq. The experimentation followed a randomized complete block design with a split plot arrangement. The zinc levels showed significant differences for growth traits, and the 50 mg Zn L^{-1} level exhibited the highest average characteristic for the vascular bundle thickness (232.1 µ). However, the manganese (60 mg Mn L^{-1}) exceeded the measurement of the lower stomata width (13.50 µ), and its comparative treatment (15.74 µ) outperformed the rest of the variants. The mung bean genotype Black Indian outshone the rest of the cultivars for most traits, such as lower stomata length (16.29 µ), but it did not differ significantly from the cultivar Green Vc6089a10 for the mentioned trait. The interactions of foliar application of zinc, manganese, and mung bean cultivars significantly influenced all these growth parameters.

Keywords: Mung bean (*Vigna radiata* L.), zinc (Zn), manganese (Mn), leaf epidermis, stomata

Key findings: : Zinc and manganese levels and their interactions in mung bean cultivars significantly influenced all the growth parameters. The mung bean genotype Black Indian outperformed most cultivars for most traits.

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INTRODUCTION

Mung bean (*Vigna radiata* L.) belongs to the family Fabaceae, one of the large-flowering food legume crops that contribute to ensuring the food requirements of the world's growing population. Mung bean contains a high percentage of protein (29%) as an animal feed, whether in seeds or green fodder form. Despite the importance of this crop, it has a low productivity in Iraq compared with global production (Uddin *et al.,* 2021).

Manganese is one of the essential elements required for photosynthesis as it participates with chlorine in the photolysis process of water to obtain electrons to form energy compounds (ATP) and the reduction of NADP to NADPH + H in the light reaction. Manganese is also vital in building the chlorophyll molecule. In addition to other elements, it regulates the osmotic potential of the plant cell and helps process protein formation through its involvement in the nitrate reduction process, providing keto acids in the Krebs cycle in association with ammonia and amino acid formation (Tabasum *et al.,* 2010).

Similarly, zinc is an essential micronutrient compulsory for plant growth and development. Zinc is also a catalyst for evaluating numerous compounds, such as proteins and many biochemical pathways, i.e., carbohydrates, converting sugars into starch, and carbon and protein metabolism. It also affects fertility and the production of high-quality pollen grains and vitality; therefore, zinc is a preferred addition to crop plants during the flowering phase (Faruque *et al.,* 2000).

In *Vigna radiata* L., a high percentage of fallen flowers and pods, reaching more than 60% of total flowers, negatively affect crop productivity. Therefore, studying all possible means is necessary to solve pods-shattering problems to increase the fertility rate and knots in flowers to raise productivity. Using micronutrients, such as different manganese and zinc concentrations, can reveal the effects of these concentrations on the plant's stem and leaf epidermis anatomical characteristics to improve mung bean crops' quality. Applying different concentrations of manganese and zinc in crops could increase the plant's efficiency by absorbing various nutrients from the soil and transporting them to the leaves for food, improving the crop's growth and productivity. The presented study determined the anatomical variations in the stem and leaf epidermis of *V. radiata* L. with foliar application of manganese and zinc.

MATERIALS AND METHODS

Field experiment procedure

The field experiment commenced in the fall of 2022 at the College of Agriculture, District Ramadi, Anbar Governorate, Iraq. The soil physical and chemical properties used for the experiment are available in Table 1. The foliar application, with three concentrations each of zinc (0, 30, and 60 mg Zn L^{-1}) and manganese (0, 25, and 50 mg Mn L^{-1}), helped to determine their effects on the physiological, anatomical, and chemical characteristics of three mung bean cultivars, i.e., Indian green cattle VC6089A10 (V1), Indian green cattle VC6173B1319 (V2), and Black Indian Gold Star (V3). The experiment had a randomized complete block design (RCBD) with a split-plot arrangement and three replications. Zinc (Zn) concentrations occupied the main plots, the manganese (Mn) concentrations on the subplots, and the mung bean cultivars occupied the sub-subplots. Zinc sulfate ($ZnSO_4$) was the formulation used as a source of Zinc (Zn), while manganese sulfate ($MnSo_4$) was the source of manganese (Mn).

Soil preparation operations, such as plowing, smoothing, and leveling, ensued, with the experimental land divided into small experimental units with an area of 7 m with two dimensions (3.5 m × 2 m) containing five rows. The row length was 3.5 m, with rows and plant spacing of 40 and 25 cm, respectively, to obtain a crop density of 80,000 plants ha^{-1}. The three mung bean cultivars came from the Department of Agricultural Research, Ministry of Agriculture, Iraq. Seed sowing of all mung bean cultivars transpired on June 17, 2022, at a 2–3 cm depth, by placing 2–3 seeds per pot,

followed by experimental land irrigation. After three days of germination, the failed pots received patching, and later, plant thinning continued in two stages to ensure a single plant per pot.

The experimental land fertilization used triple superphosphate fertilizer (P_2O_5 46%) at the rate of 75 kg P ha^{-1} before sowing while adding nitrogen as urea fertilizer (N 46%) at 40 kg N ha^{-1} had two split doses, with the first dose occurring at germination and the second dose at the beginning of the flowering stage (Al-Fahdawi, 2019). Crop service operations, such as irrigation and weeding, progressed whenever needed. The zinc and manganese fertilization with foliar application proceeded in two batches: the first after 30 days of planting and the second after 50 days of mung bean planting. The control treatment used distilled water only. The mung bean plants of three cultivars sustained spraying before sunset using a 16-liter sprinkler and adding 0.15 ml^{-1} of Al-Zahi as a dispersant. It reduced the surface tension of water and ensured complete wetness of the foliage, increasing the efficiency of the spray solution in penetrating leaves' outer surface (Abu-Dahi *et al.,* 2001).

Leaf epidermis study

The plant leaves' epidermis scraping began in the middle between the apex and the base using a sharp anatomical scalpel. After completion of the scraping process, sample washing with distilled water followed before placing them in a Petri dish with sodium hypochlorite solution (industrial minor) at 5% concentration for five minutes and then washing again with distilled water. Transferring the sample to another Petri dish containing 10% KOH ensured soaking for 5-10 minutes, with the solution replaced with 70% ethyl alcohol for 10-15 minutes.

Later, the transferred sample to another Petri dish containing 1% safranin dye was kept for 30-45 minutes, washing it afterward. Sections with distilled water acquired ethyl alcohol in a descending series (70%, 95%, and 100%, respectively) at each concentration for 10 minutes and then transferred to a Petri dish containing xylene for 10 minutes. From there, samples placed on a slide mixed with a water droplet combined with a drop of xylene and covered with a cover slide are ready for examination through a microscope. The scrutiny used a KRÜSS compound microscope, and imaging employed an AmScope camera (Model MU 1000). The said process followed the method according to Al-Khazraji and Aziz (1989) with some modifications according to Al-Hadeethi *et al.* (2020).

Preparation of transverse sections of a stem

The collected plant samples had their stem cut from the middle to prepare them for manual slicing. Preparing the sections proceeded by hand sectioning following the procedure of Hutchinson (1954) with some modifications as follows (AL-Hadeethi *et al.*, 2020):

- The selected stems and leaves, cut into small pieces, had a length ranging from 5–7 cm from an area located approximately in the middle. Cutting the sample, held in a vertical position between the thumb and forefinger and in a flat position that was not inclined until finished, using a sharp slicing blade into thin pieces. Obtaining a thinner cross-sectional section and vivid contours, cutting some plant sections incurs slicing under a dissecting microscope.
- Transferring the sections to a solution of industrial minor diluted with distilled water at 5% concentration to remove the chlorophyll dye took five to ten minutes.
- The sections, stained with safranin (prepared by dissolving 1 g of the dye in 99 ml of ethyl alcohol at a 50% concentration) for one to two hours, underwent washing with 70% ethyl alcohol to remove the excess dye.
- The sections attained another transfer to 90% ethyl alcohol for five minutes.
- From there, the sections again moved to alcohol with a 95% concentration and absolute alcohol, respectively, for two minutes.
- The sections transferred to a mixture of absolute alcohol and xylene in a 1:1 volume ratio remained for two minutes.

- Then, the sections' transfer to pure xylene remained for two minutes.
- Then, transferring the sections to a slide of a bottle had a drop of water + a drop of xylene received another slide cover gently placed on it, avoiding the formation of bubbles in the section.
- The final examination continued with a KRÜSS compound microscope, with the imaging done utilizing an AmScope camera, Model MU 1000.

RESULTS AND DISCUSSION

The shapes of stomata in the various mung bean cultivars are visible in Figures 1-3. The stomata type was paracytic. The results revealed significant differences in the stomata length in the upper epidermis of the triple treatment among the genetic structures of the cultivar Gold Star with different concentrations of zinc and manganese (Table 1). The mung bean cultivar Indian black Mach (Gold Star), with a zinc concentration of 50 mg L^{-1} and manganese at 0 mg L^{-1}, achieved the highest percentage of genetic compositions (19.88%). The zinc treatment and genetic composition also achieved meaningful differences at the 50 mg L^{-1} Zn concentration for the cultivar Gold Star, with the utmost genetic composition percentage (17.39%). The treatment of manganese concentration at 0 mg L^{-1} for mung bean cultivar green Mach VC6089A10 achieved the maximum proportion of genetic composition (18.01%) (Table 1, Figure 1). These differences could be due to variations in the genotype. The promising results agree with past findings, which reported observed differences among various *Vicia faba* cultivars by treating with potassium and zinc (Ali *et al.,* 2009; Hasan *et al.,* 2018).

The results enunciated that different concentrations of zinc and manganese resulted in remarkable variations in the stomata width in the upper epidermis of the triple treatment among the genetic structures of mung bean cultivars (Table 1). The cultivar green Indian vc6173b1319, with a zinc concentration of 25 mg L^{-1} and manganese concentration of 60 mg L^{-1}, showed the highest percentage of genetic composition (16.25%). The zinc treatment and genetic composition also achieved notable variances at the 0 mg L^{-1} Zinc concentration for mung bean cultivar Indian Green vc6089a10, with the maximum fraction genetic composition (13.94%). The manganese treatment at 30 mg L^{-1} concentration for the cultivar Gold Star achieved the utmost percentage of genetic composition (13.80%) (Table 1, Figure 1). These results were analogous to past findings by treating chickpeas with varied salicylic and ascorbic acid concentrations, increasing the stomatal length and width (Farjam et al., 2015).Prominent differences were visible in the stomatal length in the lower epidermis of the triple treatment among the genetic structures of the mung bean cultivars with the different zinc and manganese concentrations. The cultivar Indian Green vc6173b1319, with a 0 mg L^{-1} zinc concentration and manganese at 30 mg L^{-1}, achieved the highest percentage of genetic composition (18.68%). The combined effect of zinc and genetic composition also revealed the significant differences at 0 mg L^{-1} zinc for mung bean cultivar Indian Green vc6173b1319, with the utmost genetic composition proportion (16.79%). Likewise, the manganese treatment at a 0 mg L^{-1} concentration for the cultivar Gold Star achieved the maximum genetic composition percentage (17.20%). These results agree with previous findings of Nair *et al*. (2013), Hasan *et al.* (2018) and Mahmoud *et al*. (2023), who studied the anatomical and physiological traits of broad bean (*Vicia faba* L.) seedlings after applying the salicylic acid and salt stress (Table 2, Figure 2).

Noteworthy disparities were evident for the stomatal width in the lower epidermis of the triple treatment among the genetic structures of mung bean cultivars with applications of different concentrations of zinc and manganese. The cultivar green Indian vc6089a10, with a zinc concentration of 50 mg L^{-1} and manganese concentration of 30 mg L^{-1}, attained the highest percentage of genetic composition (15.76%). The zinc treatment and genetic makeup also acquired significant differences at the 25 mg L^{-1} zinc concentration for the mung bean cultivar Gold Star, with the

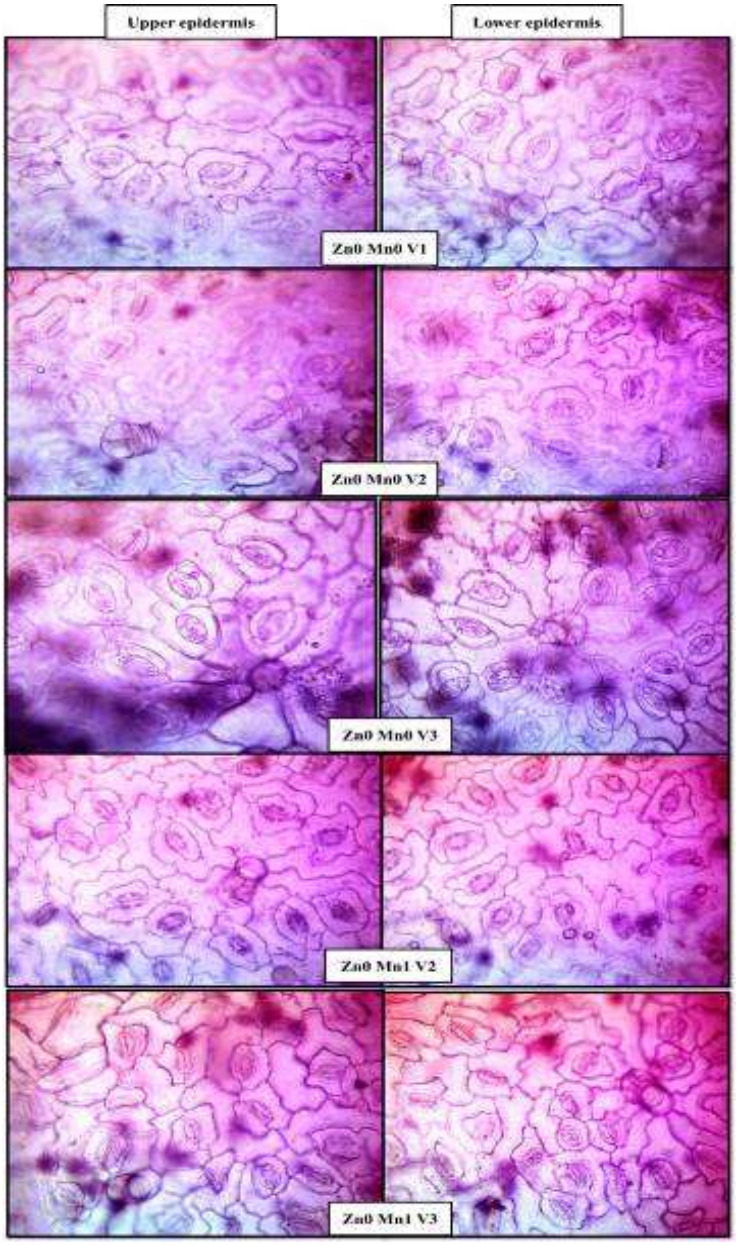

Figure 1. The shape of the stomata in the upper and lower epidermis.

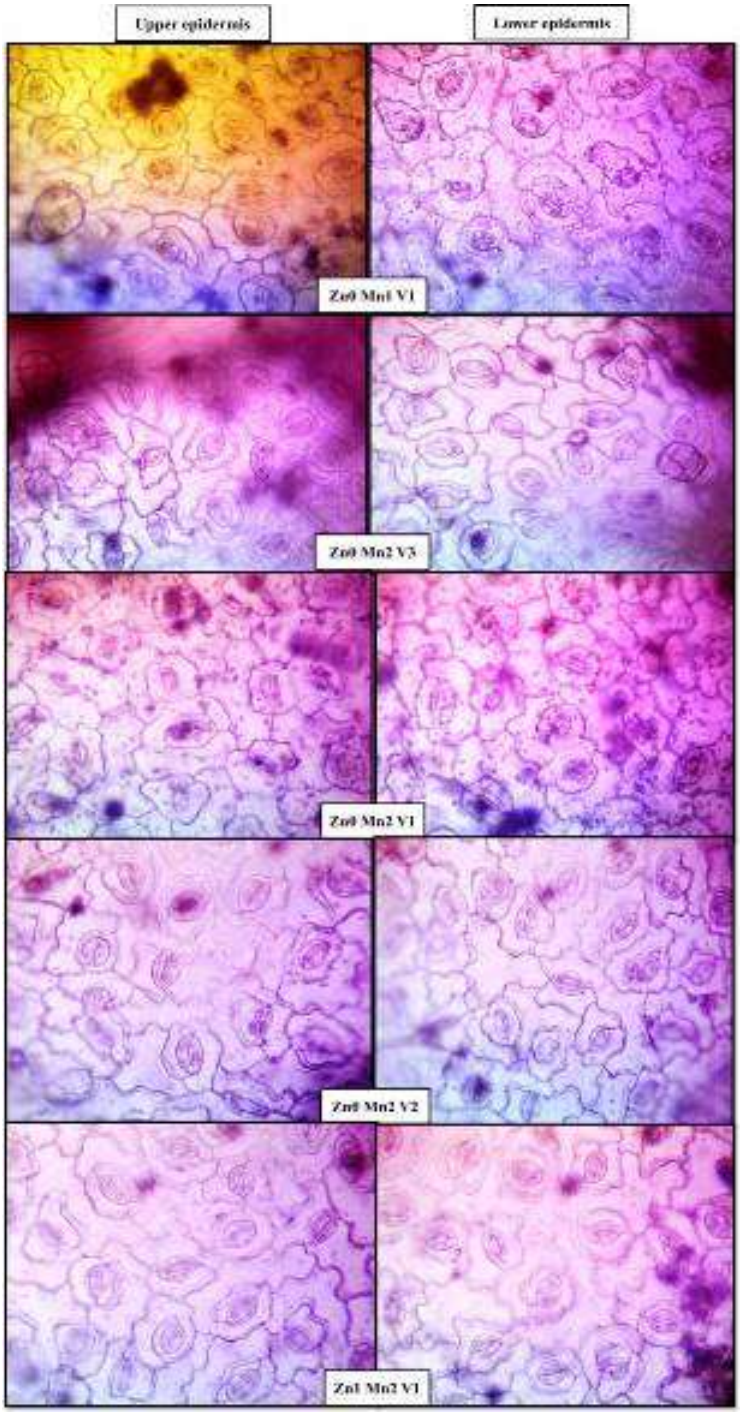

Figure 2. The shape of the stomata in the upper and lower epidermis.

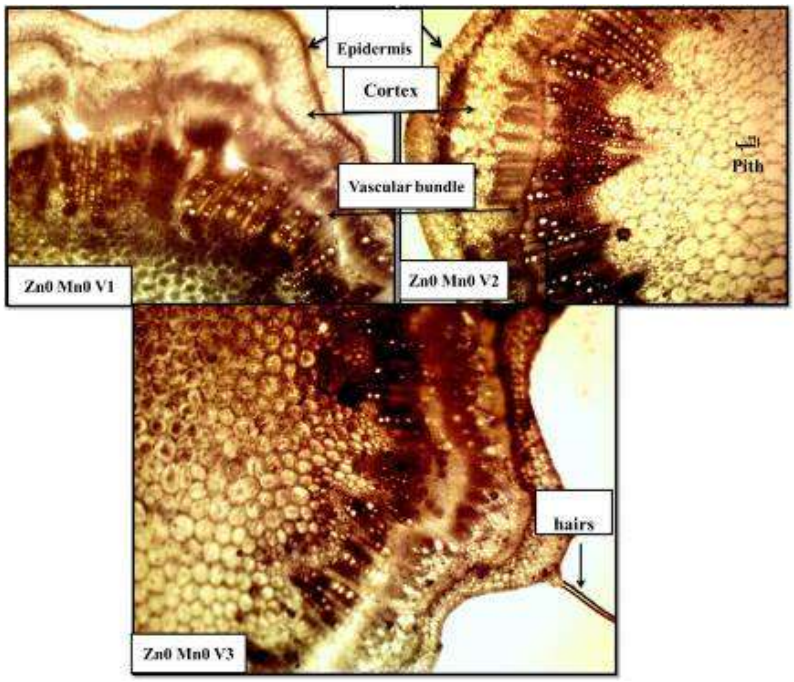

Figure 3. The shape of the vascular bundles in the species.

Table 1. Measurements of the stomata length in the upper epidermis (µm).

Zinc concentration (mg L^{-1})	Manganese concentrations (mg Mn L^{-1})	Genetic composition			Zn × Mn
		V1	V2	V3	
0	0	17.78	10.51	10.00	12.77
	30	12.58	14.43	15.28	14.10
	60	14.65	13.46	18.41	15.51
25	0	17.58	14.15	14.50	15.41
	30	14.20	15.70	15.38	15.09
	60	11.71	17.67	17.93	15.77
50	0	18.65	18.61	19.88	19.05
	30	13.97	14.38	15.12	14.49
	60	13.36	12.20	17.17	14.24
$LSD_{0.05}$	3.45				3.01
	Zinc concentration (mg L^{-1})	Genetic composition			Zn average
		V1	V2	V3	
V × Zn	0	15.01	12.80	14.57	14.12
	25	14.50	15.84	15.94	15.43
	50	15.33	15.07	17.39	15.93
$LSD_{0.05}$	3.02				N.S
	Mn concentration (mg L^{-1})	Genetic composition			Mn average
		V1	V2	V3	
Mn × V	0	18.01	14.43	14.79	15.74
	30	13.58	14.84	15.26	14.56
	60	13.24	14.44	17.84	15.18
$LSD_{0.05}$		1.44			0.70
Cultivars means		14.94	14.57	14.94	
$LSD_{0.05}$		0.91			

Table 2. Measurements of the stomata length in the lower epidermis (µm).

Zinc concentration (mg L^{-1})	Manganese concentrations (mg Mn L^{-1})	Genetic composition			Zn × Mn
		V1	V2	V3	
0	0	13.10	17.18	16.72	15.67
	30	13.35	18.68	17.82	16.62
	60	18.55	14.50	16.88	16.64
25	0	17.95	10.51	18.27	15.58
	30	15.28	10.00	14.43	13.24
	60	12.97	15.25	17.43	15.22
50	0	13.25	13.46	16.62	14.44
	30	17.60	15.35	13.27	15.41
	60	11.50	14.27	15.13	13.63
$LSD_{0.05}$	3.42				N.S
	Zinc concentration (mg L^{-1})	Genetic composition			Zn average
		V1	V2	V3	
V × Zn	0	15.00	16.79	17.14	16.31
	25	15.40	11.92	16.71	14.68
	50	14.12	14.36	15.01	14.49
$LSD_{0.05}$	2.09				N.S
	Mn concentration (mg L^{-1})	Genetic composition			Mn average
		V1	V2	V3	
Mn × V	0	14.77	13.72	17.20	15.23
	30	15.41	14.68	15.17	15.09
	60	14.34	14.67	16.48	15.17
$LSD_{0.05}$		2.01			N.S
Cultivars means		14.84	14.36	16.29	
$LSD_{0.05}$		0.92			

Table 3. Measurements of thickness of the vascular bundles in the stem (µm).

Zinc concentration (mg L^{-1})	Manganese concentrations (mg Mn L^{-1})	Genetic composition			Zn × Mn
		V1	V2	V3	
0	0	124.2	304.2	160.2	196.2
	30	146.8	185.9	179.0	170.6
	60	132.6	200.1	174.5	169.1
25	0	262.4	134.0	126.2	174.2
	30	227.1	100.9	183.0	170.3
	60	251.9	158.0	230.8	213.6
50	0	305.6	151.8	220.6	226.0
	30	250.9	274.1	253.3	259.4
	60	226.4	185.7	220.4	210.8
$LSD_{0.05}$	73.08				N.S
	Zinc concentration (mg L^{-1})	Genetic composition			Zn average
		V1	V2	V3	
V × Zn	0	134.5	230.1	171.2	178.6
	25	247.1	131.0	180.0	186.0
	50	261.0	203.8	231.4	232.1
$LSD_{0.05}$	44.99				31.69
	Mn concentration (mg L^{-1})	Genetic composition			Mn average
		V1	V2	V3	
Mn × V	0	230.7	196.6	169.0	198.8
	30	208.3	187.0	205.1	200.1
	60	203.6	181.3	208.5	197.8
$LSD_{0.05}$		41.11			N.S
Cultivars means		214.2	188.3	194.2	
$LSD_{0.05}$		N.S.			

highest percentage of genetic composition (14.10%). The manganese treatment at 0 mg L^{-1} for the cultivar Gold Star achieved the utmost genetic composition fraction (14.43%). Ali *et al.* (2009) also reported the Impact of spraying with potassium, zinc, and *Artemisia inculta* extracts on the flowering, setting, and anatomical features of *Vicia faba* L. plants (Table 2, Figure 2).

The results of the vascular bundles showed notable alterations in the thickness of the stem vascular bundle of the triple treatment among mung bean cultivars with different zinc and manganese concentrations (Table 3, Figure 3). The cultivar green Indian vc6089a10, with a zinc concentration of 50 mg L^{-1} and manganese concentration of 0 mg L^{-1}, obtained the maximum percentage of genetic composition (305.6%). The zinc treatment (50 mg L^{-1}) and genetic makeup of the cultivar Gold Star also achieved substantial differences, with the highest genetic composition proportion (231.4%). The manganese treatment at a 0 mg L^{-1} treatment for the mung bean cultivar Indian green vc6089a10 exhibited the topmost percentage of genetic composition (230.7%). The results agree with Hasan *et al.* (2018), stating that increasing the salicylic acid and elements also boost the thickness of the vascular bundles of the stem remarkably compared with the control treatment.

Metals are among the critical factors that negatively influence human beings and the environment, and their release into the surroundings causes toxicity to plants with their continual exposure to potentially toxic metals (Sanita-di-Toppi and Gabbrielli, 1999; Cakmak *et al.,* 2000; Al-Hadeethi, 2020, 2021). The researchers found proline accumulation is a widespread process among higher plants in response to zinc and other metal stress factors when treated at the Zn level (1 mM), with the rise of proline mainly observed in the roots of *Triticum aestivum* (Li *et al.,* 2013). Similarly, the same findings were parallel for *Solanum lycopersicum* tissues with an increase in Zn concentrations (0.05, 0.1, 0.15, and 0.2 mM) (Al-Khateeb and Al-Qwasemeh, 2014) and in *Vigna unguiculata* seedlings treated with Zn (0.25 and 0.5 mM) (Basha and Selvaraju, 2015; Khalaf and AL-Hadeethi, 2019).

The knowledge of Zn uptake and hyper-accumulation mechanism in plants is a considerable advancement in the present era. Some plants have developed a wide range of strategies for physiological adaptation to tolerate and hyper-accumulate the Zn in their aboveground parts. Several genes belonging to different families (HMA, ZIP, YSL, and MTP) have become distinct as being involved in Zn tolerance and hyper-accumulation, and transcriptomic analysis of contrasting populations has clarified the evolutionary mechanism of the Zn hyper-accumulation characteristics.

CONCLUSIONS

The interactions of zinc and manganese foliar applications and mung bean cultivars significantly influenced all growth parameters. However, the manganese treatment (60 mg L^{-1}) exceeded the characteristic of the lower stomatal width. The mung bean genotype Black Indian outperformed most cultivars for most growth traits.

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