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Zinc effect on growth rate, chlorophyll, protein and mineral contents of hydroponically grown mungbeans plant (*Vigna radiata*)

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Abstract Four varieties of mungbeans (Ramazan, Swat mungI, NM92 and KMI) from different research stations of KPK (Khyber Pukhtunkhwa) in Pakistan were grown hydroponically in pots containing sand giving nutrient solutions with and without Zn. Each variety was applied with Zn solutions at three levels i.e. 0, 1 and 2 μM concentrations. Plant samples were taken 2 months after transplant and the effect of Zn supply was observed on plant growth rate, protein, minerals and chlorophyll contents of mungbean leaves. Plant growth, chlorophyll contents, crude proteins and Zn contents were noted to be higher when greater supply of zinc doses was applied. Plant phosphorous contents declined with supply of Zn from 1 μM to 2 μM compared to the control signifying a Zn/P complex foundation possibly in roots of plant, preventing the movement of P to plant. Plant copper and Mg contents increased whereas Fe showed competitive behavior with Zinc while K, Na and Mn plant contents were non-significantly depressive with Zn increase from control to 2 μM. Zinc application at 2 μM concentrations in solution culture turned out to be the best treatment for improving the growth and quality parameters of mungbean.

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1. Introduction

Green revolution since the past few decades has increased the production of daily used food crops. Foods that were rich in protein and cheap in price were a demand for the poor living in developing countries (Ali et al., 1997). In this regard, pulses were found more versatile and appealing in providing protein rich diets, easy cultivation, long time storage and low price

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(Thirumaran and Seralathan, 1988; Rachie and Robert, 1974). Mungbean (*Vigna radiata*) also called green gram is an important summer-growing, pulse crop in Pakistan (Ahmad et al., 2003). The unique and common feature of mungbean is the root nodules that contain aerobic bacteria called rhizobia which fix atmospheric nitrogen in the root and thus enhance soil fertility (Ashraf et al., 2003). As far as medical applications are concerned mungbeans are used in the prevention of cancer and are also known to exhibit antimicrobial and insecticidal activities (Pookpakdi and Pataradilok, 1993).

Among other essential parameters, appropriate supply of micronutrients is also essential for proper growth and yield of crops. Their deficiency in soil is a large and growing problem in the developing world (Singh, 2009). When micro-nutrients become limited, water, fertilizers and other high-energy production inputs may be wasted. In Pakistan zinc scarcity in soil is the first most widespread problem. In the KPK (Khyber Pukhtunkhwa) province of Pakistan the extent of zinc deficient soils ranges from 21% to 77%. 42% of agricultural fields of Mansehra and Swat have zinc deficiency. On average 37% of fields are deficient in zinc (Khattak et al., 1995).

Zinc deficiency in plants affect photosynthesis due to altered chloroplast pigments (Kosesakal and Unal, 2009). The most visible zinc deficiency symptoms are short internodes and a decrease in leaf size and delayed maturity (Brown et al., 1993).

Hydroponics is the growing of plants without soil in nutrient solutions (Resh, 2001). Although it is not adopted on a large scale yet it is favored owing to its controlled conditions of nutrient availability for plant growth. Hydroponics technology can be adopted extensively for studies including nutrient uptake and their effect on interactions with other available nutrients (Chaoui et al., 1997). In this regard a simple sand holding system was developed and used for the study of zinc effect on physiochemical parameters of mungbean plants which would help to establish an effective dose of zinc for mungbean varieties cultivated in various regions of KPK, Pakistan.

2. Materials and methods

All the reagents used in this work were of analytical grade and were used as such. Double distilled water was used for solution preparation.

2.1. Sample collection

Samples of four certified high yielding and disease resistant varieties of mungbeans were obtained from various Agricultural Research Stations situated in Khyber Pukhtunkhwa Province of Pakistan. Among them NM-92 (V3) and KMI (V4) were obtained from Karak research station, mung swat1 (V2) from Mingora and Ramazan (V1) was obtained from NIFA (Nuclear institute for food and Agriculture) Peshawar. Seeds of each variety were sifted from dirt and malformed kernels and were then subjected to further studies.

2.2. Seed disinfection and germination

Seeds were then surface sterilized with 1% bleach ($NaHClO_4$) for 10 min. After bleaching the seeds were rinsed with distilled water thrice to remove excess of chlorine and then allowed to germinate on moist filter paper in dark at 25 °C for 2 leaf stage.

2.3. Hydroponic system

After two leaf stages of mungbean plants, culturing media were established which consisted of approximately 02 l of 12 plastic pots (03 treatments of each variety) in which sand was taken as the supporting material for small seedlings. The sand was washed properly with water in order to remove all the clay particles. Four small holes were made at the bottom of plastic pots; linen cloth was used to cover the holes. All the pots were then put in another plastic vessel. One tungsten bulb of 200 W and 3 tube lights (50 W) were fitted over the system for the supply of light. The germinated seedlings were then transplanted in these plastic pots in the hydroponic system.

The pots were divided into three groups where in each group 4 different cultivars were grown. To one group no $ZnSO_4$ solution was applied and to the second and third groups 1 μM and 2 μM $ZnSO_4$ solution was applied respectively.

2.4. Nutrient solution

Half strength Hoagland nutrient solution was used (Spomer et al., 1997). Macronutrient contents of nutrient solution given in Table 1 were prepared from the salts of KH_2PO_4, K_2SO_4, KCl, $Ca(NO_3)_2$, $MgSO_4$ while micronutrients were prepared from FeEDTA, $ZnSO_4$, $CuSO_4$, H_3BO_3, $MnSO_4$ and (NH_4) $6MoO_{24}$. Stock solutions were prepared and diluted to the required concentrations. Freshly prepared aqueous 0.666 M Fe EDTA was added to each pot for accomplishment of the iron requirement of the mungbean plants. The solution was changed after every 3 days.

2.5. Chlorophyll and protein determination

Plant samples were taken two months after transplant for growth measurement and analysis. The analysis of mungbean leaves for the chlorophyll content was performed on High Performance Liquid Chromatography, by the method of Heinonen (1990). Protein was determined by estimating the nitrogen content by the Kjeldahl method (Association of Official Analytical Chemists et al., 1990). The procedure consists of three basic steps, digestion of the sample in sulfuric acid with a catalyst which results in conversion of nitrogen to ammonia, distillation of the ammonia into a trapping solution; and quantification of the ammonia by titration with a standard solution. The percent of nitrogen contents and crude protein was calculated by using the relation:

$$\% \ N = \frac{(S - B) * N * 0.014 * D * 100}{W * V} \quad (1)$$

$$\% \ Crude \ Protein = \%N * F \quad (2)$$

where S denotes sample reading, B is the blank reading, D is the dilution factor; W weight of sample, V is the volume of titrant consumed and F is equal to 6.25.

2.6. Mineral analysis

The samples were digested with nitric acid and perchloric acid to release minerals for analysis. The inorganic phosphorous in the digested solution was determined by "molybdenum blue complex" reported by Murphy and Riley (1962). The blue

Table 1 Composition of nutrient solution.

Reagents	Stock molarity (C_1)	Final molarity (C_2)	ml of S. solution (V_1)
$Ca(NO_3)_2$	2 M	2.0 mM	0.500
KH_2PO_4	1 M	0.5 mM	0.250
K_2SO_4	1 M	0.7 mM	0.375
KCl	1 M	0.01 mM	0.005
$MgSO_4$	1 M	0.65 mM	0.325
Mn $SO_4.2H_20$	9.67 mM	1.00 μM	0.052
$ZnSO_4$	7.60 mM	1.00 μM	0.658
$CuSO_4$	0.14 mM	0.20 μM	0.714
H_3BO_3	46.00 mM	2.00 μM	0.020
$(NH_4)_6MoO_{24}$	0.05 mM	0.02 μM	0.200
FeEDTA	666 mM	20.0 μM	0.015

color of the phosphorous complex was measured on a spectrophotometer at 880 nm wavelength and the amount of unknown phosphate was determined from a standard plot of absorbance vs concentration (Fig. 1).

Zinc, iron, copper, magnesium and manganese were analyzed by using atomic absorption spectrophotometer model Perkin Elmer 3100 while sodium and potassium ions were measured using a flame photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK) according to the standard methods of Association of Official Analytical Chemists et al. (1990).

2.7. Statistical analysis

A completely randomized block design was used for the statistical analysis of the data. The analysis of variance of each variable was computed by the standard procedure (M-Stat C). Treatment means were compared by determining the least significant difference (LSD) at 5% level of probability ($P = 0.05$).

3. Results and discussion

3.1. Effect of Zn on plant height

Data pertaining to plant height of mungbeans treated with different Zn concentrations is presented in Table 2 and Fig. 2. The data indicated that maximum height was noted in all varieties of mung bean plants treated with 2 μM of Zn followed by 1 μM and control. Plant heights with 1 μM and 2 μM Zn treatments were statistically non-significant, but on the average were significantly 23.4% higher than that of control. Heights attained by V_1, V_2 and V_3 were similar, but were 8% higher than V_4. The plant height was positively correlated with zinc treatments ($r \geqslant 0.5$). A similar effect was reported by Alam and Shereen (2002) who observed the effect of different levels of zinc and phosphorous on wheat during water culture experiment and found that wheat shoot length was increased in almost all treatments as compared to the control. However, the result observed in the present case is in contrast to those reported by Ashok et al. (2010) on mungbean growth in soil. Comparing the literature observations of mungbean grown in soil with our system suggest an increase in tolerance limit of mungbean plant when grown hydroponically. Absorbed Zn content translocation to different parts of plant fulfills the plant requirement for proper plant growth.

3.2. Effect of Zn on chlorophyll content

Table 3 presents the data regarding chlorophyll contents of mungbean plants treated with different Zn doses. Mean chlorophyll contents ranged from 45.69 to 184.4 mg kg^{-1}. The data revealed that maximum chlorophyll content was found in all varieties treated with 2 μM of Zn. The chlorophyll content with 1 μM of zinc was statistically similar to that with 2 μM of zinc application and on the average was 100% more than the control. As regards varietal effects it was found that chlorophyll content of V_4 was statistically at par with V_3 but significantly 49 and 303% higher than that of V_2 and V_1 respectively. The correlation of chlorophyll content with external zinc treatments was however, negligible ($r < 0.5$). A similar behavior was also reported by Khalil et al. (1998) on beans grown hydrophonically. His studies reflected that zinc deficient leaves appeared light green due to the low concentration of chlorophyll. Hisamitsu et al. (2001) investigated that zinc deficiency disrupted the chlorophyll synthesis. Increased chlorophyll contents are due to zinc which acts as a structural and catalytic component of proteins, enzymes and as co-factor for normal development of pigment biosynthesis (Balashouri, 1995). Unlike the above findings, field studies in corn suggested that Zn inhibited the chlorophyll production by interfering with Fe metabolism, but not by lowering the Fe content of the leaves (Rosen et al., 1977). The results of soil study are not compatible with solution culture which may be due to the fact that in a hydroponics system delivery of a perfectly balanced nutrient solution to the roots takes place

Table 2 Plant height (cm) on dry weight basis in mungbean varieties at different concentrations of Zn in solution culture.

Zn treatments	V1	V2	V3	V4	Mean ± St. dv
Control	19.60b	19.93d	19.53bc	13.03e	18.02b 3.33
1 μM	22.94a	22.60a	22.70a	20.73 cd	22.24a 1.02
2 μM	23.18a	23.00a	23.20a	21.03bc	22.60a 1.05
Mean ± St.d v	21.91a	21.84a	21.81a	20.27b	
	2.00	1.67	1.99	4.53	

V1 = Ramazan, V2 = Swat mungI, V3 = NM92, V4 = KMI.
St. dv = standard deviation.
The mean followed by similar letter (s) are not significantly different at $P = 0.05$.

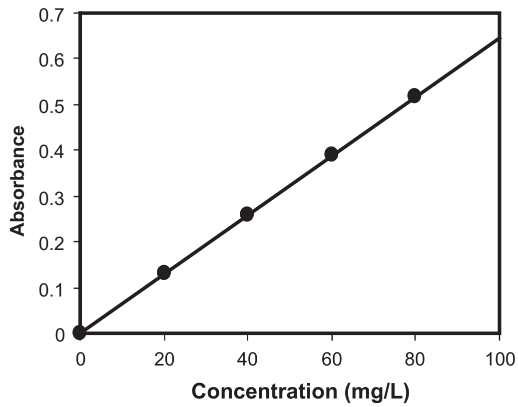

Figure 1 Standard curve for the determination of phosphate concentration at 880 nm.

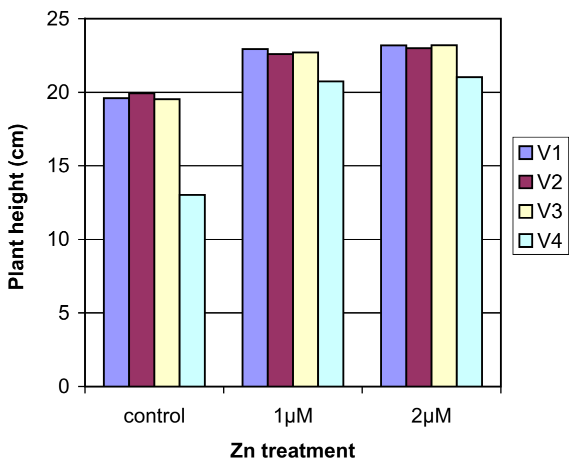

Figure 2 Plant height as a function of Zn treatments.

in a highly soluble form which enables the plant to absorb food with very little effort in contrast to soil where the roots must look for the nutrients and extract them.

3.3. Effect of Zn on crude protein content

The mean crude protein contents (Table 4) of mungbean varieties were non-significantly different from each other and showed a range of 15.52–18.02%. On average a gradual increase to the extent of 28.2 to 72.3% was noted in crude protein contents in all the varieties compared to control with an increase in the dose of Zn. Correlation between different zinc treatments and crude protein was positive (r = 0.896). These findings were in accordance with Hisamitsu et al. (2001). They observed that zinc deficiency affects nitrogen metabolism in corn plant. Krishna (1995) also reported a significant positive effect of zinc treatment on crude protein content in the seeds of mungbean. Zinc is required as structural and catalytic components of protein and enzymes for normal growth and development (Broadley et al., 2007). In contrast Sagardoy et al. (2009) observed the antagonistic effect of Zn along with N in sugar beet (*Beta vulgaris L.*) plants grown in hydroponics.

3.4. Effect of Zn on P and Fe contents

Fig. 3 shows that the mean phosphorous contents of Mungbean varieties ranged from 3.1 to 6.45 g/kg. All the varieties showed a maximum of phosphorous contents in the control which drops with external Zn application. Phosphorous content of V_2 was maximum, followed by that of V_3 and V_4 statistically at par in between and the least in V_1. Tissue P of V_2 was higher by 57.7%, 52.4% and 108% than V3, V4 and V1. Zn treatment also affected tissue P significantly and got decreased as the Zn concentration increased. P was maximum in the control and decreased by 29.9 and 41.7% with 1 μM and 2 μM Zn respectively. Correlation between tissue P and Zn treatments was also negative (r = −0.676). Zn and P are observed to interact and may interfere with the availability and utilization of each other. High Zn uptake efficiency may depress root phosphorous uptake and may also involve in a high rate of Zn transport from roots to shoot via the xylem, and this may hinder P translocation from roots to shoot. These conclusions are supported in the literature (Zhu et al., 2001; Keram et al., 2012).

It is also observed that like P, Zn application has an adverse effect on Fe contents and Fe uptake in Mungbean plants (Table 5). Plant Fe was reduced by 7 and 22% from control with supply of 1 to 2 μM Zn. The decrease of Fe may be due to competitive interactions with Zn which probably occur at the absorption sites of plant roots. Similar conclusions were reported by Loneragan and Webb (1993), Rajaie et al. (2009). Zn strongly influences the iron metabolic function in plants, if one is present in excess the uptake of other may depress (Francois and Goodin, 1972). The same phenomenon is followed in the present system as the concentration of external Zn is increased.

Table 3 Chlorophyll contents (mg kg^{-1}) on dry weight basis in mungbean varieties at different concentrations of Zn in solution culture.

Zn Treatments	V1	V2	V3	V4	Mean ± St. dv
Control	35.7f	73.45de	93.12 cd	105.93c	78.55b 30.63
1 μM	36.81f	145.30b	210.82a	221.01a	153.5a 84.71
2 μM	64.54e	146.07b	210.57a	226.08a	161.9a 73.52
Mean ± St. dv	45.69c 16.34	123.6b 41.71	171.5a 67.88	184.4a 67.95	

V1 = Ramazan, V2 = Swat mungI, V3 = NM92, V4 = KMI.
St. d = standard deviation.
The mean followed by similar letter (s) are not significantly different at P = 0.05.

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Table 4 Percent crude protein (dry weight basis) in mungbean varieties at different concentrations of Zn in solution culture.

Zn Treatments	V1	V2	V3	V4	Mean ± St. dv
Control	12.90f	11.76f	13.95ef	11.54f	12.54c 1.11
1 μM	13.12f	16.45de	17.62bcd	18.12 cd	16.08b 2.25
2 μM	20.54ab	22.86a	20.99a	22.05abc	21.61a 1.05
Mean ± St. dv	15.52a	17.02a	18.02a	17.24a	
	4.35	5.57	3.52	4.32	

V1 = Ramazan, V2 = Swat mungI, V3 = NM92, V4 = KMI.
St. d = standard deviation.
The mean followed by similar letter (s) are not significantly different at $P = 0.05$.

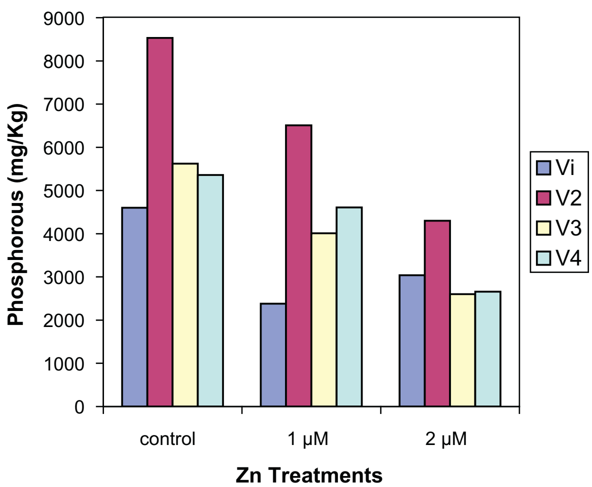

Figure 3 Plant Phosphorous contents as a function of Zn treatments.

Table 5 Mean values (mg/kg) of nutrients contents for Mungbean plant.

Zn (μM)	Na	K	Mg	Mn	Fe	Cu
Control	940	25537	3570	244.55	144.04	15.75
1	870	23547	4070	206.02	133.8	22.55
2	880	21957	3610	219.77	11.46	44.86

3.5. Effect of Zn on plant Zn contents

The results presented in Fig. 4 show the mean Zn contents of mungbean varieties which range from 55.97 to 127.01 mg/kg. The average Zn contents of V_4 are significantly higher by 126.9%, 13.5% and 27.7% than V_1, V_2 and V_3. Perusal of the data demonstrated further that plant Zn contents increased significantly with Zn application in the rooting media, giving a maximum increase of 496.6% with 2 μM of Zn followed by 404.9% with 1 μM Zn. The zinc uptake was positively correlated ($r = 0.787$) with zinc treatments. Similar data were also reported by Zhao et al. (1998). They studied the relationship between Zn and P in the Zn hyperaccumulator *Thlaspi caerulescens* using hydroponic culture and investigated that total Zn contents in the shoots increased with the increased application of external zinc.

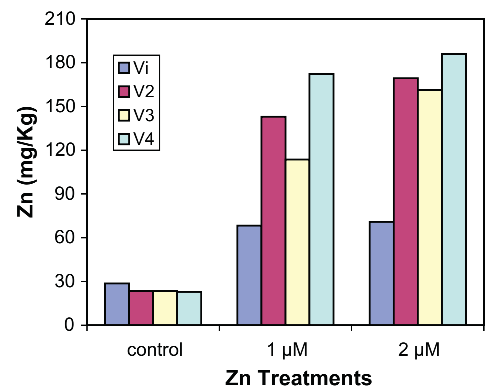

Figure 4 Plant Zinc contents as a function of Zn treatments.

3.6. Effect of Zn on plant Na, K, Mg, Mn and Cu contents

Table 5 presents the mean data regarding the effect of Zn on Na, K, Mg, Mn and Cu contents of mungbean plant which shows that Zn has a non-significant depressing effect on Na, K and Mn while Cu contents increase with increasing Zn levels to 2 μM. Whereas, the Mg effect is somewhat non-uniform, it increases sharply with 1 uM Zn supply over control and then declines with 2 μM Zn supply. The lower concentration of Mg might be due to the physiological response of the plant to the highest Zn concentration in solution which may have affected the uptake system and thus lowered the apparent concentration. Bonnet et al. (2000) also observed similar results for Mg contents by Ryegrass grown in solution culture. In contrast the friendly behavior of Cu with Zn supply in our case is anomalous to those observed by Bowen et al. (1979) who suggested on observation of data in their studies that both Cu and Zn are absorbed through same mechanism and might suppress the other if one is present in excess.

4. Conclusions

All mungbean varieties attained a greater plant height at 2 μM Zn solution. Increase was the highest in Ramazan followed by Swat mung1 and the least in KM1. Zn application also increased plant chlorophyll and protein contents in control. The chlorophyll content in NM92 and KM1 was higher than that of Swat mung1 and Ramazan respectively. The plant protein content was maximum in swat mung1 and minimum in Ramazan. NM92, Swat mung1 and KM1 varieties showed a higher percent protein than Ramazan. Plant P, K and Na

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decreased with Zn supply while Mg was increased only upto 1 μM Zn supply. Plant Zn and Cu contents were also increased with Zn application. However, plant Mn and Fe were decreased with Zn supply.

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