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 Pakhtunkhwa) 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 l M 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 l M to 2 l M 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 l M. Zinc application at 2 l M 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.
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 requirement of the mungbean plants. The solution was changed after every 3 days.

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 KH₂PO₄, K₂SO₄, KCl, Ca(NO₃)₂, MgSO₄ while micronutrients were prepared from FeEDTA, ZnSO₄, CuSO₄, H₃BO₃, MnSO₄ and (NH₄)₂ 6MoO₄. 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:

\[
\% \text{ Crude Protein} = \frac{\text{Sample Weight}}{\text{Dilution Factor}} \times 6.25 \times \frac{V}{W} 
\]

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 phosphorus in the digested solution was determined by “molybdenum blue complex” reported by Murphy and Riley (1962). The blue
color of the phosphor 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 mungbean plants 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 ($r \geq 0.5$). 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 hydroponically. 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 (Balashouiri, 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>
<thead>
<tr>
<th>Zn treatments</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>V4</th>
<th>Mean ± St. dv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.60b</td>
<td>19.93d</td>
<td>19.53bc</td>
<td>13.03e</td>
<td>18.02b 3.33</td>
</tr>
<tr>
<td>1 μM</td>
<td>22.94a</td>
<td>22.60a</td>
<td>22.70a</td>
<td>20.73 cd</td>
<td>22.24a 1.02</td>
</tr>
<tr>
<td>2 μM</td>
<td>23.18a</td>
<td>23.00a</td>
<td>23.20a</td>
<td>21.03bc</td>
<td>22.60a 1.05</td>
</tr>
<tr>
<td>Mean ± St. dv</td>
<td>21.91a</td>
<td>21.84a</td>
<td>21.81a</td>
<td>20.27b</td>
<td>21.90a 4.53</td>
</tr>
</tbody>
</table>

V$_1$ = Ramazan, V$_2$ = Swat mungI, V$_3$ = NM92, V$_4$ = KMI.
St. dv = standard deviation.
The mean followed by similar letter (s) are not significantly different at $P = 0.05$. 

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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 \( V_3, V_4 \) and \( V_1 \). 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 \( \mu \)M and 2 \( \mu \)M 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 \( \mu \)M 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>
<thead>
<tr>
<th>Table 3 Chlorophyll contents (mg kg(^{-1})) on dry weight basis in mungbean varieties at different concentrations of Zn in solution culture.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zn Treatments</strong></td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>1 ( \mu )M</td>
</tr>
<tr>
<td>2 ( \mu )M</td>
</tr>
<tr>
<td>Mean ± St. dv</td>
</tr>
</tbody>
</table>

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 \( P = 0.05 \).
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 V4 are significantly higher by 126.9%, 13.5% and 27.7% than V1, V2 and V3. 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 lM of Zn followed by 404.9% with 1 lM 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.

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 µM 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

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

<table>
<thead>
<tr>
<th>Zn Treatments</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>V4</th>
<th>Mean ± St. dv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.90f</td>
<td>11.76f</td>
<td>13.95ef</td>
<td>11.54f</td>
<td>12.54c 1.11</td>
</tr>
<tr>
<td>1 µM</td>
<td>13.12f</td>
<td>16.45de</td>
<td>17.62bcd</td>
<td>18.12 cd</td>
<td>16.08b 2.25</td>
</tr>
<tr>
<td>2 µM</td>
<td>20.54ab</td>
<td>22.86a</td>
<td>20.99a</td>
<td>22.05abc</td>
<td>21.61a 1.05</td>
</tr>
<tr>
<td>Mean ± St. dv</td>
<td>15.52a</td>
<td>17.02a</td>
<td>18.02a</td>
<td>17.24a</td>
<td>15.88a 1.35</td>
</tr>
</tbody>
</table>

V1 = Ramazan, V2 = Swat mung1, V3 = NM92, V4 = KM1. St. d = standard deviation. The mean followed by similar letter (s) are not significantly different at P = 0.05.
decreased with Zn supply while Mg was increased only up to 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.

References


