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RESEARCH ARTICLE

Effect of Zinc deficiency on growth and biochemical characters of barnyard millet lines

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ABSTRACT

Zinc (Zn) deficiency is one of the important abiotic factors limiting crop productivity worldwide and also a nutritional disorder affecting human health. Barnyard millet, one of the minor millets, which is known for its high nutritional value compared to other major cereals. To observe the effects of Zn on growth and biochemical parameters of two contrasting barnyard millet lines MDU1 and ACM-16-5, a hydroponic experiment was conducted. The barnyard millet lines selected for this study differ from each other in Zn accumulation in their seeds. The research was comprised of three treatments *viz.,* control, minimal Zn deficiency (25% of control), and Zn deficiency (completely devoid of zinc). The experiment was conducted in a completely randomized design with three replications. The sample collection for biochemical analysis and growth parameters recordings was done after ten days of stress imposition. A significant impact of Zn deficiency was observed between the two barnyard millet lines. Comparing the performance of MDU1 and ACM-16-5, MDU1 performed better than ACM-16-5 due to the efficient uptake and utilization of micronutrients, particularly Zn, under control and minimal Zn deficit condition. It is clear from our findings that zinc influence the morphological and physiological processes of MDU1 and ACM-16-5.

Keywords: *Zinc, hydroponics, Zn deficiency, root growth.*

INTRODUCTION

Zinc (Zn) is an essential micro-nutrient required for plant growth and development (Marschner 1993). In plants, the average concentration of Zn ranges from 20 to 150 ppm. Zinc deficiency occurs in crop plants when its level falls below 20 ppm and toxicities will arise when the Zn leaf concentration exceeds 400 ppm (Tisdale *et al*. 1993). Zn is involved in a number of plant physiological processes such as hormone regulation like tryptophan synthesis which is a precursor of IAA, signal transduction via the mitogen-activated protein kinases (Lin *et al*. 2005; Hansch and Mendel 2009), repairing processes of PS II complex during photo-inhibition (Bailey *et al*. 2002; Hansch and Mendel, 2009) and maintaining CO_2 concentration in mesophyll. Zn is the second most used transition metal, and it won't undergo a redox state change (i.e., gain or loss of electrons) like Fe and Cu. Deficiency of Zn in plants is associated with chlorosis in younger leaves, necrotic spots, bronzing, rosetting in dicots, stunting, dwarf and malformed leaves. It is the only metal present in all six enzyme classes such as oxidoreductase, transferase, hydrolases, lyases, isomerases and ligases.

Zinc is taken up mainly as a divalent cation (Zn^{2+}) by plant roots. Also, plants uptake Zn in the form of organic ligand-Zn complexes. Based on the secretion of ligands by plant roots, two physiological strategies are involved in the uptake of Zn. Strategy I involves the efflux of organic acids, reductants and H^+ ions, that enhance the solubility of Zn-complexes and release Zn^{2+} ions for absorption by root epidermal cells. Strategy II involves the efflux of phytosiderophores from the roots, which forms stable complexes with Zn and allows the subsequent influx of Zn into the root epidermal cells. Plants that belong to the poaceae family adopt strategy II for Zn uptake. (Neha Gupta *et al*., 2016).

Furthermore, the accumulation of heavy metals like Zn is a complex physiological trait that is governed by the collective expression of uptake, transport, distribution and sequestration in different plant parts (Singh *et al*., 2016). The differences in the ability of the cultivars in Zn uptake and the concentrations between organs, tissues and

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intracellular compartments within the plant system was caused by variations in Zn uptake, translocation and utilization, synergistic and antagonistic effect of other mineral nutrients with Zn and differences in plant root system for Zn utilization (Tisdale *et al*. 1993). This fluctuation arises due to the differential expression of metal transporter proteins and intracellular binding sites in a particular organ. In the present study, the differential response of barnyard millet lines under control and Zn deficit condition was studied with the two contrasting lines MDU 1 (high seed Zn accumulator) and ACM 16-5 (low seed Zn accumulator) for Zn accumulation in hydroponics.

MATERIAL AND METHODS

Plant materials

Two contrasting lines (MDU1 and ACM-16-5) of barnyard millet, with different Zn accumulation levels, were used in this study, the former being high seed Zn accumulating line and latter is a low seed Zn accumulator (Unpublished data).

Plant cultivation

Following surface sterilization of seeds with deionized water, seeds were germinated for 14 days in the sand for easy separation of roots during transplanting. Then, uniform seedlings were transplanted to the solution culture (Kimura B solution). The Kimura B solution contained the following macronutrients (mM/L): $(NH_4)_2SO_4$ (0.36), $MgSO_4{\cdot}7H_2O$ (0.27), KNO_3 (0.18), $CaNO_3.H_2O$ (0.35), KH_2PO_4 (0.14); and micronutrients such as HCL (1.4×10^{-3}), H_3BO_3 (6×10^{-3}), $MnSO_4.H_2O$ (2×10^{-3}) $(Na)_2MoO_2{\cdot}2H_2O$ (4×10^{-4}), $ZnSO_4{\cdot}7H_2O$ (2×10^{-3}), $CuSO_4.5H_2O$ (4×10^{-4}), and Fe(II)-EDTA (6×10^{-2}), with the pH adjusted to 5.2. HCl or NaOH was used to maintain the pH (5.2) of the nutrient solution.

Fourteen days old seedlings were transplanted to medium containing control solution (with $ZnSO_4.7H_20$) and maintained for ten days. After the growth of seedlings in control solution, a set of plants were subjected to minimal Zn deficiency stress (25% of control) (5×10^{-4}mM/L) and complete Zn deficiency stress (without $ZnSO_4.7H_20$). All control and stressed plants were grown concurrently for ten days after treatment was imposed and harvested at the same time. The nutrient solution was renewed every four days. In this experiment, all parameters were measured after imposing Zn deficiency for ten days.

Measurement of growth and biochemical parameters

Shoot length: The length of the shoot was measured from the collar region to the tip of the longest leaf and expressed in cm.

Root length: Root length was measured from the base of the stem to the tip of the longest root and expressed in cm.

Total chlorophyll content: Total chlorophyll content in leaves was estimated using the method describes by Hiscox and Israelstam (1949) and expressed in mg g^{-1} fresh weight.

Photosynthetic rate: Leaf exchange measurements were performed using Portable Photosynthesis System (PPS) (Model LI-6400, LICOR inc., Lincoln, Nebraska, USA). Totally, three measurements were taken in the same leaf. Leaves were inserted in a $3cm^2$ leaf chamber and PPFD at 1200 µmol photons $m^{-2}s^{-1}$ and relative humidity (50-55%) were set in the instrument. The readings were taken between 9 am to 11.30 am. Photosynthetic rate was expressed in µmol CO_2 $m^{-2}s^{-1}$.

Nitrate reductase activity: Nitrate reductase activity was estimated as per the method described by Nicholas *et al*. (1976) and expressed as µg NO_2 $g^{-1}hr^{-1}$ fresh weight.

Statistical analysis

Significant differences between MDU1 and ACM-16-5 under different treatments were evaluated using Student's t-test ($p<0.01$, $p<0.05$) are shown.

RESULTS AND DISCUSSION

Effect of Zn deficiency on shoot and root length (cm) of barnyard millet lines

Shoot length (cm) of MDU1 and ACM-16-5 grown under control, minimal Zn and complete Zn deficiency was recorded and showed in Figure 1A.

Table 1. Shoot length of barnyard millet lines MDU1 and ACM-16-5 grown under control, minimal Zn deficiency and complete Zn deficiency

	MDU1	ACM-16-5
Control	46 0.9	44.4 0.7
Minimal Zn deficiency	45.4 0.2	42.9 0.9*
Zn deficiency	41.5 0.2	36 0.3**

Significant differences between MDU1 and ACM-16-5 was analyzed by Student t-test (*P<0.05,**P<0.01)are shown

Significant differences in shoot length were observed between MDU1 and ACM-16-5 in minimal and complete Zn deficiency but not in control. Under Zn deficiency, the high Zn accumulating barnyard millet line MDU1 (Unpublished data) had better shoot length compared to ACM-16-5. Deficiency of Zn in the culture solution and reduced Zn content in ACM-16-5 might be the reason for reduced shoot length. Zn deficiency causes reduced auxin biosynthesis, a hormone that is responsible for plant growth stimulation. The reports of Alloway (2004), Brennan (2005) and Hafeez *et al*. (2012) stated that

Zn is highly essential for tryptophan biosynthesis, which is a precursor of Indole-3-Acetic acid and also has an active role in the synthesis of a vital growth hormone auxin.

Table 2. Root length of barnyard millet lines MDU1 and ACM-16-5 grown under control, minimal Zn deficiency and complete Zn deficiency

	MDU1	ACM-16-5
Control	35.3 1.1	24.2 1.5**
Minimal Zn deficiency	31.6 1.2	21.1 1.0**
Complete Zn deficiency	23.9 1.3	17.6 0.1**

Significant differences between MDU1 and ACM-16-5 was analyzed by Student t-test (*P<0.05,**P<0.01)are shown

Since Zn is a co-factor for various enzymes, it induces the activity of the enzyme, which is involved in cell reproduction and enlargement (Rion and Alloway, 2004).

Table 3. Total chlorophyll content of barnyard millet lines MDU1 and ACM-16-5 grown under control, minimal Zn deficiency and complete Zn deficiency

	MDU1	ACM-16-5
Control	2.7 2.1	2.6 1.5*
Minimal Zn deficiency	2.4 2.5	2.1 1.0*
Zn deficiency	1.8 2.3	1.4 0.7**

Significant differences between MDU1 and ACM-16-5 was analyzed by Student t-test (*P<0.05,**P<0.01)are shown

Significant differences between the root length of barnyard millet lines MDU1 and ACM-16-5 was observed in all the treatments (Figure 1B).

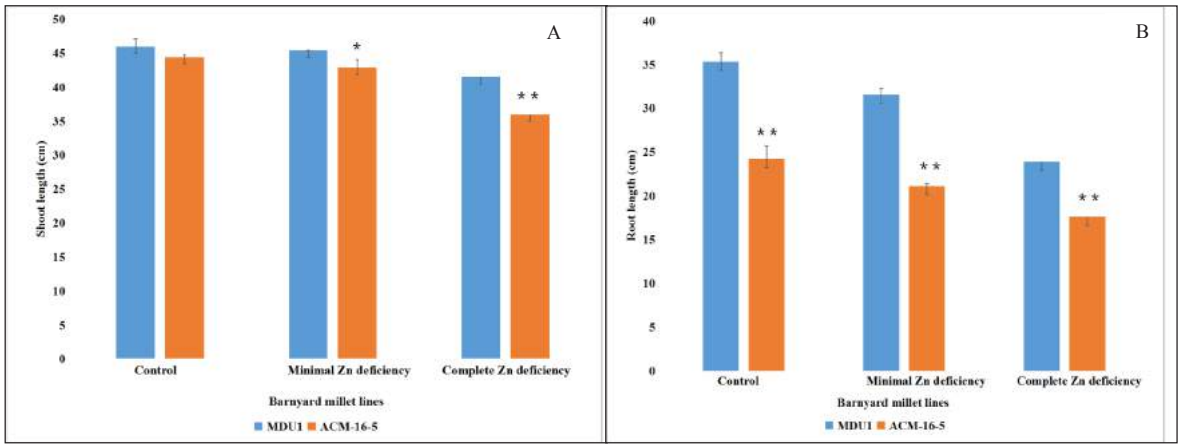

Figure 1A&B. Shoot length (A) and Root length (B) of barnyard millet lines MDU1 and ACM-16-5 grown under control, minimal Zn deficiency and complete Zn deficiency conditions. Data represent mean± SE of mean of four independent replications. Significant differences between MDU1 and ACM-16-5 was analyzed by Student's t-test (*P<0.05, P<0.01) are shown.

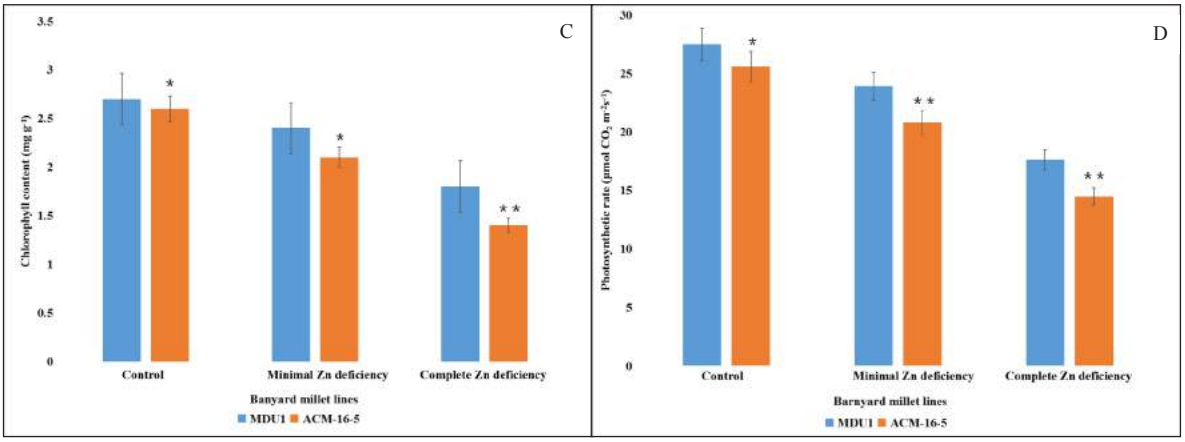

Figure 1C&D. Total chlorophyll content (C) and Photosynthetic rate (D) of barnyard millet lines MDU1 and ACM-16-5 grown under control, minimal Zn deficiency and complete Zn deficiency conditions. Data represent mean± SE of mean of four independent replications. Significant differences between MDU1 and ACM-16-5 was analyzed by Student's t-test (*P<0.05, P<0.01) are shown.

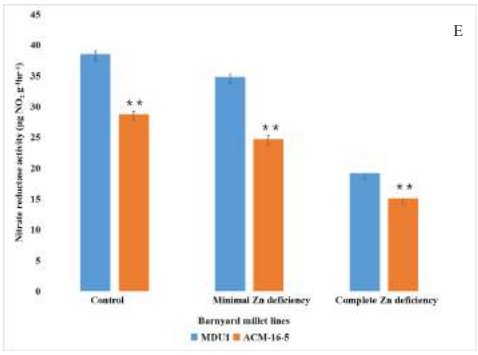

Figure 1E. Nitrate reductase activity of barnyard millet lines MDU1 and ACM-16-5 grown under control, minimal Zn deficiency and complete Zn deficiency

conditions. Data represent mean± SE of mean of four independent replications. Significant differences between MDU1 and ACM-16-5 was analyzed by Student's t-test (*P<0.05, P<0.01) are shown.

Compared to control and minimal Zn deficiency condition, the root length was reduced in complete Zn deficiency condition, irrespective of lines studied.

Table 4. Photosynthetic rate of barnyard millet lines MDU1 and ACM-16-5 grown under control, minimal Zn deficiency and complete Zn deficiency

	MDU1	ACM-16-5
Control	27.5 1.7	25.6 1.5*
Minimal Zn deficiency	23.9 1.5	20.8 0.9**
Zn deficiency	17.6 0.8	14.5 0.8**

Significant differences between MDU1 and ACM-16-5 was analyzed by Student t-test (*P<0.05,**P<0.01)are shown

Even under complete Zn deficiency condition, in barnyard millet line MDU1 showed significant root growth which is due to internal Zn content. As reported by Cakmak (2000), Zn is involved in the biosynthesis of growth-promoting hormone such as Indole-3-Acetic Acid (IAA), in turn, auxin is involved in development of better root architecture by promoting primary root elongation, lateral root primordial formation, lateral root elongation and tropic growth (Cakmak, 2000; Yu *et al*., 1999). Zinc also serves as an activator of many enzymes involved in cell division and elongation (Teale *et al*. 2006).

Table 4. Photosynthetic rate of barnyard millet lines MDU1 and ACM-16-5 grown under control, minimal Zn deficiency and complete Zn deficiency

	MDU1	ACM-16-5
Control	38.5 0.6	28.8 0.5**
Minimal Zn deficiency	34.8 0.4	24.7 0.6**
Zn deficiency	19.2 0.3	15.1 0.4**

Significant differences between MDU1 and ACM-16-5 was analyzed by Student t-test (*P<0.05,**P<0.01)are shown

Effect of Zn deficiency on chlorophyll content, photosynthetic rae and nitrate reductse activity of barnyard millet lines

The chlorophyll content of barnyard millet lines MDU1 and ACM-16-5 grown in control, minimal Zn deficiency condition and complete Zn deficiency condition were showed in Figure 1C. The total chlorophyll content was varied significantly between the lines across all the treatments. Even though significant reduction of chlorophyll content was

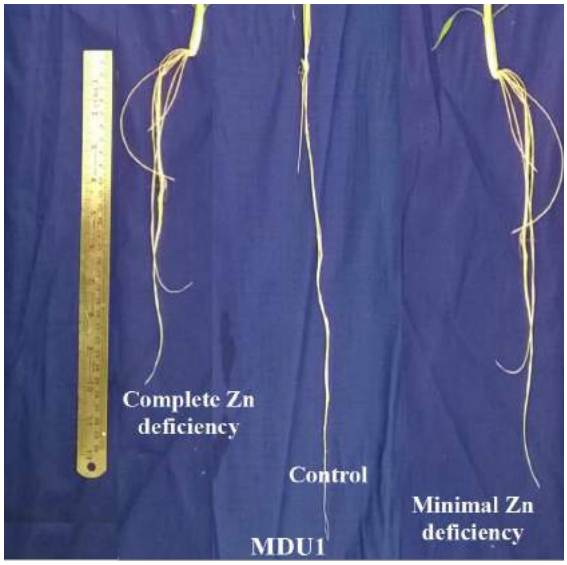

Figure 2. Root length of barnyard millet lMDU1 grown under control, minimal Zn deficiency and Complete Zn deficiency

observed in all the treatments, the maximum reduction was found under complete Zn deficit condition. The barnyard millet line ACM-16-5 was highly affected due to its inability to accumulating more Zn in their plant system compared to MDU1. Zn is an essential element required for maintaining the enzyme activity and ion transport system, which preserves the structural integrity of cellular membranes and macro-molecules involved in the electron transport chain (Dang et al., 2010). Its deficiency increases free radicals and disrupts the membrane integrity of the chloroplast, thereby reducing the photosynthetic capacity Fu et al. (2015). Damages in the thylakoid membrane due to Zn deficiency leads to changes in the chloroplast ultrastructure. Zn-deficient plants usually have reduced leaf chlorophyll (Chl) concentration and lower Chl a:b ratio, which indicates damage to the intrinsic quantum efficiency of the photosystem-II (PSII) units (Chen et al. 2008a). Zn deficiency affects the activity of enzymes such as carbonic anhydrase, which is essential for the activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Srivastava and Gupta, 1996; Storey 2007), catalyzing the diffusion of CO_2 through the cell to the chloroplasts (Hatch and Slack, 1970). Zn

deficiency reduces plant growth by decreasing photosynthetic rate (Henriques, 2001), disorganization of chloroplast membrane and thylakoids, reduces photochemical efficiency of PSII (Donnini et al. 2013).

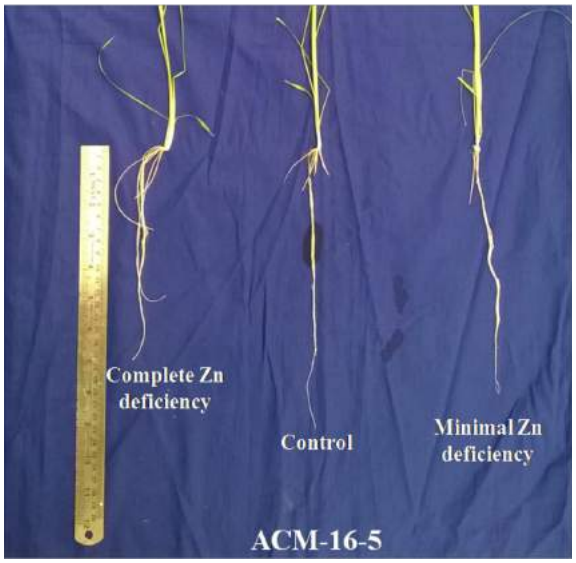

Figure 3. Root length of barnyard millet lineACM-16-5 grown under control, minimal Zn deficiency and Complete Zn deficiency

The effect of control, minimal Zn deficiency and complete Zn deficiency on the photosynthetic rate of barnyard millet lines MDU1 and ACM-16-5 are shown in Figure 1D. It was obvious from the figure that the photosynthetic rate was varied significantly among treatments. The maximum reduction of photosynthetic rate was reorded in plants under Zn deficiency, particularly in line ACM-16-5. As reported by Fu *et al*. (2015), the reactive site of photosynthesis is chloroplast. Zn deficiency leads to extensive damage of the chloroplast membrane and disorganization of thylakoids, which finally resulted in reduced photosynthetic rate. Also, Zinc deficiency reduces the capacity of stomatal response for carbon fixation, in turn, reduces the photosynthetic rate by decreasing the inter-cellular CO_2 concentration (Sharma *et al*. 1994). The above findings was similar to Sharma *et al*. (1995) on the significant role of Zn in the regulation of the stomatal aperture, which is responsible for maintaining a high K^+ content in guard cells. In addition, Zn deficiency reduces the activity of carbonic anhydrase, which is attributed to reduced net photosynthetic rate (Hacisalihoglu *et al*. 2003). Sasaki *et al*. (1998) and Marschner (1995) reported that, in Zn-deficient plants, a decrease in both CO_2 assimilation and Rubisco activity was primarily due to ROS-induced damage to the photosynthetic apparatus.

Nitrate reductase activity was highly influenced by Zn deficiency and it is shown in Fig 1E. Significant differences were observed between the treatments and also within the lines. Compared to other treatments, the maximum reduction of nitrate reductase enzyme was found under complete Zn deficiency. Seethambaram and Das (1986) also reported that the nitrate reductase enzyme activity was lowered under Zinc deficiency. This might be due to the result of decreased net photosynthesis, results in the shortage of NADH, by which the NR enzyme reduces nitrate to nitrite after accepting electrons from NADH. Seethambaram (1983) also reported that the shortage of ferredoxin due to lowered electron transport under zinc deficiency would lead to the suppression of nitrate reductase. Nason *et al*. (1951) observed that Zn deficiency indirectly influenced the activity of nitrate reductase by less nitrate uptake.

CONCLUSION

Results from our work indicated that the barnyard millet line MDU1 performed better even under Zn deficit condition compared to ACM-16-5. The differential response of the two lines under control, minimal and complete Zn deficit condition could be due to differences in the Zn uptake, transport of absorbed Zn from root to shoot and in Zn sequestration. The tight regulation of Zn absorption under minimal Zn deficiency could pave the way for the survival of root cells and Zn homeostasis for the normal functioning of physiological processes.

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