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GROWTH RESPONSE OF COTTON CULTIVARS TO ZINC DEFICIENCY STRESS IN CHELATOR-BUFFERED NUTRIENT SOLUTION

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Abstract

Growth response of eight cotton cultivars to Zn stress was assessed in chelator-buffered nutrient solution. Pre-germinated seedlings were grown in half strength modified Johnson's nutrient solution at adequate and deficient levels of Zn. Zinc deficiency was induced by adding 50 µM di-ethylene tri-amine penta acetic acid (DTPA) in addition to all micronutrient concentrations. Shoot and root growth for the various cotton cultivars was significantly different at both levels of Zn supply in the growth medium. A typical Zn deficiency symptom of inward curling of top leaves was evident in cotton cultivars grown with chelator-buffered nutrient solution (Zn deficient). Two cotton cultivars viz., FH-900 and VH-137, produced higher biomass and better Zn utilization. Zinc concentration in cotton shoots grown at deficient level of Zn was less than its critical concentration of 20 mg kg^{-1} which was several folds lower than its concentration estimated for those grown at adequate Zn level. The study also depicted successful induction of Zn deficiency in cotton in hydroponics by DTPA, indicating its suitability for screening crop cultivars for micronutrient stress particularly for Zn deficiency.

Introduction

Cotton is an important fiber crop grown in several countries of the world. It suffers from Zn deficiency on alkaline calcareous soils (Rashid & Rafique, 1997). Applied Zn participates in several reactions in soil which reduce its availability for plant uptake (Rahmatullah *et al.,* 1985). A small fraction (< 2 %) of total Zn (80 mg kg^{-1}) is extractable by common soil test procedures (DTPA, AB-DTPA). A significant proportion of Zn resides in soil matrix (Rahmatullah *et al.,* 1988) and some of it is in bio-available form.

Exploitation of plant genetic capacity for better nutrient uptake and utilization is a promising tool that has been reported to cope with mineral nutrient stress in soil (Baligar *et al.,* 1990). The ubiquitous presence of Zn as a laboratory contaminant in conjunction with its very small plant requirement has made it quite difficult to impose Zn deficiencies using conventional nutrient solution techniques (Brown, 1986). A reliable, inexpensive and fast growing technique to differentiate Zn efficient cultivars would be an essential component of any breeding effort (Rengel & Graham, 1995).

The advent of chelator-buffered nutrient solution (Parker *et al.,* 1995) represents a major step-forward in studying plant micronutrient activities, which can constantly be maintained around plant roots, thus mimicking the situation occurring in a soil (Yang *et al.,* 1994). Hydroponics culture satisfies many requirements of a mass screening program for breeding lines tolerant to Zn by providing a homogenous, uniform growth medium that can easily be controlled and maintained (Epstein, 1972).

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Keeping in view the above, we investigated the suitability of DTPA buffered nutrient solution to induce Zn deficiency in cotton cultivars for their screening in hydroponics.

Materials and Methods

The chelator (DTPA)-buffered nutrient solution was employed to induce Zn deficiency in eight cotton cultivars viz., CIM-443, CIM-446, FH-900, FH-901, Krishma, MNH-93, NIAB-98 and VH-137 in a wire-house. One week old pre-germinated seedlings were transplanted in foam plugged holes (one plant per hole) made on thermo-pal sheets floating on continuously aerated 200 L half strength modified Johnson's nutrient solution (Johnson *et al.*, 1957) taken in two polyethylene lined iron tubs. In one of the tubs, 50µM DTPA extra to micronutrient concentration (µM of Fe + Zn + Cu + Mn) was added to induce Zn deficiency while the other tub was kept as control with adequate Zn supply. The solution pH was maintained at 5.5 (± 0.5) daily with 1*N* NaOH or 1*N* HCl. The experiment was laid out in a completely randomized factorial design (CRD) with 6 replicates.

Plants harvested after 40 days growth period, were washed with distilled water, air-dried and separated into shoots and roots for estimating their dry matter yield. Shoot samples were fine ground in a Wiley Mill and mixed thoroughly. The samples were then digested in di-acid mixture of nitric acid and perchloric acid (3:1) (Miller, 1998). Zinc concentration in the digested samples was estimated by atomic absorption spectroscopy (A Analyst 100, Perkin Elmer, Norwalk CT 06859, USA). Relative reduction in shoot dry matter (SDM) due to Zn deficiency (Zn stress factor) was calculated on percent basis for each cultivar using the following formula:

$$\text{Zinc stress factor (Zn SF), \%} = \frac{\text{SDW}_{\text{adequate}} - \text{SDW}_{\text{deficient}}}{\text{SDW}_{\text{adequate}}} \text{ X } 100$$

The data were statistically analyzed using computer software MSTAT-C (Russell & Eisensmith, 1983) according to standard procedures described by Steel & Torrie (1980).

Results and Discussion

Typical Zn deficiency symptoms with little leaves and inward curling of young leaves (Fig. 1) were obvious on cotton plants grown in DTPA chelator-buffered nutrient solution indicating the successful use of DTPA to assess micronutrient deficiencies in crops.

Shoot growth is an important parameter indicating ultimate economic yield and was extensively used to study plant responses for genotypic variability in tolerance to nutrient stress at seedling stage in screening experiments (Fageria *et al.*, 1988, McNair, 1993). Similar is the case with root growth as it affects nutrient uptake and transport in plants.

Zinc supply in the root medium had a significant ($p<0.01$) main and interactive effect on biomass production (Figs. 2 & 3) of various cotton cultivars. In this experiment, variable SDM production of cultivars (Fig. 2) and significant Zn XC interaction indicated that different cultivars accumulated SDM differently at both the treatments. Such cultivars-by-environment interactions are important in crop cultivar development (Kang,

Fig. 1. Characteristic Zn deficiency symptoms ("little leaf" and inward curling) in cotton plants grown in chelator (DTPA) buffered nutrient solution.

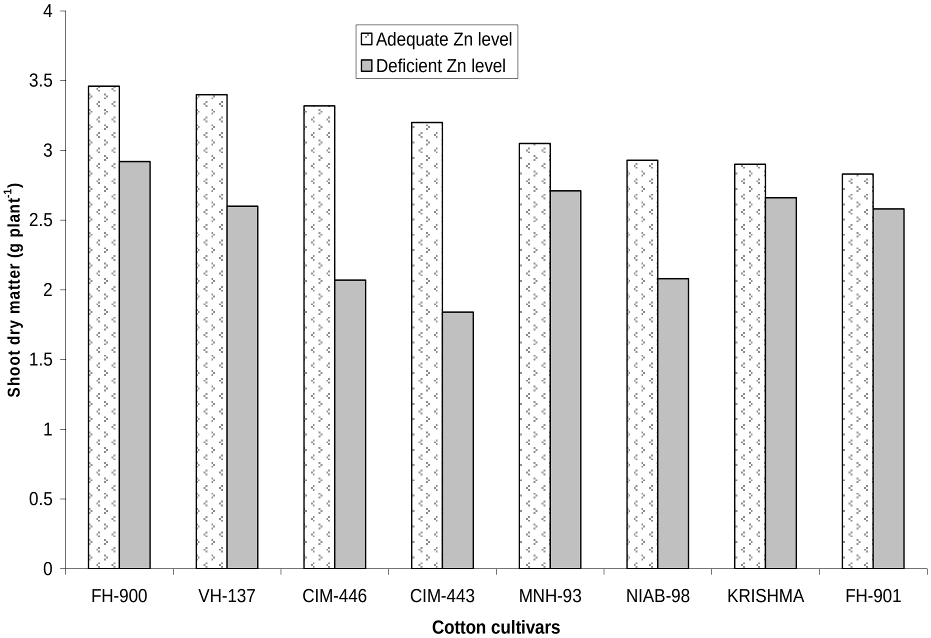

Fig. 2. Shoot dry matter (SDM) of eight cotton cultivars grown at adequate and deficient Zn level.

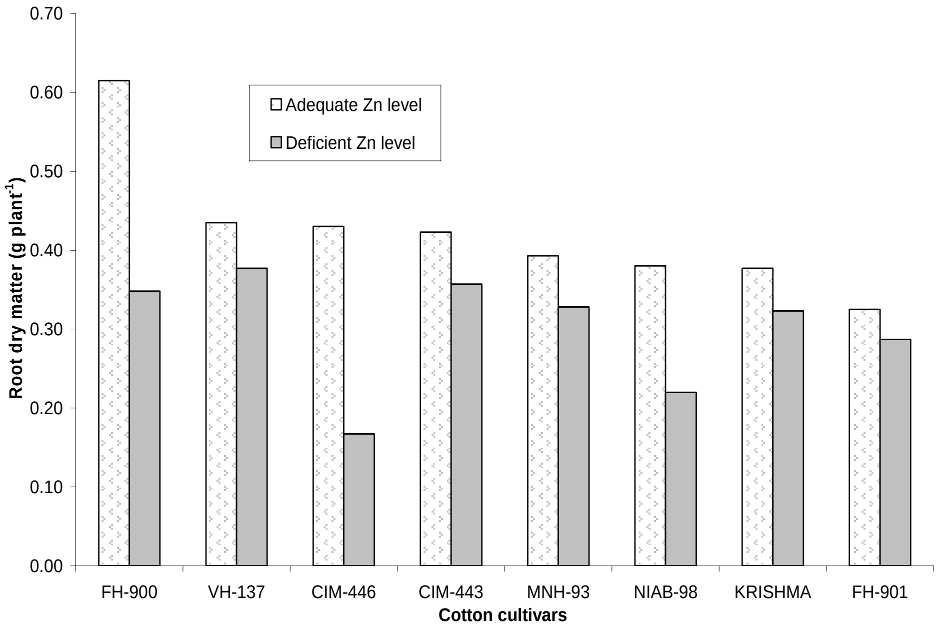

Fig. 3. Root dry matter (SDM) of eight cotton cultivars grown at adequate and deficient Zn level.

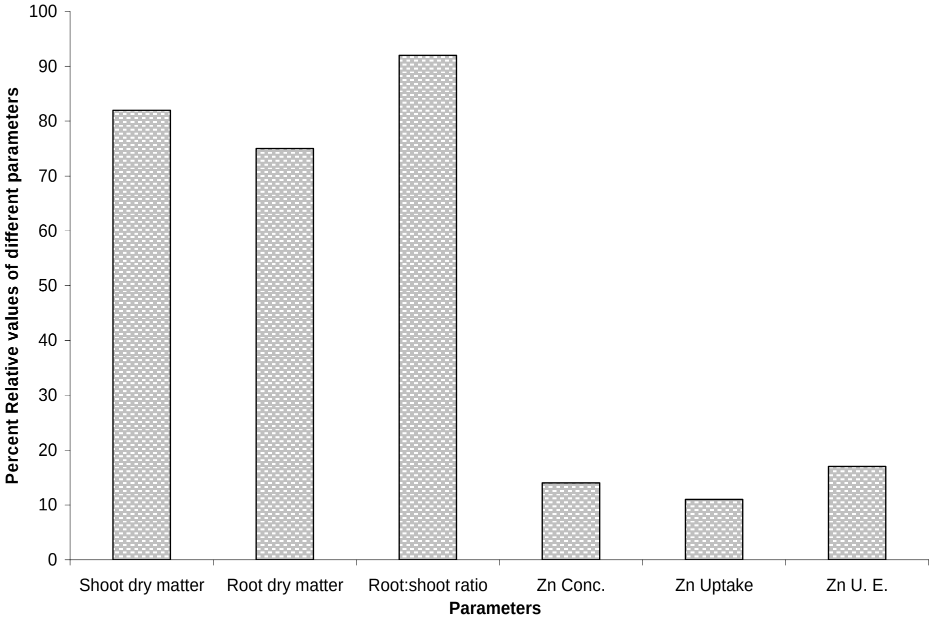

Fig. 4. Relative values of different parameters of cotton cultivars grown at deficient Zn level, to their control considered as 100.

Table 1. Zinc concentrations, Uptake and Utilization in shoot of cotton cultivars grown with deficient and adequate Zn levels. (Average of six repeats)

Cultivars	Shoot Zn concentration ($\mu g\ g^{-1}$)		Zn uptake ($\mu g\ g^{-1}$)		Zn utilization efficiency (g2 SDW μg^{-1}Zn)	
	Adequate Zn level	Deficient Zn level	Adequate Zn level	Deficient Zn level	Adequate Zn level	Deficient Zn level
FH-900	110	12.2	382	35.62	31.94	4.18
VH-137	101	14.7	344	38.22	29.76	5.65
CIM-446	91	14.5	303	30.02	27.47	7.00
CIM-443	88	14.3	283	26.31	27.66	7.77
MNH-93	106	13.0	326	35.23	35.02	4.80
NIAB-98	90	14.2	265	29.54	30.82	6.83
KRISHMA	109	12.5	317	33.25	37.69	4.70
FH-901	109.	15.0	309	38.70	38.62	5.81

Means followed by the same letter(s) in each column are statistically non-significant ($p < 0.05$)
N.S* = Non-significant

Table 2. Correlation matrix between different parameters of cotton cultivars.

	SDM	RDM	RSR	Zn Conc.	Zn Uptake
RDM	0.75 **				
RSR	-0.08	0.58			
Zn Conc.	0.59	0.57	0.20		
Zn Uptake	0.74**	0.68*	0.15	0.97**	
Zn U. E	0.32	0.39	0.29	0.93**	0.82**

*: Significant at probability <0.05
**: Highly Significant at probability <0.01

1998). On an average, SDM of plants decreased by 18.5 % due to Zn deficiency. Cakmak *et al.*, (1998) also reported similar decrease in SDM with chelator buffering of Zn. Shoot dry matter ranged between 1.72 to 3.48 g plant^{-1} at adequate and deficient Zn supply. Cultivar FH-900 produced maximum SDM at both Zn levels. Differential ability of cultivars to accumulate SDM with deficient Zn supply affirmed the existence of genetic variation for their tolerance to Zn deficiency.

Relative reduction in SDM (ZnSF) is a useful parameter in assessing relative tolerance of crop cultivars to Zn deficiency (Babikar, 1986; Yaseen *et al.*, 2000). The cultivars with negative and/or smaller values of ZnSF such as MNH-93 and FH-901 are useful and may be used as a basis for planning long-term screening experiments and selection for Zn deficiency tolerant cotton cultivars. Significant differences for ZnSF (Fig. 5) were exhibited among cotton cultivars. The maximum relative reduction in SDM (%) due to Zn deficiency was exhibited by CIM-443 (40.4 %) while Krishma and FH-901 showed negligible reduction in SDM.

Root dry matter (RDM) of cotton cultivars (Fig. 3) was significantly ($p<0.01$) decreased due to Zn deficiency. Significant ($p<0.01$) differences among cotton cultivars were observed in RDM production at both Zn levels. At adequate Zn supply in the root medium, CIM-443 produced maximum RDM (0.615 g plant^{-1}) while minimum (0.167 g plant^{-1}) was produced by NIAB-78. Babikar (1986) and Yaseen *et al.*, (2000) reported similar reduction in root dry matter in rice due to Zn deficiency. Root dry matter, shoot Zn concentration, Zn uptake, and Zn utilization efficiency had significant ($p<0.01$) and positive correlation with SDM production in all cotton cultivars (Table 2).

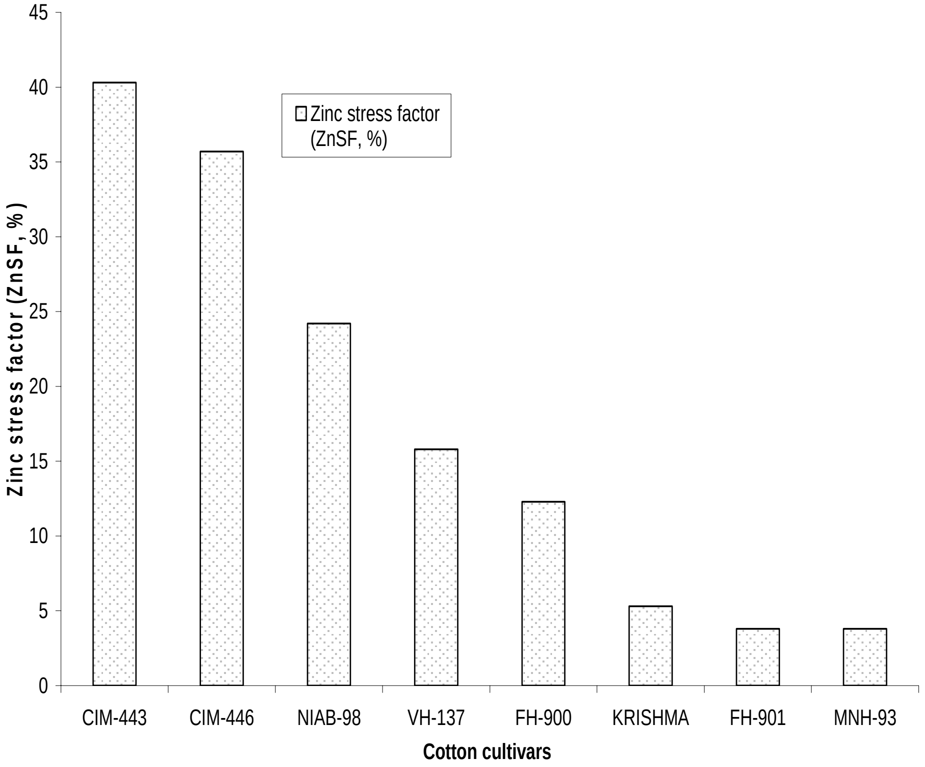

Fig. 5. Relative change in shoot dry matter (Zn SF, %) of eight cotton cultivars due to Zn deficiency.

All the cultivars differed significantly ($p<0.01$) for shoot Zn concentration at adequate Zn supply. Mean Zn concentration (Table 1) in shoots of plants grown with DTPA-chelator-buffered nutrient solution was 8-fold lower than those grown with adequate Zn. Considering 20-200 µg g^{-1} the sufficiency range of Zn in cotton shoot at about this ontogenic stage (Jones *et al*., 1991; Reuter *et al*., 1997), all the cultivars grown with DTPA-chelator-buffered nutrient solution showed internal Zn-deficiency as the shoot Zn concentration range in Zn-deficient treatment was 11.7 to 15 µgg^{-1} (Table 2). This indicates that DTPA can be used successfully in hydroponics to induce Zn deficiency in cotton. Shoot Zn concentration was significantly ($p<0.01$) and positively correlated with SDM, RDM and Zn uptake. Shoot Zn uptake decreased 9 fold in cotton cultivars grown in DTPA buffered nutrient solution compared to those grown at adequate Zn supply. The cultivars differed significantly ($p<0.01$) for Zn uptake at both Zn levels and CIM 443, CIM446 and NIAB 98 exhibited maximum shoot Zn uptake (Table 1). Rengel & Graham (1995) also observed a linear increase in uptake of Zn by wheat cultivars with an increase in solution Zn concentration.

Zinc levels had significant ($p<0.01$) effect on Zn utilization efficiency (ZnUE) (Table 1) which may be helpful in identifying plant cultivars that can yield better under low concentration of a particular nutrient (Siddiqi & Glass, 1981; Gerloff & Gableman, 1983). Averaged over both treatments, ZnUE was 6 fold higher in plants with Zn deficiency compared to adequate Zn supply. At adequate Zn supply, FH-900 exhibited maximum ZnUE and NIAB-78 exhibited minimum. At deficient Zn level, VH-137 was the most efficient Zn user, while CIM-446 was the least efficient. Cultivars, which were efficient in Zn utilization, were also efficient accumulator of biomass under adequate as well as deficient Zn supply.

Conclusion

Addition of DTPA successfully induced Zn deficiency causing a sharp decrease in most of the parameters studied in this experiment compared to control (Fig. 4). The present study suggests that chelator-buffered nutrient solution containing DTPA can be successfully used to induce Zn deficiency in cotton grown in nutrient solution. Cotton cultivars showed differential growth response to Zn deficiency stress, however, validation of these results need further field experimentation.

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