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Full Length Research Paper

Role of arbuscular mycorrhizal fungus (*Glomus intraradices*) – (fungus aided) in zinc nutrition of maize

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A greenhouse experiment was conducted to assess the nutritional improvement of maize plants (variety COHM5) inoculated with and without arbuscular mycorrhizal (AM) fungus (*Glomus intraradices* Schenck and Smith) under varying levels of Zn concentration (0, 1.25, 2.5, 3.75 and 5.0 mg kg^{-1}). Growth (leaf area index and root morphological attributes) and nutrient status (Zn and P concentrations) were measured at 45 and 75 days after sowing (DAS). Maize grains were analyzed for Zn and tryptophan concentrations. The increasing levels of Zn fertilization have no impact on the total mycorrhizal colonization, but the hyphal growth decreased slightly. Mycorrhizal inoculation consistently increased the root morphological attributes regardless of Zn fertilization. Mycorrhizal inoculation and Zn fertilization exhibited prominent increase in the leaf area index and chlorophyll content of plants and the response to mycorrhizal inoculation was higher at lower levels of Zn fertilization. The mycorrhizal dependency decreased gradually with increase in Zn levels. Mycorrhizal plants had higher P and Zn concentrations in both the stages in comparison to non-inoculated plants. Maize grains of AM+ plants had higher Zn and tryptophan concentrations. The plant available Zn concentration in mycorrhizal fungal inoculated soil was higher than non-inoculated soils, which directly contributed to increase in Zn status of mycorrhizal plants. In conclusion, mycorrhizal symbiosis enhances Zn supply to the host plants by extensive root development enabling the plant to maintain higher nutritional status and produce grains with rich tryptophan concentrations.

Key words: Arbuscular mycorrhiza, chlorophyll, maize, nutritional quality, zinc deficiency.

INTRODUCTION

Soil Zn deficiency is a world wide problem in the crop production, affecting the growth of the crops in over 50% of agricultural lands (Alloway 2001). Zinc deficiency is closely associated with 80% grain yield reduction, beside reduction in grain Zn content and other nutrients in cereal crops (Cakmak et al., 1998). The high dependency on foods cooked with reduced Zn content brings out malnutrition and Zn deficiency among people, who are estimated to be over two billion globally (Welch and Graham 2002). In order to ensure nutritional security of human beings, the production of cereals with rich grain nutritional quality should be enhanced. Zinc fertilization, a common remedial practice have use efficiency rarely exceeding 1%, while remaining Zn is fixed in the soil due to alkaline pH and higher free lime status (Mandal and Mandal 1986). However, the total Zn content in majority of the arable lands is reported high, but mostly they are present in the unavailable form. The magnitude of Zn deficiency is high in calcareous soils of arid and semi arid tropical regions, where major portion of added Zn gets fixed in the soil (Marschner 1995). The imbalanced use of fertilizers and non-addition of organic manures also cause widespread soil Zn deficiency (Singh et al., 2005). There has to be some mechanism to transforming tightly bound Zn into plant available forms.

Arbuscular mycorrhizal (AM) fungi are known to improve the availability of nutrients especially phosphorrus, moving them to the root surface by diffusion (Jakobsen et al., 1992). It is also reported that mycorrhizal symbiosis enhances absorption of relatively immobile micronutrients such as Zn and Cu (Faber et al., 1990; Kothari et al., 1991; Li et al., 1991; Liu et al., 2000; Ryan and Angus, 2003). The micronutrient improvement in mycorrhizal plants is always associated with rhizosphere

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acidification (Dodd et al., 1987), more external mycelium in the soil (Jakobsen et al., 1992) and soil biochemical changes (Subramanian and Charest, 2007). Besides, host plants retain the large green leaf area (Subramanian et al., 1997) and chlorophyll concentration (Subramanian and Charest, 1995; Augé, 2001) under the water deficit conditions. Liu et al. (2000) have indicated that the external mycelium of mycorrhizal fungus contribute significantly to the tune 60% towards host plant Zn nutrition under deficient conditions. These data suggest that mycorrhizal association may play an important role both under drought and nutrient deficient conditions. It is proved that mycorrhizal symbiosis improves Zn nutrition in maize as a secondary consequence of P nutrition. We hypothesised that the maize's responses to mycorrhizal inoculation differ with respect to varying degrees of Zn deficiency. To address this question, the changes in nutrient status, morphological and physiological attributes of maize plants that were inoculated with and without mycorrhizal fungi and subjected to varying levels of Zn fertilization, were measured at 45 and 75 days after sowing (DAS).

MATERIALS AND METHODS

Experimental soil

The physical characters of experimental soil were Alfisol, sandy loam in texture with neutral pH (7.4) and free from salinity (0.04 dSm^{-1}). The soil had less quantity of nutrients, which include organic carbon (0.4%), available N (1.23g kg^{-1}), available ($NaHCO_3$-extractable) P (0.058 g kg^{-1}), available K (1.6 g kg^{-1}) and available Zn (DTPA extractable) (0.63 mg kg^{-1}). The experimental soil was collected from the field that was kept fallow for more than three years to avoid indigenous mycorrhizal population and propagules in the root portions of previous crop. The examination of indigenous mycorrhizal status in the experimental soil revealed very less indigenous viable spore population (<10 spores in 100 g^{-1} soil).

Greenhouse experiment

The greenhouse experiment was conducted at the Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore. The climatic conditions viz. temperature (24 - 28°C), light intensity (800 - 1000 µmols provided by natural light), relative humidity (60 - 65%) and 12 h photoperiod were maintained in the greenhouse. The experiments had 10 treatments with six replications in a randomized block design. In six replications, each three were kept for sampling at 45 and 75 DAS. One plant per pot was maintained throughout the experiment. Maize (var. COHM5) plants were grown in earthen pots of 10 kg capacity (30 cm diameter and 30 cm height) with and without Arbuscular Mycorrhiza (AM) inoculation. The mycorrhizal strain (*Glomus intraradices* Schenck and Smith; TNAU-03-06) was cultured in maize plants and propagules comprised of infected root bits and spores were blended in sterile vermiculite. The vermiculate based mycorrhizal inoculum was applied 5 cm below the seeds prior to sowing at the rate of 10 g per pot as a thin layer. Zn fertilization was done at five concentrations (0, 1.25, 2.5, 3.75 and 5.0 mg kg^{-1}) in the form of $ZnSO_4$ in addition to regular NPK dose of 0.068:0.03:0.03 g NPK kg^{-1} soil. The pots were watered daily to maintain the moisture content of the soil to the saturation. The roots and shoots were sampled from each plant at 45 and 75 DAS and analyzed for nutrient concentrations. The root architecture of mycorrhizal (AM+) and non-mycorrhizal (AM-) plants was evaluated measuring root length (vertical), spread (horizontal) and volume. Leaf area index (LAI) to assess the leaf area of the AM+ and AM- plants was calculated using leaf area meter (LICOR Model LI 8000). The leaf chlorophyll content was analysed extracting 500 mg fresh leaves with 50 ml acetone, and measuring the absorbance at 663 nm (Bruinsma 1963).

Mycorrhizal colonization

The stages of mycorrhizal colonization were described as functional and vigorous colonization at 45 and 75 DAS respectively. Maize plants were pulled out from the pots without damaging the roots. The roots were repeatedly washed with tap water until they are free from dirts and soil particles. The root segments of 1 cm size in 100 numbers were cut per treatment, and estimated for mycorrhizal colonization following Dalpé (1993). Before mounting the root segments on slides, they were bleached with 2.5% KOH, acidified in 1% HCl and stained in 0.05% tryphan blue solution (tryphan blue 0.5g, glycerol 500 ml, 1% HCl 50 ml and distilled water 450 ml) and destained. Root segments were observed under the 10 x lens microscope for the presence of any of the mycorrhizal structures such as arbuscules, vesicles, external hyphae and spores.

Nutrient analysis

Plants: The shoots and residual roots were dried in hot air oven at 70°C for 48 h. The collected plant samples weighing 0.5 g were digested in triple acid mixture (9:2:1 nitric acid: sulphuric acid: perchloric acid) for nutrient analyses. The colorimetric examination of plant extract to measure the phosphorous (P) level was taken following vanadomolybdo phosphoric acid yellow colour method (Piper, 1966). The plant samples diluted to 50 ml using distilled water were fed to an Atomic Absorption Spectrometer (Varian Spectra AA 220, Australia) to determine Zn concentration. Similarly, Zn level in grains was also analyzed. The total Zn and P levels in the plant were calculated multiplying the nutrient concentration in the samples with plant dry mass. The powdered grain sample was prepared to measure the concentration of tryptophan following the protocol described by Sadasivam and Manickkam (1996). Mycorrhizal dependency, a measure of plants response to mycorrhizal inoculation in accumulating Zn in their roots was calculated using the formula from Hetrick et al. (1992).

$$\text{Mycorrhizal dependency (\%)} = \frac{\text{Zn content (AM+)} - \text{Zn content (AM-)}}{\text{Zn content (AM+)}} \times 100$$

Soil: Soil nutrient analyses were conducted to find the concentrations of Zn and Phosphorous in the soils. The DTPA soil test was done with DTPA extractant (13.1 ml triethanolamine, 1.967 g DTPA and 1.47 g $CaCl_2$ are mixed together, made up to 1 L with pH adjusted to 7.3) to extract Zn from the soil (Lindsay and Norvell, 1978). Available phosphorus in the soils was estimated by extraction with sodium bicarbonate ($NaHCO_3$) following Olsen et al. (1954).

Statistical analysis

A one-way analysis of variance (ANOVA) was done for all data and comparisons among means were made using LSD (least square difference) test, calculated at $P \leq 0.05$. Linear correlation

Table 1. Percentage of mycorrhizal colonization examined in the arbuscular mycorrhiza inoculated (AM+) and non-inoculated (AM-) root segments of maize crop plants at 45 and 75 days after sowing (DAS) under different Zn levels. Values in parentheses indicate the standard error. The levels of significance for ANOVA, * = P ≤ 0.05; ** = P ≤ 0.01; NS = Not significant.

Zn levels (mg kg^{-1})	45 DAS						75 DAS					
	Arbuscules		Vesicles		External mycelium		Arbuscules		Vesicles		External mycelium	
	AM-	AM+	AM-	AM+	AM-	AM+	AM-	AM+	AM-	AM+	AM-	AM+
0	17.8 (1.35)	40.2 (0.86)	22.4 (0.92)	67.0 (1.54)	15.2 (1.52)	46.8 (1.24)	13.0 (0.812)	33.5 (0.583)	26.0 (1.88)	43.7 (1.06)	10.5 (0.860)	41.2 (1.07)
1.25	18.2 (1.56)	38.8 (1.15)	17.0 (1.00)	62.6 (0.871)	15.0 (1.00)	44.4 (2.03)	8.5 (0.509)	30.7 (0.927)	17.5 (1.58)	48.0 (0.58)	11.0 (0.509)	42.0 (1.02)
2.5	21.2 (0.96)	40.2 (0.86)	30.0 (1.30)	64.0 (1.67)	23.0 (1.58)	40.4 (1.60)	7.2 (0.447)	36.0 (0.509)	16.5 (1.14)	48.0 (2.42)	10.0 (1.02)	42.5 (0.509)
3.75	17.8 (0.66)	45.2 (1.28)	26.2 (1.49)	63.8 (1.24)	17.0 (2.00)	40.4 (1.35)	8.2 (1.07)	29.5 (0.860)	26.7 (1.42)	37.0 (3.27)	9.5 (0.860)	35.5 (0.707)
5.0	18.2 (1.20)	45.2 (1.28)	26.2 (1.31)	63.4 (1.56)	16.8 (2.37)	38.2 (0.927)	5.5 (0.860)	32.7 (0.871)	18.5 (0.707)	52.2 (3.31)	11.5 (1.39)	33.5 (1.01)
Mean	18.6	41.9	24.4	64.2	17.4	42.0	8.48	32.5	21.0	45.8	10.5	34.9
ANOVA: M (Mycorrhizal treatment), Z (Zn levels)												
M	**		**		**		**		**		**	
Z	**		**		**		**		**		**	
M x Z	NS		NS		NS		NS		NS		NS	

coefficients (r) were calculated between Zn and P content in the roots and shoots of the maize plants. Statistical procedures were carried out with the IRRI stat (IRRI, Manila Philippines) software package.

RESULTS AND DISCUSSION

Mycorrhizal colonization

A significant increase in the proportion of mycorrhizal colonization in Arbuscular Mycorrhiza inoculated roots (AM+) from non-inoculated (AM-) maize plants at all the five levels of Zn fertilisation (Table 1). The higher proportion of mycorrhizal colonization in the AM+ maize plants was consistent across mycorrhizal structures such as arbuscules, vesicles and external mycelium, and across time of sample collection (45 and 75 days after sowing (DAS)). However, a significant reduction in the mycorrhizal colonization was recorded in the samples collected 75 days after sowing. The increase of soil Zn application had no influence with mycorrhizal colonization in both the AM+ and AM- maize plants, where as negative correlation between Zn concentrations and mycorrhizal colonization was recorded elsewhere (Gildon and Tinker 1983; Boyle and Paul 1988). Ortas et al. (2002) proved that such negative correlation was confined to P alone, and an increase of Zn up to 5 mg kg^{-1} had no effect on mycorrhizal colonization in the roots. The readily available Zn from applied fertilizer (2002) proved that such negative correlation was confined to P alone, and an increase of Zn up to 5 mg kg^{-1} had no effect on mycorrhizal colonization in the roots. It was also reported that Arbuscular Mycorrhizal colonization increased even at the highest level level of contamination of 250 mg Zn kg^{-1} soil suggesting the potential of mycorrhiza in reduction of metal contamination (Audet and Charest 2006).

Nevertheless, the development of mycorrhizal structures such as arbuscles and vesicles had no changes at varying Zn levels, but the growth of external mycelium was decreased at higher levels of Zn application in AM+ plants. The readily available Zn from applied fertilizer could

Table 2. Root morphological attributes viz. root length, spread and volume measured in the arbuscular mycorrhiza inoculated (AM+) and non-inoculated (AM-) maize crop plants at 45 and 75 days after sowing (DAS) under different Zn levels. Values in parentheses indicate the standard error. The levels of significance for ANOVA, * = P ≤ 0.05; ** = P ≤ 0.01; NS = Not significant.

Zn levels (mg kg^{-1})	45 DAS						75 DAS					
	Root length (cm)		Root spread (cm)		Root volume (ml)		Root length (cm)		Root spread (cm)		Root volume (ml)	
	AM-	AM+	AM-	AM+	AM-	AM+	AM-	AM+	AM-	AM+	AM-	AM+
0	39.2	52.1	10.0	13.5	21.3	26.4	44.3	58.4	14.2	17.6	29.3	35.3
	(0.577)	(1.73)	(0.230)	(0.288)	(0.750)	(1.09)	(2.30)	(1.73)	(0.536)	(0.440)	(1.45)	(0.881)
1.25	44.1	53.3	10.6	14.3	23.0	28.2	49.2	60.1	14.9	17.3	31.0	38.3
	(2.30)	(1.73)	(0.288)	(0.230)	(0.866)	(2.07)	(1.73)	(2.30)	(0.200)	(0.491)	(1.73)	(1.45)
2.5	44.1	53.4	11.1	14.8	24.5	30.7	51.5	62.2	15.6	17.2	34.3	40.3
	(1.73)	(2.30)	(0.230)	(0.346)	(0.866)	(1.32)	(2.30)	(2.88)	(0.702)	(0.450)	(1.45)	(2.02)
3.75	45.3	54.2	11.8	15.2	26.7	33.0	53.2	63.4	16.2	18.7	37.3	43.0
	(1.73)	(2.30)	(0.288)	(0.404)	(0.981)	(1.15)	(1.73)	(2.30)	(0.416)	(0.448)	(2.02)	(1.73)
5.0	45.3	54.2	12.1	16.0	28.6	34.2	53.2	65.3	16.3	19.1	40.3	45.0
	(1.15)	(1.73)	(0.346)	(0.346)	(1.03)	(0.405)	(2.30)	(3.46)	(0.731)	(0.378)	(2.02)	(2.88)
Mean	43.6	53.4	11.1	14.7	24.8	30.6	50.3	61.8	15.5	18.0	34.4	40.4
ANOVA: M (Mycorrhizal treatment), Z (Zn levels)												
M	**		**		**		**		**		**	
Z	NS		**		**		*		NS		**	
M x Z	NS		NS		NS		NS		NS		NS	

cause plants not to invest more on hyphal growth for foraging immobile nutrients.

Root morphology

The measurements of root architecture variables: root length (vertical), spread (horizontal) and volume in the AM+ plants were significantly higher than non-mycorrhizal (AM-) maize plants (Table 2). Graded levels of Zn addition increased the root masses of both AM- and AM+ plants. At higher levels of Zn, the response to added Zn was more pronounced in AM- plants than AM+ plants.

The increase in root architecture measurements from mycorrhizal non-inoculated (AM-) plants to mycorrhizal inoculated (AM+) maize plants was found in both the root samples collected at 45 and 75 DAS. The extensive root growth of AM plants can be attributed to the improved P nutrition of host plants. The enhanced supply of P by mycorrhizal symbiosis has been unequivocally demonstrated (Jakobsen et al., 1992; Asmah, 1995; Hetrick et al., 1996; Smith and Read, 1997; Subramanian et al., 2006). The experimental soil had extremely low available P (0.58 mg kg^{-1}) which is a constraint to root growth where as the improved P availability to mycorrhizal plants would have resulted in vigorous root growth. Zinc applica- nutrition of host plants. The enhanced supply of P by mycorrhizal symbiosis has been unequivocally demonstrated (Jakobsen et al., 1992; Asmah, 1995; Hetrick et al., 1996; Smith and Read, 1997; Subramanian et al., 2006). The experimental soil had extremely low available P (0.58 mg kg^{-1}) which is a constraint to root growth where as the improved P availability to mycorrhizal plants would have resulted in vigorous root growth. Zinc applica-tion significantly increased (P ≤0.01) all the root morphological attributes except root length at 45 DAS and spread at 75 DAS which is in line with the observation of Nable and Webb (1993) who reported that Zn is essential for root development. The synergistic interaction between P and Zn has resulted in extensive root growth in colonized plants.

Mycorrhizal inoculated plants produced signifi-cantly (P ≤ 0.01) higher root masses irrespective of

Table 3. Dry weight of roots and shoots measured in the arbuscular mycorrhiza inoculated (AM+) and non-inoculated (AM-) maize crop plants at 45 and 75 days after sowing (DAS) under different Zn levels. Values in parentheses indicate the standard error. The levels of significance for ANOVA, * = P ≤ 0.05; ** = P ≤ 0.01; NS = Not significant.

Zn levels (mg kg^{-1})	45 DAS				75 DAS			
	Roots (mg kg^{-1})		Shoots (mg kg^{-1})		Roots (mg kg^{-1})		Shoots (mg kg^{-1})	
	AM-	AM+	AM-	AM+	AM-	AM+	AM-	AM+
0	1.95	2.62	3.38	3.50	2.70	3.37	4.56	4.90
	(0.088)	(0.103)	(0.233)	(0.202)	(0.155)	(0.129)	(0.141)	(0.101)
1.25	2.15	2.81	3.73	3.85	2.92	3.55	4.64	5.67
	(0.057)	(0.129)	(0.116)	(0.202)	(0.129)	(0.155)	(0.181)	(0.202)
2.5	2.25	2.97	3.96	4.08	3.10	3.78	5.27	5.91
	(0.083)	(0.072)	(0.233)	(0.116)	(0.103)	(0.181)	(0.080)	(0.131)
3.75	2.40	3.16	4.43	4.55	3.28	3.91	5.29	6.23
	(0.090)	(0.116)	(0.233)	(0.202)	(0.077)	(0.155)	(0.121)	(0.080)
5.0	2.52	3.20	4.55	4.66	3.51	4.00	5.85	5.11
	(0.132)	(0.129)	(0.202)	(0.116)	(0.129)	(0.103)	(0.060)	(0.060)
Mean	2.25	2.95	4.01	4.13	3.10	3.72	4.32	5.56
ANOVA: M (Mycorrhizal treatment), Z (Zn levels)								
M	**		NS		**		**	
Z	**		**		**		*	
M x Z	NS		NS		NS		NS	

Zn levels at 45 and 75 DAS (Table 3). Graded levels of Zn addition increased the root masses of both AM- and AM+ plants. At higher levels of Zn, the response to added Zn was more pronounced in AM- plants than AM+ plants. At early stage of crop growth, AM fungal inoculated plants showed an increase in root mass while the shoot masses were similar. This may be attributed to the utilization of carbon for establishment of functional symbiosis (Fitter, 1988; Jakobsen and Rosendahl, 1990). The data are in conformity with the findings of Kothari et al., 1991) who obtained no response in terms of shoot growth of maize to mycorrhizal inoculation in the early stages. Zinc nutrition favoured the dry matter production in the early stages than in later stages. Zinc by way of activating C metabolism enzymes such as carbonic anhydrase (Ohki, 1976) and amino acids, improved the photosynthetic ability (Marschner, 1986) and protein synthesis of plants and thereby plant growth.

Leaf area index and chlorophyll content

Leaf area index (LAI), measured in AM+ plants at 45 and 75 DAS were significantly (P ≤ 0.01) higher than AM- plants (Table 4 and Figure 1) at both stages of observation. Mycorrhizal colonization would have stimulated leaf expansion as a result of improved Zn nutritional status (Cakmak et al., 1998) or better water use efficiency (Subramanian and Charest 1995). As expected, zinc application progressively increased the LAI of both inoculated and uninoculated plants. The chlorophyll content of AM+ plants at 45 DAS was also significantly higher than AM- plants (Table 4 and Figure 2). The response to added Zn was more pronounced in AM- plants than AM+ plants. The increase in chlorophyll content of AM plants can be attributed to enhanced Zn nutrition as Zn promotes the development of photosyn thetic pigments (Misra et al., 2005).

Plant nutritional status

Mycorrhiza-inoculated maize plants had significantly ($P \leq 0.01$) higher Zn and P concentrations than uninoculated (AM-) plants (Table 5 and 6). The increase in Zn and P concentration in AM inoculated plants can be attributed to the extensive root development and hyphae that reduce the distance for diffusion of nutrients thus enhancing the nutrient absorption. AM+ shoots had higher Zn concentration (30.2 and 34.7 mg kg^{-1}) than AM- shoots (29.1 and 33.9 mg kg^{-1}), at 45 and 75 DAS, respectively. The enhanced acid phosphatase activities in mycorrhizal plants (Dodd et al. 1987) assist in releasing P which in turn transported by the external mycelium (Jakobsen et al. 1992) and resulted in enhancement of nutritional status of the host plants. The hyphal transport of Zn to the host plant has been reported as 60% (Liu et al. 2000). Increasing levels of Zn significantly (P ≤ 0.01) increased the Zn concentrations of roots and shoots at 75 DAS and roots alone at 45 DAS.

There was a strong positive relationship between Zn and P contents in the roots (r = 0.95; $P < 0.001$) and the

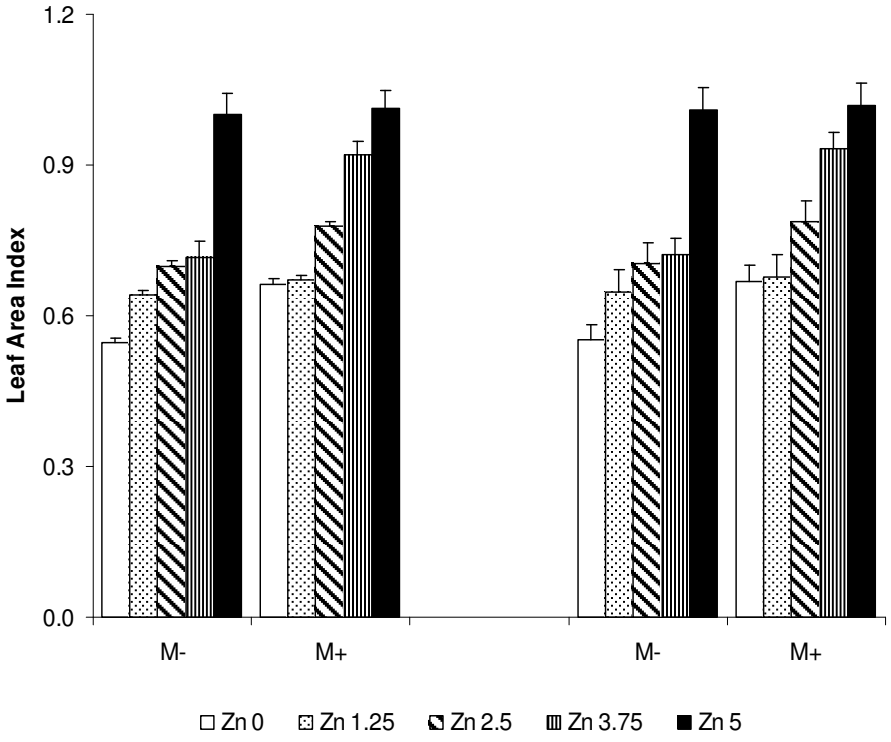

Figure 1. Leaf area index (LAI) measured in the arbuscular mycorrhiza inoculated (AM+) and non-inoculated (AM-) maize crop plants at 45 and 75 days after sowing (DAS) under different Zn levels. Error bars represent standard errors of three replications

Table 4. The levels of significance for ANOVA for Leaf Area Index and chlorophyll content (mg g^{-1}) under different Zn levels (Z) with (AM+) or without (AM-) arbuscular mycorrhizal fungal inoculation. ** P ≤ 0.01; NS, not significant.

	M	Z	M x Z
Leaf Area Index			
45 DAS	**	**	**
75 DAS	**	**	**
Cholorophyll content			
45 DAS	**	**	NS

shoots (r = 0.96; P < 0.001), which confirms the synergistic interaction among P and Zn aided by mycorrhizal colonization (Figure 3). Mycorrhizal dependency based on Zn contents was reported to be 19.7, 16.0, 16.2, 17.6 and 14.7% in Zn fertilization levels from 0, 1.25, 2.5, 3.75 to 5.0 mg kg^{-1} soil respectively.

AM inoculated plants produced grains with significantly (P ≤ 0.01) higher Zn concentration in comparison to non-noculated plants (Table 7). Mycorrhizal plants assimilated greater amounts of Zn and translocated to grains which assisted in enrichment of tryptophan in grains. Grains of AM inoculated plants had significantly higher concentrations of tryptophan than non-inoculated plants (Table 7). The role of Zn in tryptophan biosynthesis has been well established (Cakmak et al., 1989; Brown et al., 1993).

Soil nutrient status

Overall, available Zn concentration was recorded high in the soil collected from mycorrhiza-inoculated maize plants (AM+) (Table 8). With the progression of plant growth stages, AM- soil had lower DTPA Zn status while AM+ soil had consistently higher available Zn. Mycorrhizal inoculation increased the DTPA-Zn by 74% in comparison to AM- soil even at the highest dose of Zn applied (5 mg kg^{-1}) in the soil. The soil from mycorrhizal inoculated maize plants (AM+) had more available phosphorous (P) than from (AM-) maize plants. The increase in P concentration was independent of Zn levels. Mycorrhiza-inoculated plants were reported to release organic acids that reduce rhizospheric pH, and facilitate nutrients availability (Koide and Kabir, 2000).

Conclusion

To conclude, mycorrhizal inoculation in the maize plants improves the root architectural attributes, plant nutritional

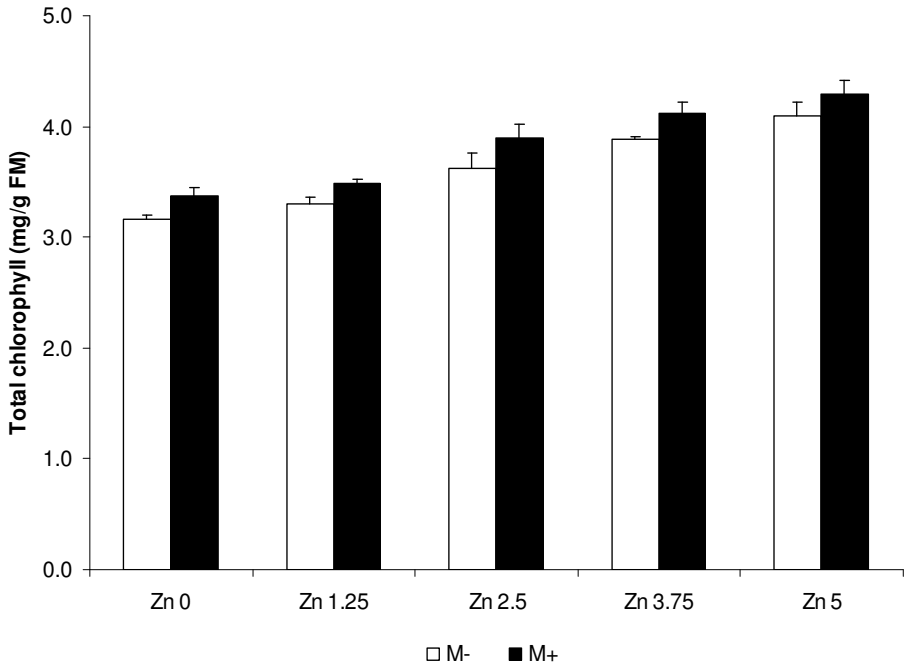

Figure 2. Total chlorophyll content in the arbuscular mycorrhiza inoculated (AM+) and non-inoculated (AM-) maize crop plants at 45 and 75 days after sowing (DAS) under different Zn levels. Error bars represent standard errors of three replications.

Table 5. Zinc (Zn) concentration examined in the roots and shoots of arbuscular mycorrhiza inoculated (AM+) and non-inoculated (AM-) maize crop plants at 45 and 75 days after sowing (DAS) under different Zn levels. Values in parentheses indicate the standard error. The levels of significance for ANOVA, * = P ≤ 0.05; ** = P ≤ 0.01; NS = Not significant.

Zn levels (mg kg^{-1})	45 DAS				75 DAS			
	Roots (mg kg^{-1})		Shoots (mg kg^{-1})		Roots (mg kg^{-1})		Shoots (mg kg^{-1})	
	AM-	AM+	AM-	AM+	AM-	AM+	AM-	AM+
0	33.3 (0.86)	35.5 (0.0029)	27.8 (0.577)	28.5 (0.520)	33.5 (0.346)	35.9 (0.520)	32.6 (0.404)	33.7 (0.404)
1.25	33.6 (0.009)	35.9 (0.0029)	28.4 (0.693)	29.1 (0.520)	33.9 (0.520)	36.4 (0.693)	33.1 (0.346)	34.1 (0.577)
2.5	34.3 (0.0029)	35.6 (0.0029)	28.9 (0.751)	29.9 (0.693)	34.5 (0.866)	37.1 (0.635)	33.9 (0.404)	34.9 (0.520)
3.75	35.3 (0.0029)	37.2 (0.0029)	29.8 (0.520)	31.6 (0.462)	35.2 (0.289)	37.8 (0.462)	34.8 (0.231)	35.4 (0.231)
5.0	35.6 (0.0029)	37.2 (0.666)	30.4 (0.808)	31.7 (0.404)	35.6 (0.520)	38.1 (0.577)	35.0 (0.520)	35.6 (0.289)
Mean	34.4	36.3	29.1	30.2	34.5	37.1	33.9	34.7
ANOVA: M (Mycorrhizal treatment), Z (Zn levels)								
M	**		**		**		**	
Z	NS		**		**		**	
M x Z	NS		NS		NS		NS	

Table 6. Phosphorous (P) concentration examined in the roots and shoots of arbuscular mycorrhiza inoculated (AM+) and non-inoculated (AM-) maize crop plants at 45 and 75 days after sowing (DAS) under different Zn levels. Values in parentheses indicate the standard error. The levels of significance for ANOVA, * = P ≤ 0.05; ** = P ≤ 0.01; NS = Not significant.

Zn levels (mg kg^{-1})	45 DAS				75 DAS			
	Roots (mg kg^{-1})		Shoots (mg kg^{-1})		Roots (mg kg^{-1})		Shoots (mg kg^{-1})	
	AM-	AM+	AM-	AM+	AM-	AM+	AM-	AM+
0	6.45 (0.16)	8.12 (0.12)	16.8 (0.23)	18.7 (0.40)	38.9 (0.46)	41.1 (0.35)	21.1 (0.35)	23.2 (0.29)
1.25	6.52 (0.18)	8.33 (0.16)	17.4 (0.35)	19.1 (0.35)	39.4 (0.17)	41.8 (0.40)	22.2 (0.23)	24.1 (0.35)
2.5	6.63 (0.19)	8.42 (0.17)	17.7 (0.23)	19.3 (0.52)	40.3 (0.52)	42.3 (0.23)	23.1 (0.40)	24.5 (0.23)
3.75	6.68 (0.18)	8.52 (0.12)	18.1 (0.52)	19.0 (0.17)	40.6 (0.35)	42.6 (0.29)	23.5 (0.23)	24.8 (0.40)
5.0	6.71 (0.17)	8.54 (0.17)	18.2 (0.35)	19.2 (0.29)	40.5 (0.40)	42.4 (0.23)	23.7 (0.35)	24.6 (0.23)
Mean	6.60	8.39	17.6	19.1	39.9	42.0	22.7	24.2
ANOVA: M (Mycorrhizal treatment), Z (Zn levels)								
M	**		**		**		**	
Z	NS		NS		**		**	
M x Z	NS		NS		NS		NS	

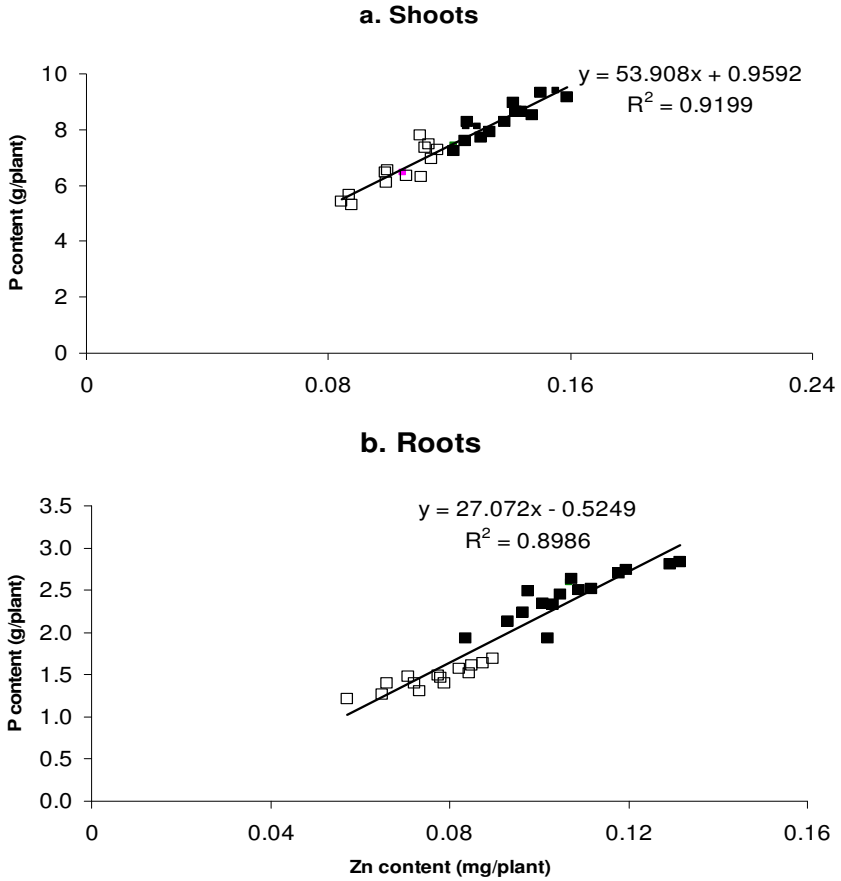

Figure 3. Correlations between Zn and P content in the shoots (a) and roots (b) of the arbuscular mycorrhiza inoculated (AM+) and non-inoculated (AM-) maize crop plants.

Table 7. Concentrations of Zn and tryptophan measured in the grains of arbuscular mycorrhiza inoculated (AM+) and non-inoculated (AM-) maize crop plants under different Zn levels. Values in parentheses indicate the standard error. The levels of significance for ANOVA, * = P ≤ 0.05; ** = P ≤ 0.01; NS = Not significant.

Zn levels (mg kg^{-1})	Grain Zn concentration (mg kg^{-1})		Tryptophan concentration (mg kg^{-1})	
	AM-	AM+	AM-	AM+
0	39.8 (0.404)	41.5 (0.289)	76.0 (1.04)	80.2 (1.27)
1.25	40.4 (0.231)	42.0 (0.408)	78.0 (0.577)	80.8 (0.462)
2.5	41.2 (0.208)	42.7 (0.404)	78.6 (1.27)	84.2 (1.039)
3.75	42.2 (0.340)	43.8 (0.305)	80.0 (0.693)	86.0 (0.924)
5.0	42.6 (0.289)	44.1 (0.346)	81.0 (0.577)	88.4 (1.39)
Mean	41.2	42.8	78.7	83.9
ANOVA: M (Mycorrhizal treatment), Z (Zn levels)				
M	**		**	
Z	**		**	
M x Z	NS		NS	

Table 8. Available zinc (Zn) and phosphorus (P) concentrations examined in the soils of arbuscular mycorrhiza inoculated (AM+) and non-inoculated (AM-) maize crop plants at 45 and 75 days after sowing (DAS) under different Zn levels. Values in parentheses indicate the standard error. The levels of significance for ANOVA, * = P ≤ 0.05; ** = P ≤ 0.01; NS = Not significant.

Zn levels (mg kg^{-1})	Available Zn (mg kg^{-1})				Available P (kg ha^{-1})			
	45 DAS		75 DAS		45 DAS		75 DAS	
	AM-	AM+	AM-	AM+	AM-	AM+	AM-	AM+
0	1.08 (0.219)	1.43 (0.029)	1.07 (0.110)	1.44 (0.092)	5.93 (0.029)	6.19 (0.058)	5.98 (0.046)	6.25 (0.040)
1.25	1.73 (0.202)	1.85 (0.069)	1.72 (0.075)	1.86 (0.139)	5.94 (0.035)	6.22 (0.052)	6.00 (0.046)	6.28 (0.075)
2.5	2.67 (0.353)	3.23 (0.116)	2.62 (0.104)	3.25 (0.115)	5.94 (0.052)	6.24 (0.035)	6.20 (0.040)	6.30 (0.052)
3.75	3.13 (0.433)	3.87 (0.060)	3.02 (0.156)	4.01 (0.173)	5.96 (0.017)	6.26 (0.023)	6.30 (0.035)	6.31 (0.035)
5.0	3.23 (0.245)	5.63 (0.364)	3.06 (0.222)	5.79 (0.179)	6.25 (0.023)	6.25 (0.058)	6.21 (0.023)	6.30 (0.035)
Mean	2.37	3.20	2.29	3.27	5.94	6.23	6.14	6.29
ANOVA: M (Mycorrhizal treatment), Z (Zn levels)								
M	**		**		**		**	
Z	**		**		NS		**	
M x Z	**		**		NS		*	

status and availability of soil nutrients at Zn fertilization levels. While such favourable changes enhance maize plants to survive during Zn deficient conditions, they assure nutritional security providing nutrients enriched grains.

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