

IMPACT OF ZINC FERTILIZATION ON GAS EXCHANGE CHARACTERISTICS AND WATER USE EFFICIENCY OF COTTON CROP UNDER ARID ENVIRONMENT

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Abstract

Gas exchange characteristics play an important role in the growth and development of cotton plants. Therefore, field experiments were conducted for consecutive two years during 2004 and 2005 to study the effects of Zn fertilization @ 0 (control), 5.0, 7.5, 10.0 and 12.5 kg Zn ha⁻¹ on gas exchange characteristics of cotton cv. CIM-473. The soil of the experimental site was alkaline in nature, moderately calcareous, silt loam with low Zn (DTPA -extractable Zn, 0.5 mg kg⁻¹ soil). The net photosynthetic rate (P_N), transpiration rate (E) and stomatal conductance (g_s) increased while intercellular CO₂ (C_i) decreased with the increasing Zn levels during both years of study. The water use efficiency (P_N/E) was improved by 32% under the highest Zn dose compared to control. There were positive correlations between Zn doses and P_N (0.98**), E (0.97**), g_s (0.88**), WUE (0.94**), while a negative correlation was observed with C_i (-0.95**). The addition of Zn fertilizer caused beneficial influence on cotton plant by enhancing its water use efficiency and gas exchange parameters.

Introduction

Alkaline-calcareous nature of the soils in arid- and semi-arid regions of the world is conducive to zinc (Zn) deficiency (Rashid & Ahmad, 1994). The deficiency of Zn in many crop plants has been reported in many countries like Australia, India, China, Turkey and Pakistan (Sillanpaa, 1982; Fageria *et al.*, 2002; Singh *et al.*, 2005; Rashid & Ryan, 2008). Low Zn availability in these soils is attributed to Zn fixation by free CaCO₃. The precipitation of Zn in alkaline soil solution environment and low Zn replenishment is because of low soil organic matter content generally < 1% (Lindsay, 1972; Rashid & Ryan, 2004).

Zinc deficiency induces reduction in net photosynthesis by 50 to 70% depending on plant species and extent of deficiency (Seethambaram & Das, 1985; Pandey & Sharma, 1989; Shrotri *et al.*, 1989; Hu & Sparks, 1991; Brown *et al.*, 1993). Zinc is a component of plant carbonic anhydrase (CA) enzyme (Tobin, 1970) and it is located in the chloroplast and cytoplasm in C₃ plants (Okhi, 1976). A decline in CA activity has been reported in plants due to Zn deficiency (Okhi, 1976; Read & Graham, 1980). Earlier researchers (Nelson *et al.*, 1969; Hatch & Slack, 1970) reported that CA may be directly involved in photosynthesis, facilitating the diffusion of CO₂ through the liquid phase of the cell to the chloroplast. Sharma *et al.*, (1995) observed that Zn deficiency caused decrease in CA activity, stomatal aperture and transpiration in leaves of cauliflower.

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In the last quarter of 20th century various researchers (Sharma *et al.*, 1994; Hu & Sparks 1991) revealed that stomatal conductance and transpiration rates also decreased under zinc deficiency. Zinc is involved in cellular functions such as protein metabolism, photosynthetic carbon metabolism and indole acetic acid (IAA) metabolism (Marschner, 1995). Moreover, its deficiency has been reported to cause reduction in dry matter production of many crop plants (Cakmak *et al.*, 1997; Cakmak *et al.*, 1998; Khan *et al.*, 2004; Wang & Jin, 2005). Some stresses such as salt stress or nutritional stress may affect plant water status, net CO₂ assimilation rate, stomatal conductance and transpiration rate (Ben-Rouina *et al.*, 2006). Zinc is an essential nutrient for plant growth and development therefore, the crop productivity including cotton is limited due to its deficiency (Irshad *et al.*, 2004; Rashid & Ryan, 2004).

However, reports about effects of Zn deficiency on cotton crop relating to gas exchange characteristics are limited. Hence, the present studies were undertaken to quantify the effects of zinc fertilizer on gas exchange characteristics and water use efficiency in cotton crop under an arid environment.

Materials and Methods

Field experiment was conducted for consecutive two years during the cotton cropping seasons 2004 and 2005, at the Experimental Farm of the University College of Agriculture, Bahauddin Zakariya University (BZU), Multan (longitude: 71°, 30.79' E; latitude: 31°, 16.4' N; altitude: 128 m). The soil used belonged to Sultanpur series. It was coarse silty, hyperthermic, Typic Haplocambids (Anonymous., 1998), porous, friable, moderately calcareous, weakly structured, and developed in arid climate in sub-recent flood plains of the Indus delta. Composite soil samples (0-15 cm depth) were collected prior to applying experimental treatments. The soil samples were analyzed following standard procedures described by Ryan *et al.*, (2001). The analytical data of soil are presented in Table 1. The soil was silt loam, non-saline, alkaline, moderately calcareous and low in organic matter.

Table 1. Physico-chemical characteristics of the soil selected for field experiment.

Characteristics	Values
Soil series	Sultan Pur
Textural class	Silt loam
Sand (%)	29
Silt (%)	53
Clay (%)	18
Organic matter (%)	0.78
CaCO ₃ (%)	5.6
pH	8.1
ECe (dSm ⁻¹)	1.8
NaHCO ₃ -P (mg kg ⁻¹)	9
NH ₄ OAc – K (mg kg ⁻¹)	162
DTPA –Zn (mg kg ⁻¹)	0.54

A widely grown cultivar of cotton (*Gossypium hirsutum* L.) cv. CIM-473 was used as test crop. Zinc was applied @ 0 (control), 5.0, 7.5, 10.0, and 12.5 kg ha⁻¹ as ZnSO₄·7H₂O (21% Zn) prior to crop sowing in a randomized complete block design with four replications. The crop was sown in May and the size of the experimental plots was 123.9 m² with plant-to-plant distance of 30 cm and row-to-row 75 cm. The basal crop fertilization included 150 kg N ha⁻¹ as urea, 60 kg P₂O₅ ha⁻¹ as triple superphosphate, 50 kg K₂O ha⁻¹ as sulphate of potash and 1.0 kg B ha⁻¹ as borax applied as full dose prior to crop sowing except N. Nitrogen was applied in three splits i.e., one-third at sowing, one-third at flowering and the remaining one-third at peak flowering.

A pre-emergence herbicide Stomp-330E was applied @ 2.5 L ha⁻¹ to control weeds. Mechanical weed control was also carried out as and when needed. As far as possible, the crop was kept free of insects pests by scheduled sprays of recommended pesticides. The crop received standard irrigation and production practices of the area. The meteorological data are illustrated in Fig. 1.

Gas exchange characteristics: Measurements of net photosynthetic rate (P_N), transpiration rate (E), intercellular CO₂ (C_i) and stomatal conductance (g_s) were made on a fully expanded third leaf from top of each plant using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). Measurements were taken at peak flowering stage from 9.00 to 11.00 hours with the following specifications/ adjustments: molar flow of air per unit leaf area 403.3 $\mu\text{mol m}^{-2}\text{s}^{-1}$, atmospheric pressure 99.9 kPa, water vapour pressure into chamber ranged from 6.0 to 8.9 mbar, PAR at leaf surface was maximum up to 1711 $\mu\text{mol m}^{-2}\text{s}^{-1}$, temperature of the leaf ranged from 30.7 to 42.0°C, ambient temperature ranged from 28.6 to 38.5°C, and ambient CO₂ concentration was 352 $\mu\text{mol mol}^{-1}$.

Statistical analysis: Data were analyzed statistically according to the methods described by Gomez & Gomez (1984).

Results

Data on net photosynthetic rate (P_N) differed significantly among different levels of Zn fertilizer (Table 2, Fig. 2). P_N increased with concurrent increase in Zn fertilizer. Averaged across the years, maximum P_N value of 26.0 $\mu\text{mol (CO}_2\text{) m}^{-2}\text{s}^{-1}$ was obtained by the application of 12.5 kg Zn ha⁻¹ as compared to the P_N values of 14.0 $\mu\text{mol (CO}_2\text{) m}^{-2}\text{s}^{-1}$ in the untreated check. Positive correlations between Zn rates and P_N were observed.

Transpiration rate (E) also differed significantly with increasing Zn rates (Table 2, Fig. 3). On an average, E increased from 3.6 to 5.1 m mol (H₂O) m⁻² s⁻¹ as the Zn level was increased from 0 to 12.5 kg ha⁻¹. A positive correlation ($r= 0.97^{**}$) was found between Zn rates and E .

Water use efficiency ($\text{WUE} = P_N/E$) was improved markedly with the increase in Zn supply (Table 2, Fig. 4). The values of P_N/E ranged from 3.8 to 5.2 $\mu\text{mol (CO}_2\text{) m mol}^{-1}$ (H₂O) during both the years. The WUE was increased upto 36.8% with the highest Zn dose compared to control. Zinc rates and WUE also had a positive relationship ($r= 0.94^{**}$).

Stomatal conductance (g_s) differed significantly among the different Zn rates (Table 2, Fig. 5). On an average, 23% increase in g_s over control plot was observed with the application of 12.5 kg Zn ha⁻¹. Averaged across the years, minimum stomatal conductance of 267 m mol m⁻² s⁻¹ was maintained in control while the maximum of 329 m mol CO₂ m⁻² s⁻¹ was recorded in treatment receiving 12.5 kg Zn ha⁻¹.

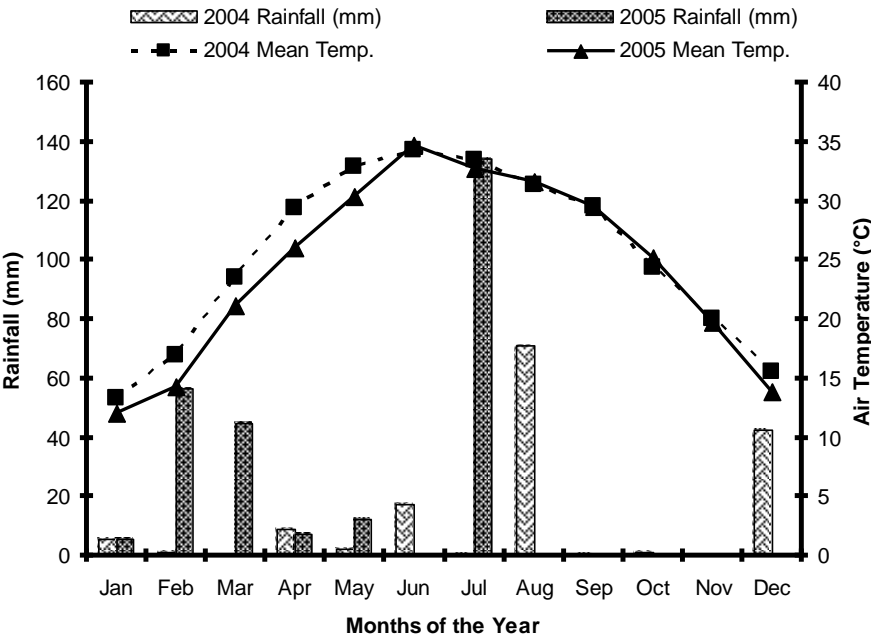


Fig.1. Mean monthly rainfall and temperature during 2004 and 2005.

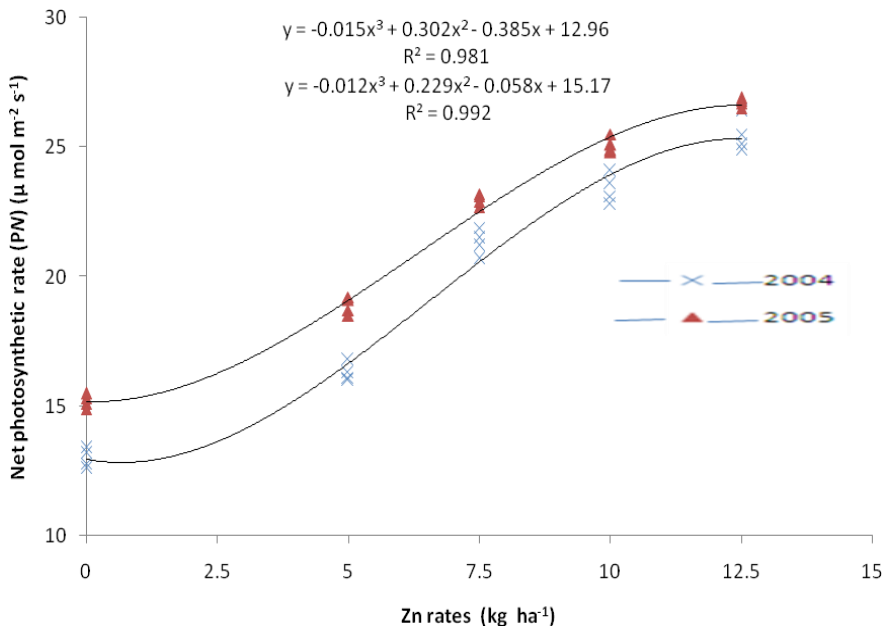


Fig. 2. Relationships between applied Zn and net photosynthetic rate.

Table 2. Effect of zinc nutrition on net photosynthetic rate (P_N) [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$], transpiration rate (E) [$\text{mmol}(\text{H}_2\text{O}) \text{ m}^{-2} \text{ s}^{-1}$], Water use efficiency ($\text{WUE} = P_N/E$) [$\mu\text{mol}(\text{CO}_2) \text{ mmol}^{-1}(\text{H}_2\text{O})$], stomatal conductance (g_s) [$\text{mmol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$] and intercellular CO_2 (C_i) [$\text{mmol m}^{-2} \text{ s}^{-1}$].

Zn rates (kg ha^{-1})	P_N		E		WUE		g_s		C_i	
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
0.0	14.2	13.8	3.6	3.7	3.9	3.8	259.5	275.7	358.5	366.5
5.0	18.7	17.9	4.2	4.0	4.5	4.4	274.2	281.2	321.2	332.8
7.5	22.6	21.9	4.6	4.4	4.9	4.9	291.0	302.2	290.8	312.8
10.0	24.6	23.9	4.8	4.8	5.1	5.0	308.0	326.5	259.8	268.7
12.5	26.2	25.8	5.0	5.2	5.2	5.0	321.0	338.0	200.7	217.2
LSD ($p \leq 0.05$)	0.98	1.2	0.1	0.2	0.2	0.1	13.4	15.5	13.2	10.4

The significant differences in intercellular CO_2 concentration (C_i) were observed among different Zn rates (Table 2, Fig. 5). The values of C_i decreased with concurrent changes in Zn nutrition. On an average, there was 44.8% decrease in C_i over control with the highest Zn rate (12.5 kg ha^{-1}). A negative relationship ($r = 0.95^{**}$) was found between C_i and Zn rates. Averaged across the years, C_i decreased from 362 to 208 $\text{mmol m}^{-2} \text{ s}^{-1}$ as the Zn level was increased from 0 to 12.5 kg ha^{-1} .

Discussion

Gas exchange characteristics like P_N , E and g_s showed significant positive increases with concurrent decrease in C_i as a result of increased Zn nutrition. Similar results have been reported by (Sharma *et al.*, 1994, 1995; Wang & Jin, 2005) who found that Zn deficiency depressed photosynthetic capacity because of decrease in g_s and increase in C_i . Since Zn deficiency has been reported to decrease carbonic anhydrase (CA) activity (Ohki, 1976; Fisher *et al.*, 1997; Cakmak & Engles, 1999), the decrease in photosynthetic activity under control plots might be due to the effect of low CA activity. Furthermore, the decrease in net photosynthesis may also partly be attributed to the decrease in chlorophyll content and abnormal structure of chloroplast as a result of Zn deficiency. Increased Zn supply probably exerted positive influence on chlorophyll formation and CA activity which ultimately promoted P_N . Carbonic anhydrase activity is well known to be involved in photosynthesis, facilitating the diffusion of CO_2 through the liquid phase of the cell to the chloroplast (Hatch & Slack, 1970; Zelitch, 1971).

The increase in transpiration rate (E) because of Zn nutrition substantiates the findings of Hu & Sparks (1991) and Sharma *et al.*, (1994) who reported that Zn deficiency caused reduction in the instantaneous transpiration efficiency of leaves. The results of this study show that Zn fertilization resulted in an increase in WUE. The improvement in WUE by Zn nutrition corroborates with the results of Khan *et al.*, (2003, 2004) and Wang & Jin (2005) that Zn supply caused increase in WUE. Hatfield *et al.*, (2002) observed that WUE could be increased from 15 to 25% by changing nutrient management practices. In the present studies WUE increased by 32% as a result of Zn fertilization. These findings verify that addition of Zn in Zn deficient soils of the arid environment may prove very beneficial by improving water use efficiency of the cotton crop.

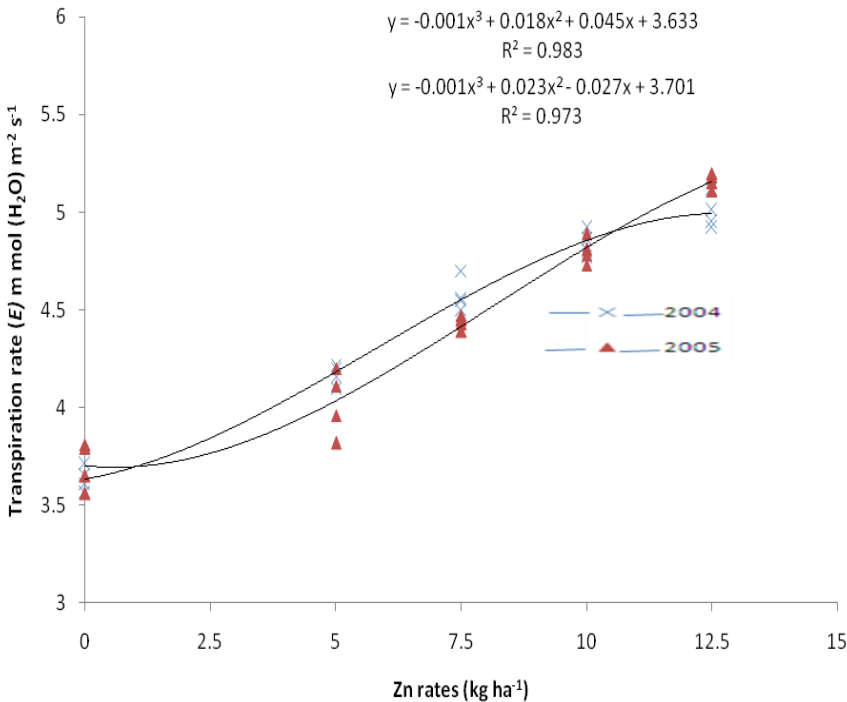


Fig. 3. Relationships between Zn applied and transpiration rate.

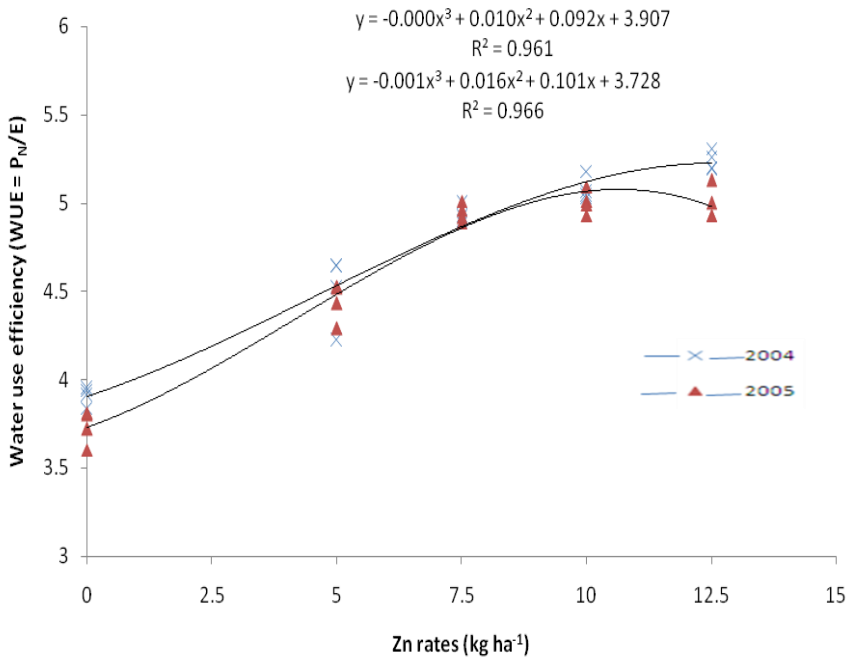


Fig. 4. Relationships between Zn applied and water use efficiency.

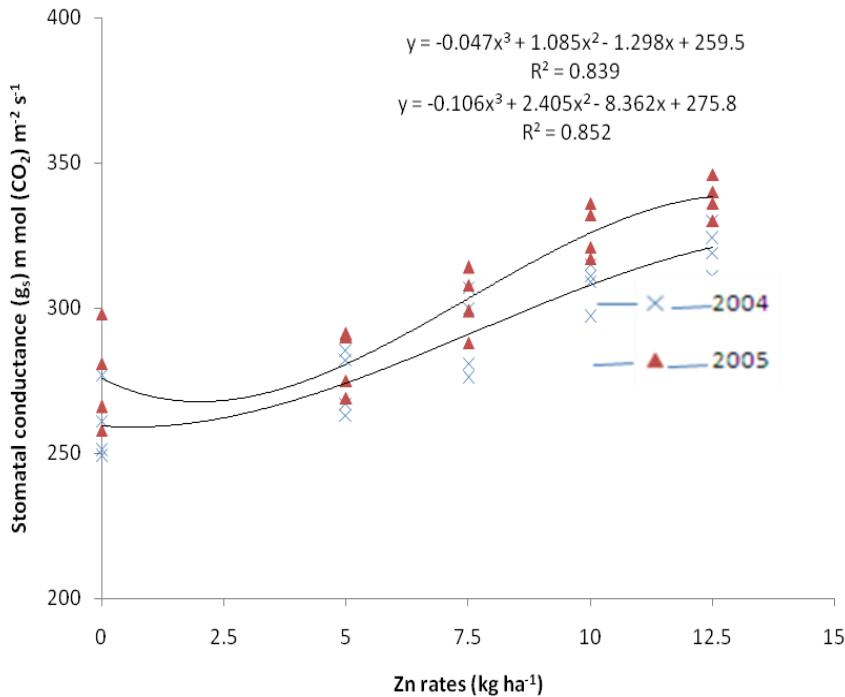


Fig. 5. Relationships between Zn applied and stomatal conductance.

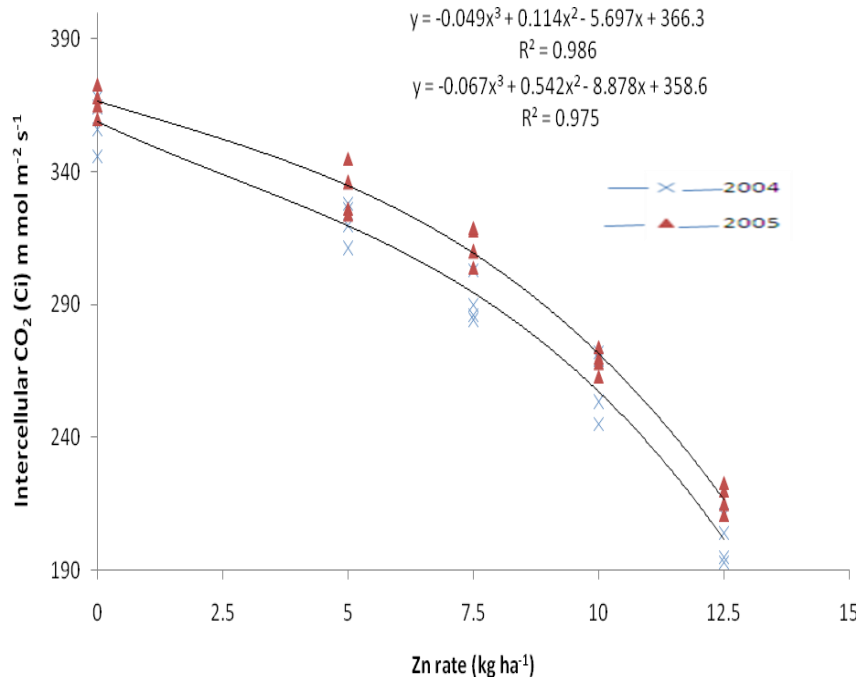


Fig. 6. Relationships between Zn applied and intercellular CO_2 .

Similar trend with varying Zn levels was also observed in case of g_s . The decreased g_s with Zn deficiency has also been reported by Khan *et al.*, (2004). Zinc nutrition caused increase in stomatal aperture as Zn is thought to be involved in stomatal regulation due to its role in maintaining membrane integrity (Khan *et al.*, 2004). Sharma *et al.*, (1995) reported the involvement of Zn in stomatal opening being the constituent of CA which is required for maintaining adequate HCO_3^- in the guard cells and also a factor affecting the K^+ uptake by the guard cells.

The decrease in C_i with Zn nutrition demonstrated the increased utilization of inter cellular CO_2 by the cotton plant. The results are in conformity to the findings of Hu & Sparks (1991) who observed that increased photosynthetic activity and stomatal conductance caused reduction in C_i . They also reported that C_i decreased as Zn concentration increased in leaves. Similar results have also been observed by Sharma *et al.*, (1994, 1995).

The correlations between P_N , E , WUE and g_s showed positive whereas C_i showed negative relationships with Zn nutrition. Gas exchange characteristics play vital role in yield simulation. The regression equations developed from the results may also help in adjusting the dose of Zn nutrient to optimize gas exchange characteristics during the cotton season for improvement in WUE and seed cotton yield under arid environment.

Conclusion

The results of the study reveal that Zn fertilizer is essential for proper maintenance of physiological processes such as P_N , g_s , E and C_i . Addition of Zn fertilizer helped in improving water use efficiency which would ultimately play a vital role in achieving sustainable cotton production in an arid environment.

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