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EFFECT OF ZINC ON CHLOROPHYLL CONTENTS, GAS EXCHANGE ATTRIBUTES AND ZINC CONCENTRATION IN RICE

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Abstract

The deficiency of Zn is one of the major human's health-related issues all over the world. Most food nutritionists suggest to take Zn fortified food to overcome the deficiency of Zn. However, our food is also becoming Zn deficient due to multiple factors, i.e., soil pH, low organic matter, high yielding varieties, over phosphorus application. In addition to the above, our farming community is also unaware of how they can examine the Zn deficiency symptoms. It is a fact that most cereals, i.e., maize and rice, are highly susceptible to Zn deficiency. Therefore, this study aimed to find out the relation of Zn deficiency with respect to chlorophyll content and gas exchange attributes that could be an important indicator for Zn deficiency or optimum level in cereals, i.e., rice. Results showed that chlorophyll contents and gas exchange attributes were significantly low in control (No Zn) over those treatments where Zn was applied at variable rates. The highest level of Zn also significantly enhanced chlorophyll a (134 and 65%), chlorophyll b (143 and 43%), total chlorophyll (142 and 60%), photosynthetic rate (102 and 59%), transpiration rate (68 and 43%) and stomatal conductance (60 and 111%) in both Super Basmati (SB) and KSK-434 rice varieties. In conclusion, chlorophyll content and gas exchange attribute reduction are major indicators of Zn deficiency in rice. For improvement in fine grains and coarse grains, rice 10 kg ha^{-1} Zn application is more economical for improvement in Zn concentration.

Key words: Chlorophyll content, *Oryza sativa* L., Photosynthetic rate, Stomatal conductance, Transpiration rate.

Introduction

The deficiency of micronutrients is developing an alarming situation all over the globe (Stephenson *et al.*, 2000). A large number of small children (1.7% = 116,000 children) died every year due to a deficiency of micronutrients (Kotecha, 2008; Black *et al.*, 2013). Among all the micronutrients, the demand for zinc is most critical after iron, especially in humans (Hambidge & Krebs, 2007; Black *et al.*, 2008). The deficiency of Zn makes the immune system quite weak. Due to such a weak immune system, humans become susceptible to many diseases (Hotz & Brown, 2004). It has been documented that about 2 billion people are Zn deficient all over the world (Streim & Oslin, 2015). The situation is critical in South Asia, where 95% of the population is Zn deficient while 0.4 million humans die every year due to Zn deficiency (Hettiarachchi *et al.*, 2004).

As a micronutrient Zn plays an imperative role in many physiological processes and energy dissipation that can improve the productivity of crops (Monnet *et al.*, 2005). Zn deficiency (ZnD) decreased plant growth by reducing the rate of photosynthesis, chloroplast thylakoids and light-harvesting complex (LHC) proteins disorganization and disintegration of chloroplast membrane. Higher accumulation of starch and carbohydrates are also major indicators of Zn deficiency, especially in rice (Suzuki *et al.*, 2006). However, alkaline pH, calcareousness of soil parent material and sandy texture are also major factors influencing the bioavailability of Zn to crops (Johnson *et al.*, 2005; Alloway, 2008). It has been proposed that each unit of increase in soil pH (5-7) is negatively correlated with Zn availability, i.e., up to 30-folds (Quijano-Guerta *et al.*, 2002). Also, the over-application of phosphatic inorganic fertilizers minimizes the soil Zn uptake by plants (Cakmak, 2008). On the other hand, intensive cultivation of high-yielding varieties is an allied factor responsible for developing Zn deficiency over old adaptive varieties (Cakmak, 2008).

To meet the deficiency of Zn in humans, many strategies have been adopted so far, i.e., use Zn fortified food, supplements intake and Zn bio-fortification in staple food. However, Zn enriched food and supplements have less adaptability and economically expensive (Cakmak, 2008). In many underdeveloped areas, cereals are widely consumed as a staple food. Thus, the concept of Zn fortification in staple food could be an effective and less costly approach to overcome the problem of Zn deficiency (Hettiarachchi *et al.*, 2004; Brown *et al.*, 2010). As a staple food, rice is consumed by 3.5 billion people worldwide (Wang *et al.*, 2007).

As in different cereals crops, rice is highly susceptible to Zn deficiency. The current experiment was performed to find out the best application rate of Zn for improvement in grain Zn concentration, gas exchange attributes and chlorophyll content in coarse and fine grain rice. It is hypothesized that improvement in gas exchange attributes and chlorophyll contents would be indicators of improvement in grain Zn concentration.

Materials and Methods

Experimental site: A field experiment was done to explore the relationship of better Zn uptake in leaves and grains, gas exchange attributes and chlorophyll content on dry seeded rice crop at 31.16º, 71.30º Department of Soil Science, Bahauddin Zakariya University, Multan. The highest temperature of the crop season was recorded in June (42°C), while the minimum temperature was recorded in the month of November (8.1°C). The total rainfall throughout the growing season was 106 mm. Pre-sowing physicochemical characteristics of soil are given in Table 1.

Table 1. Physiochemical soil characteristics.

Attributes	Units	Values
pH*s*	-	8.23
EC*e*	dSm^{-1}	3.26
Organic Matter	%	0.40
Availiable P	$mg\ kg^{-1}$	5.21
Extractable K	$mg\ kg^{-1}$	225
Texture	Clay Loam	

Experimental layout and Zn application: There were six levels of zinc, i.e., 0, 5.0, 7.5, 10.0, 12.5, 15.0 kg Zn ha^{-1} applied by using $ZnSO_4$ at the time of field plots preparation in two rice varieties arranged in a randomized complete block design (RCBD) with three replications. All Zn rates were applied in the soil in a single dose. The pre-experimental soil Zn concentration (0.42 mg kg^{-1} = 0.84 kg ha^{-1}) was kept in mind and subtracted from applied levels of Zn.

Seeds collection: Seeds of 2 rice varieties Super Basmati (SB) as fine and KSK-434 as coarse grain, were purchased from a local market.

Field Plot dimensions: In field plot of 3 m length × 3m width was made for the cultivation of fine and coarse grain rice varieties. The total area of each plot was 9 m^2.

Fertilizers: To meet the macronutrients requirement of rice crops nitrogen (N as urea), phosphorus (P as diammonium phosphate) and potassium (K as sulphate of potash) were applied at the rate of 143:88:68 kg ha^{-1} in each experimental unit as documented by Jabran *et al.*, (2012). Phosphorus was applied in a single split for better seedling's growth, K was applied in two splits, while N was applied in three splits.

Seed rate and sowing method: All broken and damaged seeds were manually screened out initially and then healthy seeds were used for sowing. Seeds were sown @ 75 kg ha^{-1} by drill method (Jabran *et al*., 2012).

Harvesting: When plants were 60 days old, gas exchange attributes and chlorophyll contents were determined. For the determination of leaves and grains, Zn concentration harvesting was done at the time of maturity. Straw and grains were also collected for straw and biological yields.

Chlorophyll contents and gas exchange characters: Chlorophyll contents were determined according to Arnon (1949) and gas exchange attributes were analyzed by the portable infrared gas analyzer (LCA-4 ADC).

Zinc analysis: Collected samples were digested using a di-acid, i.e., HNO_3: $HClO_4$ mixture (Chapman & Pratt, 1961) to analyze Zn concentration on atomic absorption spectrophotometer (Jones *et al.*, 1991).

Statistical analysis

Statistical analysis was carried out using standard statistical procedures (Steel *et al.,* 1997). Two factorials ANOVA was applied for significance determination by using Statistix 8.1 software. Treatments were compared by Tukey's test ($p \leq 0.05$).

Results

Chlorophyll a: Effect of treatments was significant for improvement in chlorophyll a contents in different rice varieties. A significant positive correlation was noted between various levels of Zn and chlorophyll a. However, among different rice varieties and chlorophyll a correlation was not significant (Table 2). Application of different Zn and rice varieties rates have significant main and interactive effects on chlorophyll a. Interaction of different rates of Zn and rice varieties was ordinal and significant for chlorophyll a (Fig. 1B). For chlorophyll a content, Zn15, Zn12.5 and Z10 remained statistically alike to each other in both SB and KSK 434. However, Zn15, Zn12.5 and Z10 differed significantly from control for chlorophyll a content in SB and KSK 434 (Fig. 1A). Application of Zn5 differed significantly better from control in SB for the synthesis of chlorophyll a. The highest increase of 134 and 65% in chlorophyll a content was noted in SB and KSK 434, respectively, over control in Zn15.

Chlorophyll b: Application of different rates of Zn significantly affects chlorophyll b in rice varieties. A significant positive correlation was noted between various levels of Zn and chlorophyll b. However, among different rice varieties and chlorophyll b, correlation was non-significant (Table 2). Different application rates of Zn and rice varieties have significant main and interactive effects on chlorophyll b. For chlorophyll b significant ordinal interaction was noted between different rates of Zn and rice varieties (Fig. 2B). For chlorophyll b content, Zn15, Zn12.5 and Z10 remained statistically alike to each other in both SB and KSK 434. However, Zn15, Zn12.5 and Z10 differed significantly from control for chlorophyll b content in SB. In case of KSK 434 Zn15 and Zn12.5 differed significantly but no significant change was noted in Zn10 over control for chlorophyll b content (Fig. 2A). Application of Zn5 and Zn7.5 also differed significantly better from control in SB for the synthesis of chlorophyll b. However, no significant change was noted among control, Zn10, Zn7.5 and Zn5 for chlorophyll b content in KSK 434. The highest increase of 143 and 43% in chlorophyll b content was noted in SB and KSK 434, respectively, over control in Zn15.

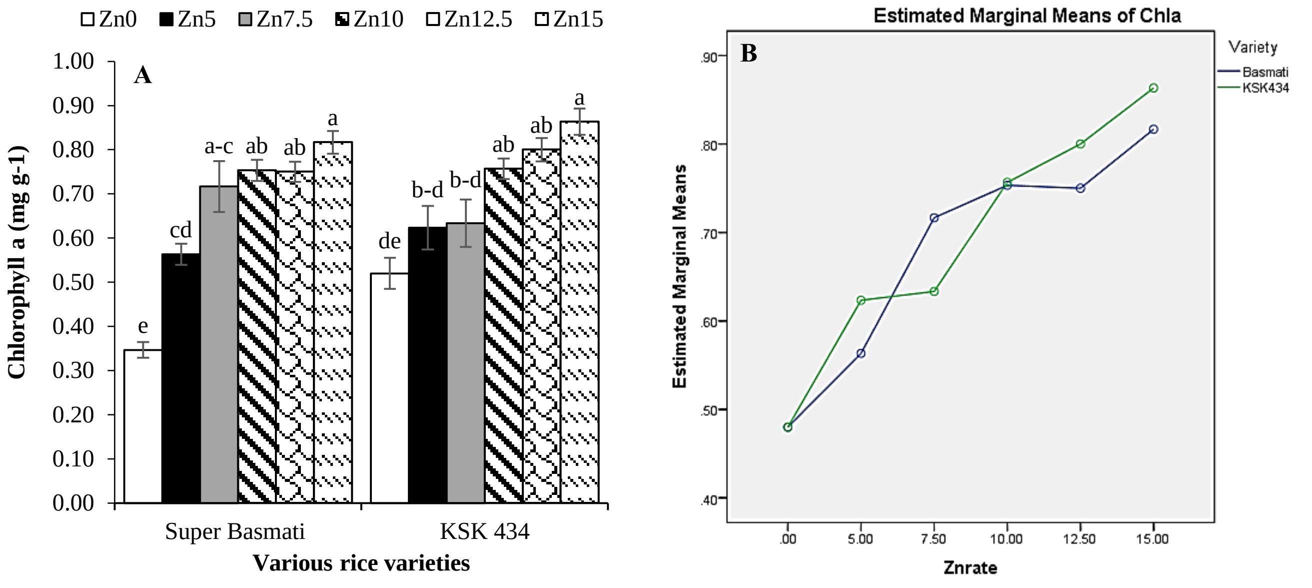

Fig. 1. Effect of various levels of Zn on the synthesis of chlorophyll a in fine grains Super Basmati and coarse grains KSK 434 rice (A). Two factorial ANOVA-based graph (B) showing the interaction of Zn application rate × rice varieties for chlorophyll a (Chla).

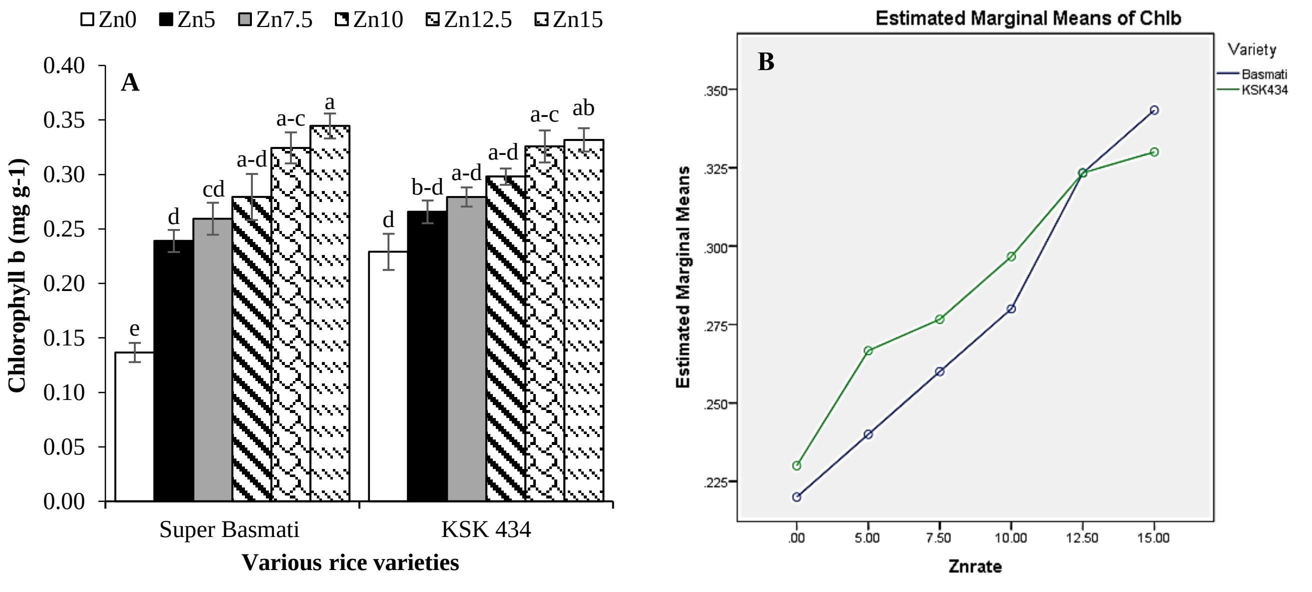

Fig. 2. Effect of various levels of Zn on the synthesis of chlorophyll b in fine grains Super Basmati and coarse grains KSK 434 rice (A). Two factorial ANOVA-based graph (B) showed Zn application rate × rice varieties for chlorophyll b (Chlb).

Total Chlorophyll: In fine and coarse rice varieties, various levels of Zn significantly affect total chlorophyll contents. A significant positive correlation was noted between various levels of Zn and total chlorophyll. However, among different rice varieties and total chlorophyll correlation was non-significant (Table 2). Different application rates of Zn and rice varieties have significant main and interactive effects on total chlorophyll. Significant ordinal interaction was observed between different rates of Zn and rice varieties for total chlorophyll (Fig. 3B). However, Zn15, Zn12.5 and Z10 differed significantly from control for total chlorophyll content in SB and KSK 434. Application of Zn5 and Zn7.5 also differed significantly better from control in SB to synthesize total chlorophyll content (Fig. 3A). However, no significant change was noted among control, Zn7.5 and Zn5 for total chlorophyll content in KSK 434. The maximum increase of 142 and 60% in total chlorophyll content was noted in SB and KSK 434, respectively, over control in Zn15.

Stomatal conductance: In both fine and coarse rice varieties, stomatal conductance was significantly affected by different levels of Zn significantly. Significant positive correlation was noted between various levels of Zn and stomatal conductance. However, among different rice varieties and stomatal conductance correlation was non-significant (Table 2). Different doses of Zn and rice varieties have significant main and interactive effects on stomatal conductance. Significant ordinal interaction was observed between different rates of Zn and rice varieties for stomatal conductance (Fig. 4B). Zn15, Zn12.5, Z10 and Zn 7.5 remained statistically alike for stomatal conductance in SB but differed significantly over control. In the case of KSK 434, Zn15 and Zn12.5 differed significantly from control. Application of Zn5 did not differ significantly over control in SB for stomatal conductance (Fig. 4A). However, significant improvement was noted where Zn7.5 and Zn5 over control for stomatal conductance in KSK 434. The maximum increase of 60 and 111% in stomatal conductance was noted in SB and KSK 434, respectively, over control in Zn15.

Transpiration rate: In different rice varieties, treatments significantly affected the transpiration rate. A significant positive correlation was noted between various levels of Zn and transpiration rate. However, among different rice varieties and transpiration rate correlation was non-significant (Table 2). Different doses of Zn have significant main and interactive effects on transpiration rate. However, rice varieties main effect did not differ significantly for the transpiration rate. Interaction of different rates of Zn and rice varieties was significant ordinal for transpiration rate (Fig. 5B). For transpiration rate, Zn15, Zn12.5, Z10 and Zn 7.5 remained statistically alike to each other in SB and KSK 434 but differed significantly over control. Application of Zn5 did not vary significantly over control in SB and KSK 434 for transpiration rate (Fig. 5A). However, a significant change was noted where Zn7.5 over control for transpiration rate in SB and KSK 434. The maximum increase of 68 and 43% in transpiration rate was noted in SB and KSK 434, respectively, over control in Zn15.

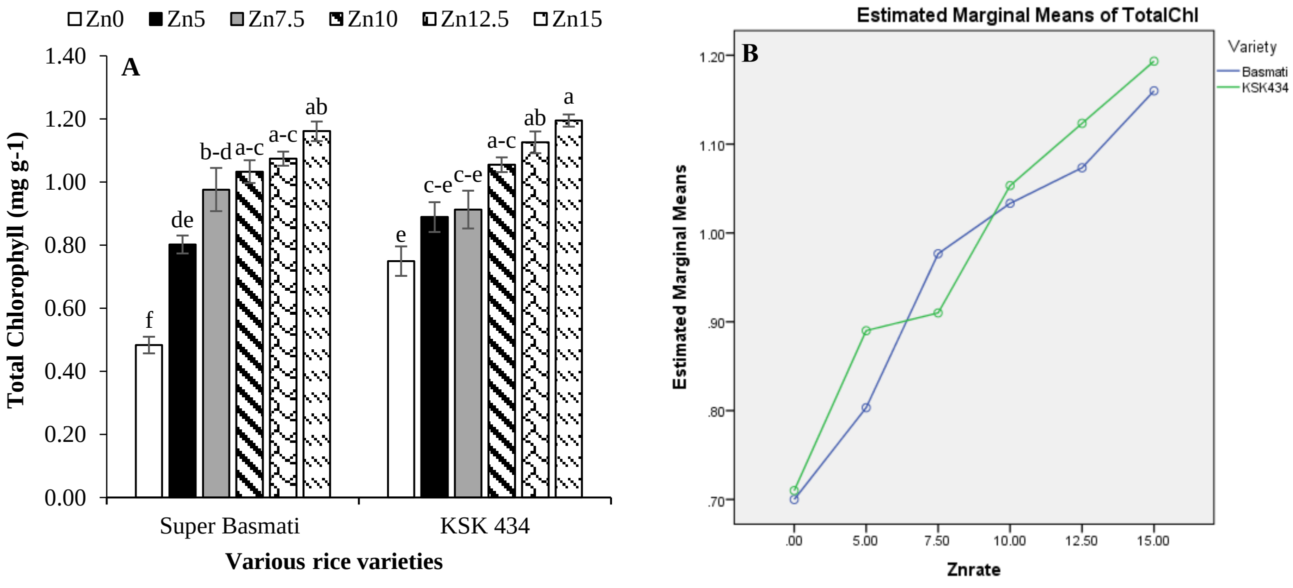

Fig. 3. Effect of various levels of Zn on the synthesis of total chlorophyll in fine grains Super Basmati and coarse grains KSK 434 rice (A). Two factorial ANOVA-based graph (B) showed Zn application rate × rice varieties for total chlorophyll (TotalChl).

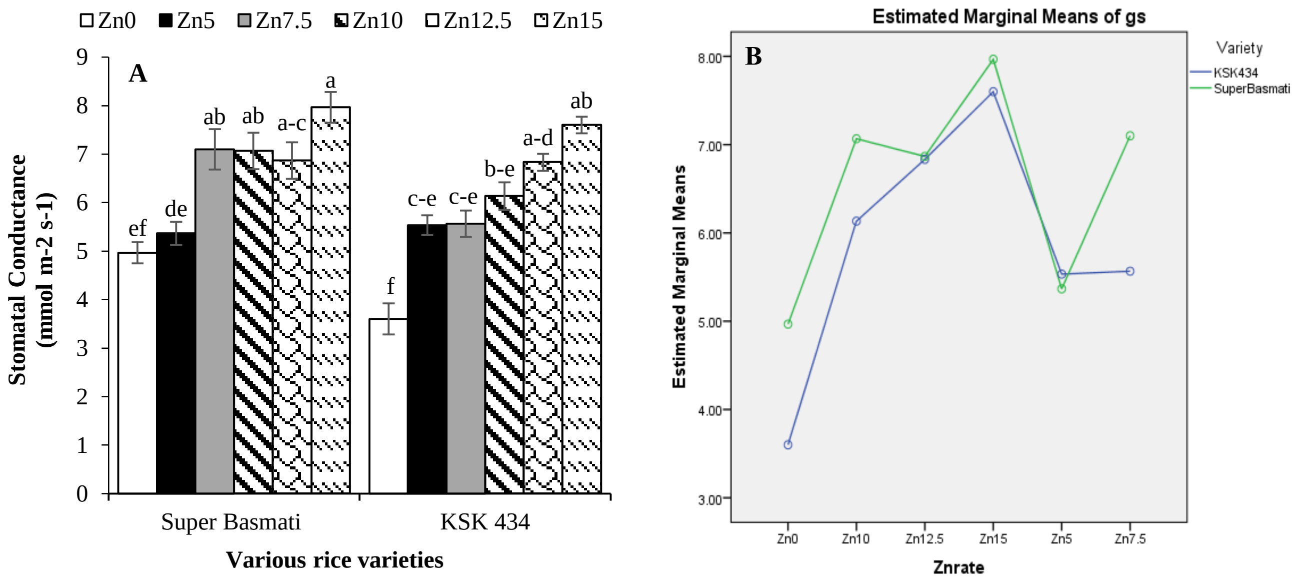

Fig. 4. Effect of various levels of Zn on stomatal conductance in fine grains Super Basmati and coarse grains KSK 434 rice (A). Two factorial ANOVA-based graph (B) showing the interaction of Zn application rate × rice varieties for stomatal conductance (gs).

Table 2. Correlation among treatments, gas exchange attributes, chlorophyll contents and Zn concentration in leaves and grains.

Parameters	Chlorophyll a	Chlorophyll b	Total chlorophyll	Photosynthetic rate	Transpiration rate	Leaves Zn	Grains Zn	Varieties	Various Zn levels
Chlorophyll b p-value	0.8743 0.0000 *								
Total chlorophyll	0.9904 0.0000*	0.9330 0.0000*							
Photosynthetic rate	0.9163 0.0000*	0.9050 0.0000*	0.9371 0.0000*						
Transpiration rate	0.8236 0.0000*	0.8482 0.0000*	0.8522 0.0000*	0.8560 0.0000*					
Stomatal conductance	0.7501 0.0000*	0.6750 0.0000*	0.7484 0.0000*	0.7219 0.0000*	0.7469 0.0000*				
Leaves Zn	0.7073 0.0000*	0.7019 0.0000*	0.7243 0.0000*	0.7558 0.0000*	0.6780 0.0000*	0.4338 0.0082*			
Grains Zn	0.8609 0.0000*	0.8403 0.0000*	0.8776 0.0000*	0.8787 0.0000*	0.8743 0.0000*	0.8415 0.0000*	0.6354 0.0000*		
Variety	0.1391 0.4185ns	0.2027 0.2358ns	0.1609 0.3487ns	0.1646 0.3373ns	0.0336 0.8458ns	-0.2668 0.1157ns	0.5641 0.0003*	-0.0881 0.6093ns	
Various Zn levels	0.8884 0.0000*	0.8671 0.0000*	0.9056 0.0000*	0.9059 0.0000*	0.9157 0.0000*	0.8595 0.0000*	0.7079 0.0000*	0.9397 0.0000*	0.0000 1.0000ns

* = Significant; ns = Non-significant

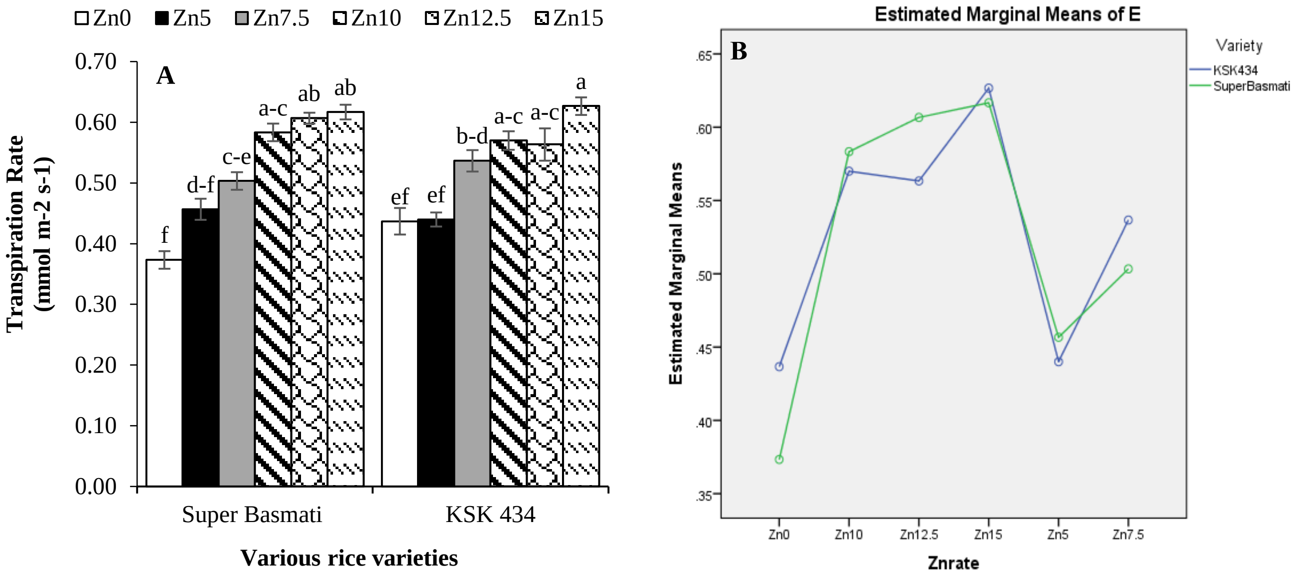

Fig. 5. Effect of various levels of Zn on transpiration rate in fine grains Super Basmati and coarse grains KSK 434 rice (A). Two factorial ANOVA-based graph (B) showing the interaction of Zn application rate × rice varieties for transpiration rate (E).

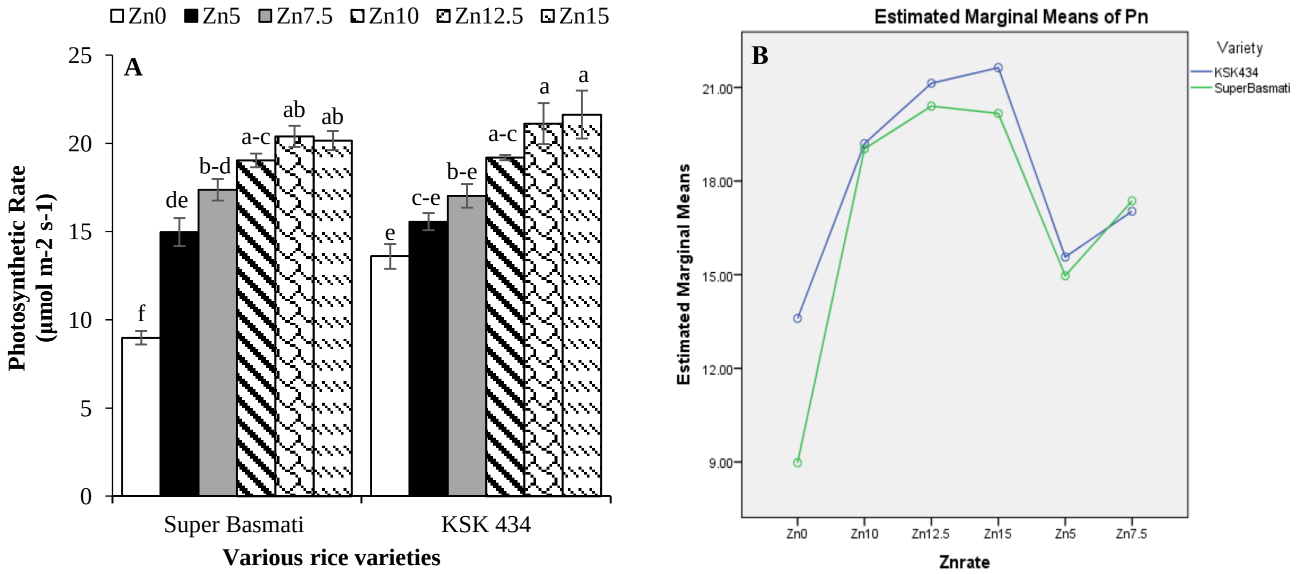

Fig. 6. Effect of various levels of Zn on the photosynthetic rate in fine grains Super Basmati and coarse grains KSK 434 rice (A). Two factorial ANOVA-based graph (B) shows Zn application rate × rice varieties for photosynthetic rate (Pn).

Photosynthetic rate: Effects of treatments were significantly different for photosynthetic rate in different rice varieties, i.e., coarse and fine. A significant positive correlation was noted between various levels of Zn and photosynthetic rate. However, among different rice varieties and photosynthetic rate correlation was non-significant (Table 2). Different doses of Zn and rice varieties showed significant main and interactive effects on the photosynthetic rate. Interaction of different rates of Zn and rice varieties was ordinal and significant for photosynthetic rate (Fig. 6B). For photosynthetic rate, Zn15, Zn12.5, Z10 and Zn 7.5 remained statistically alike to each other in SB and KSK 434 but differed significantly over control. Application of Zn7.5 and Zn5 did not differ significantly over control in KSK 434 but differed significantly in SB for photosynthetic rate (Fig. 6A). The maximum increase of 102 and 59% in photosynthetic rate was noted in SB and KSK 434, respectively, over control in Zn15.

Leaves Zn concentration: Effects of Zn differed significantly for Zn concentration in leaves in coarse and fine rice varieties. A significant positive correlation was noted between various levels of Zn and leaves Zn concentration. Similarly, among different rice varieties and leaves Zn, a significant positive correlation was present (Table 2). Different doses of Zn and rice varieties showed significant main and interactive effects on leaves Zn concentration. Interaction of variable rates of Zn and rice varieties was ordinal and significant for leaves Zn concentration (Fig. 7B). For leaves, Zn concentration, Zn15, Zn12.5 and Z10 differed significantly over control in KSK 434. In SB, the application of Zn15 and Zn 12.5 differed significantly over control for leaves Zn concentration (Fig. 7A). The maximum increase of 66 and 39% in leaves Zn concentration was noted in SB and KSK 434, respectively, over control in Zn15.

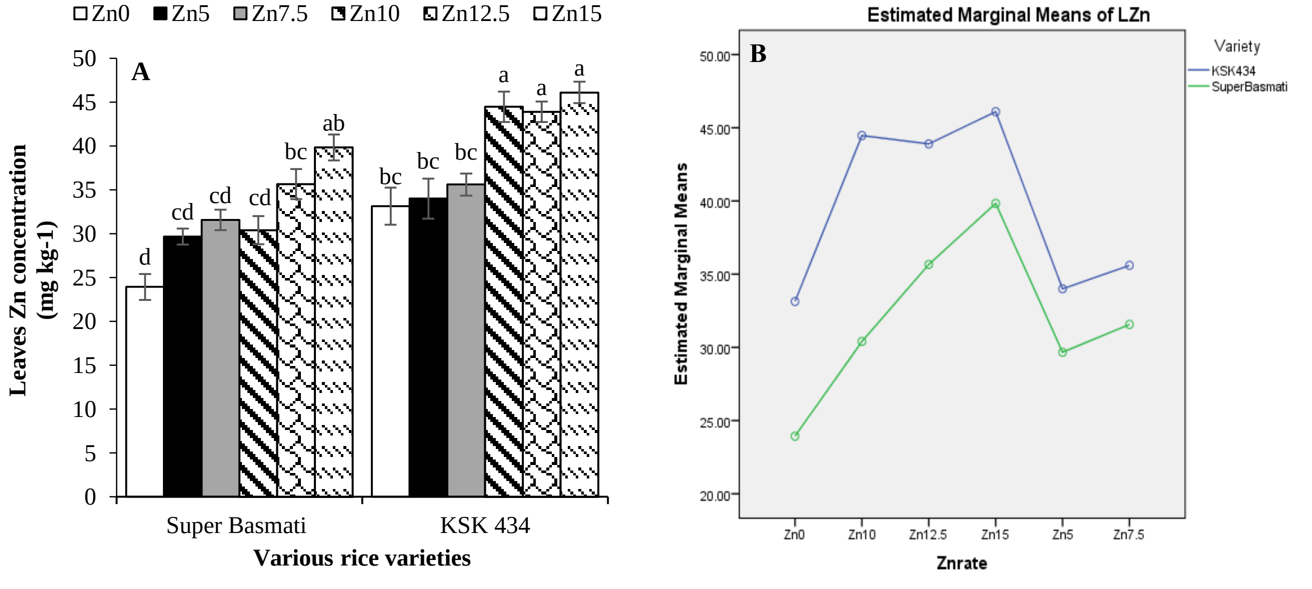

Fig. 7. Effect of various levels of Zn on leaves Zn concentration in fine grains Super Basmati and coarse grains KSK 434 rice (A). Two factorial ANOVA based graph (B) showing the interaction of Zn application rate × rice varieties for leaves Zn concentration (LZn).

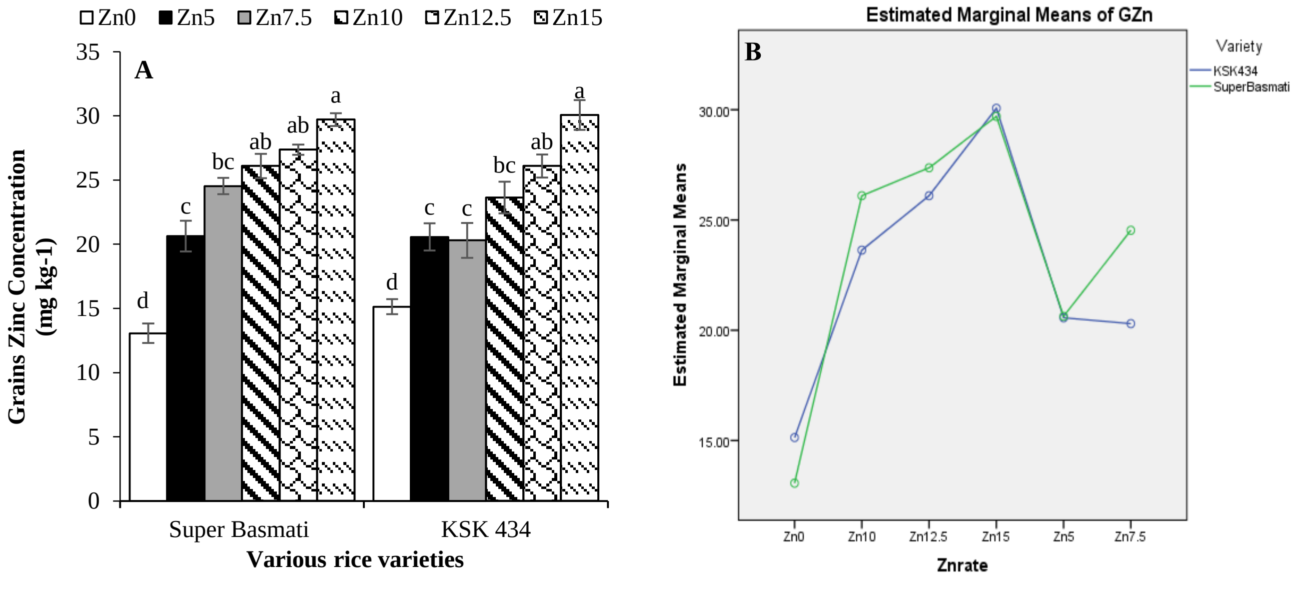

Fig. 8. Effect of various levels of Zn on grains Zn concentration in fine grains Super Basmati and coarse grains KSK 434 rice (A). Two factorial ANOVA based graph (B) showing the interaction of Zn application rate × rice varieties for grains Zn concentration (GZn).

Grains Zn concentration: For grains Zn concentration, application of different levels of Zn differed significantly. A significant positive correlation was noted between various levels of Zn and grains Zn concentration. However, Zn concentration negative correlation among different rice varieties and grains was non-significant (Table 2). Different doses of Zn and rice varieties showed significant main and interactive effects on grain's Zn concentration. Interaction of variable rates of Zn and rice varieties was ordinal and significant for grains Zn concentration (Fig. 8B). For grains, Zn concentration, Zn15, Zn12.5 and Z10 differed significantly in SB and KSK 434 over control (Fig. 8A). Application of Zn7.5 and Zn5 also differed significantly better over control in SB and KSK for grains Zn concentration. The maximum increase of 127 and 99% in grains Zn concentration was noted in SB and KSK 434, respectively, over control in Zn15.

Discussion

The current study showed that increasing the application rate of Zn significantly improved chlorophyll a, chlorophyll b and total chlorophyll from control. A significant reduction in chlorophyll contents in control validated that chlorosis is an important indicator of Zn deficiency in rice. However, the improvement in chlorophyll contents might be due to the activation of the enzymatic system in the plants. Many scientists so far documented that Zn deficiency significantly decreased the process of photosynthesis up to 50-70%. The reduction in photosynthesis depends upon the type of plant species and the severity of Zn deficiency (Brown *et al.,* 1993).

According to Tobin (1970), Zn is an important part of plant enzyme named carbonic anhydrase (CA), which is present in the chloroplast and cytoplasm, especially in C3 plants (Ohki, 1976). Due to Zn deficiency in plants, CA activity becomes restricted (Ohki, 1976; Reed & Graham, 1980). Earlier researchers (Nelson *et al.,* 1969; Hatch & Slack, 1970) documented that this CA enzyme might be involved in the diffusion of CO_2 via the liquid phase of the cell to chloroplast during photosynthesis. Sharma *et al.* (1995) argued that inhibition of CA activity due to Zn deficiency also played an imperative role in decreasing stomatal conductance and transpiration rate in leaves.

The findings of the current experiment also validated the above fact where transpiration rate and stomatal conductance was significantly decreased in control (No Zn). Under the low level of Zn, the membrane loses its integrity, due to which stomatal conductance becomes low (Khan *et al.,* 2004). Disturbance in maintaining the adequate HCO_3 in the guard cells significantly affects the uptake of K ions. Due to the limited availability of K, plants become unable to maintain turgor pressure in guard cells and ultimately, stomatal conductance is decreased (Sharma *et al.,* 1995). A significant improvement in the water use efficiency of plants under Zn fertilization is directly linked with improvement in the rate of transpiration in the plants (Wang *et al.,* 2007). Better water uptake resulted from the major cause of improvement in rice leaves and grain's Zn concentration in the current study. As plants take up water, the solubilize Zn also moved along with water.

In addition to the above, it was noted that increasing level of Zn positively affects the gas exchange attributes, i.e., photosynthetic rate, transpiration rate and stomatal conductance. The improvement in photosynthetic rate was a possibility due to the improvement in rice's chlorophyll content under the various levels of Zn. According to Ahmad *et al*., (2009), the optimum availability of Zn activates the CA enzyme and plays an imperative role in the formation of chlorophyll in plants.

Conclusion

It is concluded that Zn's better availability directly affects the gas exchange attributes and chlorophyll content in rice. Better uptake of Zn significantly enhances chlorophyll contents and gas exchange attributes. A decline in chlorophyll synthesis and reduction in gas exchange attributes are indirect indicators of Zn deficiency in plants. A better yield of rice 10 kg ha^{-1} Zn is a better and economical application rate under the current scenario.

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