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IMPACT OF ZINC AND IRON LEVELS ON KEY ENZYME ACTIVITIES AND GRAIN PROTEIN CONTENT OF RICE

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Abstract

Although all micronutrients are important but zinc (Zn) and iron (Fe) deficiency is a serious concern regarding health of plants and humans both. Therefore, an experiment was conducted to see their effect on key enzyme activities (superoxide dismutase, carbonic anhydrase, catalase and peroxidase) and total protein content in grains of two contrasting rice varieties viz., NDR359 (Zn inefficient) and PD16 (Zn efficient). Various concentrations of Zn (0, 0.25% and 0.5% $ZnSO_4$) and Fe (0, 0.5% and 1.0% $FeSO_4$) were foliarly applied on both the varieties at 30, 60 and 90 days after sowing (DAS). Regarding activity of superoxide dismutase (SOD), maximum (0.127 units min^{-1}) was recorded at 0.5% Zn+0% Fe in NDR359 while 0.5% Zn+0% Fe was found most effective (21.76 units mg^{-1}) in increasing carbonic anhydrase (CA) activity in PD16. With respect to catalase (CAT) activity, maximum was recorded at 0% Zn+1.0% Fe in NDR359 whereas regarding peroxidase (POD) activity, variety PD16 showed maximum at 0.5% Zn+0% Fe. Protein content in grains of both the varieties was found to be increased with increasing levels of both the micronutrients and maximum was recorded in PD16 at 0.5% Zn+1.0% Fe. All these combinations of Zn and Fe can be further tested and recommended for future use.

Key words: Rice, zinc, iron, enzyme activities, protein content.

Introduction

Rice (*Oryza sativa* L.) is a major cereal crop and a staple food for half of the world's population. It provides 23% of the global human per capita energy and 16% of the per capita protein (IRRI, 1997). Polishing of rice grain make it deficient in nearly all the micronutrients but Zn and Fe deficiency is most prevalent (Mathpal *et al.*, 2015). Micronutrient deficiencies affect near about 3 billion population of world (FAO, 2009). Annually death of more than 1.5 million children takes place globally because of the deficiency of vitamin A, Fe and Zn with most of those occurring in developing world and situation is worst in South Asian countries and Sub-Saharan Africa (Caulfield *et al.*, 2005). Presence of free carbonates in high concentration, low organic matter in soil, high pH of soil solution and excessive use of chemical fertilizers etc. are all potent cause of micronutrient deficiencies which ultimately not only hampering crop productivity but also deteriorating grain quality (Arif *et al.*, 2012).

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Zinc plays very important role in plant metabolism by influencing the activities of hydrogenase and carbonic anhydrase enzymes, stabilization of ribosomal fractions and synthesis of cytochrome. Plant enzymes activated by zinc are involved in carbohydrate metabolism, maintenance of the integrity of cellular membranes, protecting plant cells from reactive oxygen species, protein synthesis, regulation of auxin synthesis and pollen formation (Marschner, 1995; Cakmak, 2000). Being a part of Zn-finger transcription factors it is also involved in the development and function of floral tissues like anthers, tapetum and pollen in many plants (Kapoor *et al.*, 2000). Zinc is involved in protein, nucleic acid, carbohydrate, and lipid metabolism. Several molecules associated with DNA and RNA synthesis are also zinc metalloenzymes, such as RNA polymerases, reverse transcriptases and transcription factors (Ishimaru *et al.*, 2011). The control of gene transcription and the coordination of other biological processes regulated by proteins containing DNA-binding Zn-finger motifs. Zinc finger proteins interact via their individual fingers to three base pair subsites on the target DNA to regulate gene expression (Roy *et al.*, 2012).

Regarding importance of Fe for plants and humans, iron (Fe) is fourth most abundant and third most growth limiting nutrient on earth crust (Rout and Sahoo, 2015). It functions in various physiological processes, including ATP biosynthesis during photosynthesis and respiration, chlorophyll development and is an integral part of heme, Fe-sulfur cluster and other Fe-dependent biological reactions. Under aerobic conditions, iron is oxidized and poorly soluble in water. In many dicots and non-grass monocots, inhibition of root elongation, increase in root tip diameter and formation of additional root hairs occurs because of Fe deficiency (Schmidt *et al.,* 2000). Iron deficiency is also correlated with changes in chloroplast organization, altered expression of small and large subunits of Rubisco and decreased chlorophyll synthesis (Moseley *et al.,* 2002). Iron deficiency is one of the major agricultural problems which lead to reduced crop yields. Although, Fe is abundant in soils, but it is present in insoluble forms thus results in poor availability and utilization for plants (Kerkeb and Connolly, 2006).

Keeping in mind the deficiency status of Zn and Fe as well as their prime importance for both plants and humans the present study was planned to examine their effect on key enzyme activities in two contrasting rice genotypes.

Materials and methods

Super oxide dismutase (SOD) activity

The SOD activity was estimated in freshly harvested leaves at flowering according to the method as described by Mathpal *et al.*, 2015.

Carbonic anhydrase (CA) activity

Carbonic anhydrase (CA) activity was estimated in freshly harvested leaves at flowering after following the method as described by Mathpal *et al.*, 2015.

Catalse (CAT) activity

Catalase (CAT) activity was estimated *in vitro* by the method followed by Kato and Shimizu, 1987 with some modifications. Leaf tissue (0.2 g) was homogenized and homogenate was centrifuged at 12,000 g for 20 min. During whole extraction process 4^0C temperature was maintained. To the reaction mixture (100 mM potassium phosphate buffer (pH 7.0), 0.1 μM EDTA and 20 mM H_2O_2) 50 μl enzyme extract was added. Decrease in H_2O_2 was measured by recording reduction in absorbance at 240 nm and activity of enzyme was calculated using the extinction coefficient (40 $mM^{-1}cm^{-1}$ at 240 nm) for H_2O_2.

Peroxidase (POD) activity

Peroxidase activity was estimated *in vitro* by measurement of the purpurogallin produced, as described by Kar and Mishra, 1976. After grinding leaf tissue (0.2 g), centrifugation of homogenate was carried out for 20 min at 12,000 g. The whole of the extraction was done at 4°C. To the above described reaction mixture (125 μmol of phosphate buffer, 50 μmol of pyrogallol and 50 μmol H_2O_2) 100 μl of enzyme extract was added. The absorbance was recorded at 470 nm and activity was calculated using extinction coefficient (26.6 Mm^{-1} cm^{-1} at 470 nm) for pyrogallol and expressed as units per milligram of fresh weight (U mg^{-1} fw). One unit of POD activity was defined as 1 mmol purpurogallin produced per minute.

Protein content in grains

Extraction and estimation of total protein in dehusked rice grains was done by using the method followed by Mathpal *et al.*, 2015.

Result and Discussion

Superoxide dismutase (SOD) activity

The superoxide dismutase activity in leaves of both contrasting varieties of rice is shown in fig. 4.1. The foliar spray of 0% Zn+0.5%Fe and 0% Zn+1.0% Fe solutions decreased the SOD activity by 19.6 percent and 24.5 percent in PD16 while NDR359 showed a reduction of 9.2 percent and 12.5 percent, respectively as compared to 0% Zn+0% Fe. The variety PD16 showed a reduction of 13.2 percent and 16.9 percent in SOD activity whereas NDR359 showed a percent reduction of 11.3 and 14.7 in enzyme activity with the application of 0.25% Zn+0.5% Fe and 0.25% Zn+1. 0%Fe in comparison to 0.25% Zn+0% Fe. The foliar spray of 0.5% Zn+0.5% Fe and 0.5% Zn+1.0% Fe levels of zinc and ferrous sulphate decreased the SOD activity in PD16 by 13.9 percent and 18.2 percent while in NDR359 a percent reduction of 18.1 and 20.4 was recorded as compared to 0.5% Zn+0% Fe, respectively. Overall the maximum (0.127 units min^{-1}) SOD activity was recorded in NDR359 under 0.5% Zn+0% Fe and the minimum (0.077 units min^{-1}) for PD16 under 0% Zn+1.0% Fe level of zinc and ferrous sulphate.

The results of SOD activity clearly indicates that the

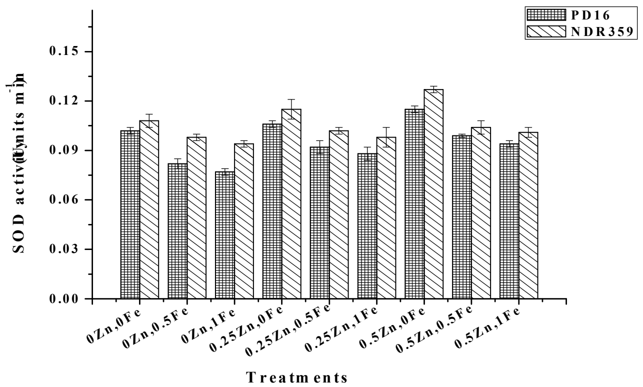

Fig 1: Effect of foliar spray of different zinc & iron levels on super oxide dismutase (SOD) activity (Units min^{-1}) in two contrasting rice genotypes. Vertical bars indicate ± standard deviation.

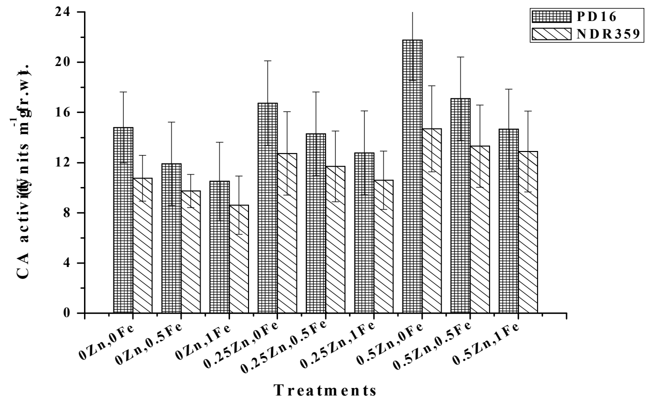

Fig 2: Effect of foliar spray of different zinc & iron levels on carbonic anhydrase (CA) activity (Units mg^{-1} fr. wt.) in two contrasting rice genotypes. Vertical bars indicate ± standard deviation.

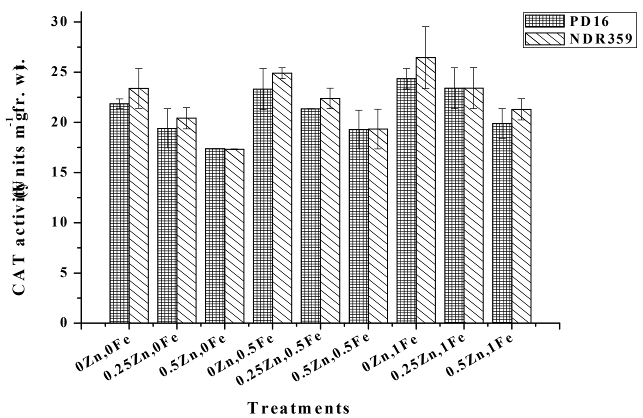

Fig 3. Effect of foliar spray of different zinc & iron levels on catalase (CAT) activity (Units mg-1 fr. wt.) in two contrasting rice genotypes. Vertical bars indicate ± standard deviation

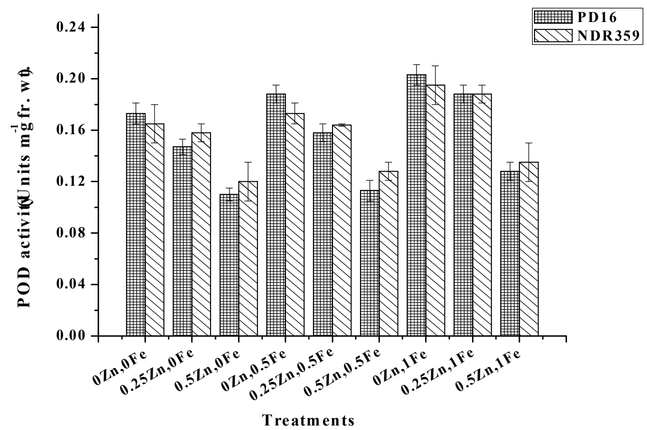

Fig 4: Effect of foliar spray of different zinc & iron levels on peroxidase (POD) activity (Units mg^{-1} fr. wt.) in two contrasting rice genotypes. Vertical bars indicate ± standard deviation.

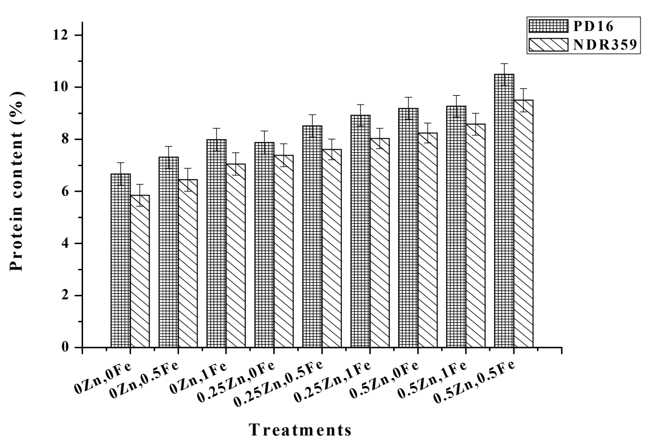

Fig 5: Effect of foliar spray of different zinc & iron levels on protein content (%) in two contrasting rice genotypes. Vertical bars indicate ± standard deviation.

enzyme activity increased with increasing zinc sulphate levels from 0% to 0.5% whereas an increase in the level of ferrous sulphate from 0% to 1.0% under a given level of zinc sulphate decreased the enzyme activity in both the varieties. The iron at higher concentration was likely to inhibits the uptake and translocation of zinc thus, making the leaves more deprived of zinc which ultimately resulted in reduced enzyme activity. Being a zinc inefficient variety, NDR359 showed higher SOD activity as compared to PD16 which was a zinc efficient variety. The possible reason of these results would be that NDR359 accumulated more zinc in leaves. Superoxide dismutase used zinc as a cofactor and zinc-SOD is the most abundant form of enzyme in the plant cell. Chen *et al.* (2003) showed the essentiality of zinc for the SOD activity. Under zinc deficiency, a reduction in the Cu/Zn SOD activity was recorded in the zinc inefficient wheat genotypes. Similarly, a reduction in the expression of Cu/Zn SOD under zinc deficient conditions in rice leaves was observed by Obata *et al.,* 1999 which showed 15% increment in the SOD activity by increasing 1% of zinc in the growth medium. Bharti *et al.,* 2013 also reported higher SOD activity under increased (20 kg Zn ha^{-1}+0.5% foliar application) levels of zinc.

Carbonic anhydrase (CA) activity

Carbonic anhydrase (CA) activity under varying levels of zinc and iron was recorded in leaves of both the varieties (fig 2). The variety PD16 showed a percent reduction of 19.0 and 28.5 while NDR359 showed a percent reduction of 14.3 and 23.9 with the application of 0% Zn+0.5% Fe and 0% Zn+1.0% Fe levels of zinc and ferrous sulphate solutions as compared to 0% Zn + 0% Fe. By the application of 0.25% Zn+0.5% Fe and 0.25% Zn+1.0% Fe a reduction of 14.3 percent and 23.9 percent was recorded in PD16 while in NDR359 a percent reduction of 7.8 and 17.3 was noted, respectively as compared to 0.25%Zn+0%Fe level of zinc sulphate. Similarly, the application of 0.5% Zn+0.5% Fe and 0.5% Zn + 1.0% Fe combination of zinc and ferrous sulphate solution decreased the enzyme activity by 21.1 percent and 32.7 percent in PD16 whereas 8.9 percent and 12.3 percent in NDR359, respectively as compared to 0.5% Zn+0% Fe. Variety, PD16 showed maximum (21.76 units mg^{-1} fr.wt.) carbonic anhydrase activity at 0.5% Zn+0% Fe whereas minimum activity (8.60 units mg^{-1} fr.wt.) was recorded for NDR359 at 0% Zn+1.0% Fe combination of zinc and ferrous sulphate.

Zinc act as a cofactor for carbonic anhydrase activity and acted as an integral component of the enzyme. Carbonic anhydrase is most important enzyme in plants as it catalyze the conversion of CO_2 into bicarbonate ion which is accepted by the plant for further CO_2 fixation. The results of the present investigation clearly showed that enzyme activity increased with increasing zinc levels indicating its dependence on zinc for activity. As antagonistic effect of zinc and iron on each other is well known, the increasing levels of iron from 0% to 1.0% ferrous sulphate at a constant level of zinc sulphate reduced the activity of enzyme in both the varieties and the effects were more profound in PD16. Sasaki *et al.* (1998) reported a reduction in the CO_2 transport rate and the carbonic anhydrase activity in response to reduced zinc supply. Similarly, in zinc inefficient wheat genotype a reduced activity of carbonic anhydrase was recorded when it was grown under zinc deficient conditions (Hacisalihoglu *et al.*, 2003).

Catalase (CA) activity

Catalase activity under various levels of zinc and iron, in both the rice varieties is shown in fig 3. Foliar spray of 0.25% Zn+0% Fe and 0.5% Zn+0% Fe zinc sulphate solution caused a reduction of 11.0 percent and 20.6 percent in PD16 while in NDR359 a percent reduction of 12.4 and 25.7 was recorded, respectively as compared to 0% Zn+0% Fe. The variety, PD16 showed a reduction of 8.5 percent and 17.5 percent whereas, NDR359 showed a reduction of 10.4 percent and 22.4 percent with the application of 0.25% Zn+0.5% Fe and 0.5% Zn+0.5% Fe level of zinc sulphate solution, respectively in comparison to 0% Zn+0.5% Fe level. The foliar application of 0.25% Zn+1.0% Fe and 0.5% Zn+1.0% Fe levels of zinc and ferrous sulphate solution decreased the enzyme activity in PD16 by 3.7 percent and 18.5 percent while in NDR359 the reduction was 11.3 percent and 19.3 percent, respectively as compared to 0% Zn+1.0% Fe level. The maximum (26.44 units mg^{-1} fr.wt.) catalase activity was recorded at 0% Zn+1.0% Fe for NDR359 while the minimum (17.32 units mg^{-1} fr.wt.) was also recorded for NDR359 under 0.5% Zn+0% Fe level.

Catalase acts as a marker enzyme for the iron in plant cell and its activity gets directly affected by the iron content as it is required as a cofactor for the enzyme activity. In the present investigation catalase activity increased with increasing levels of ferrous sulphate but by increasing concentration of zinc sulphate concentration at a constant level of ferrous sulphate decreased the enzyme activity in both the varieties. It might be due to competing behaviour of both zinc and iron with each other for uptake site at the plant cell membrane and during long distance transport through the xylem (Kabata-Pendias, 2001; Alloway, 2008). A comparison of both the varieties showed that NDR359 had higher enzyme activity as compared to PD16. The increased activity of catalase under increasing dose of iron was also reported in rice genotypes by Zhang *et al.*, (2010) and they noted about 33% increase in catalase activity with increase in iron level from 0 to 200 μM.

Peroxidase (POD) activity

Peroxidase activities measured in leaves of both the rice varieties under different levels of zinc and iron are presented in fig 4. Application of 0.25% Zn+0% Fe and 0.5% Zn+0% Fe solutions decreased the peroxidise activity as compared to 0% Zn+0% Fe level by 15.0 percent and 36.4 percent in PD16 and by 4.2 and 27.2 percent in NDR359, respectively. Foliar application of zinc and ferrous sulphate solutions in a combination of 0.25% Zn+0.5% Fe and 0.5% Zn+0.5% Fe reduced the enzyme activity in comparison to 0% Zn+0.5% Fe by 15.9 percent and 39.8 percent in PD16 and by 5.2 percent and 26.0 percent in NDR359 respectively. The application of 0.25% Zn+1.0% Fe and 0.5% Zn+1.0% Fe zinc and ferrous sulphate solutions decreased the enzyme activity by 7.9 percent and 36.9 percent in PD16 and by 3.5

percent and 30.7 percent in NDR359, respectively. Peroxidase activity was found to be maximum (0.203 units mg^{-1} fr wt.) at 0% Zn+1.0% Fe in PD16 whereas the minimum (0.110 units mg^{-1} fr. wt.) was also found at 0.5% Zn+0% Fe level in the same variety.

Like catalase, peroxidase is also another enzyme that contains iron as an integral component and required for its catalytic acivity. Iron toxicity is a nutritional disorder of rice which is associated with high ferrous iron in flooded soil. Application of excess ferrous iron was found to induce peroxidase activity in rice leaves. In the present investigation, the peroxidase activity was found to increase with increasing levels of iron sulphate and the reduction recorded in both the varieties was due to increased zinc sulphate levels. An increase in peroxidase activity in response to application of iron was reported by Fang and Kao, 2000. Similarly, use of humic acid rich organic fertilizers increased uptake and mobilization of iron by the plant which increased the activity of peroxidase (Denre *et al.*, 2013).

Protein content

Protein content in grains of two rice varieties under various zinc and iron levels is shown in fig 5. The foliar application of 0% Zn+0.5% Fe and 0% Zn+1.0% Fe solutions increased the protein content by 9.5 percent and 19.7 percent in PD16 whereas, in NDR359 the content increased by 10.2 percent and 20.5 percent, respectively over 0% Zn+0% Fe. Foliar spray of 0.25% Zn+0.5% Fe and 0.25% Zn+1.0% Fe further enhanced the protein content by 7.9 percent and 13.1 percent in PD16 and by 2.9 percent and 8.6 percent in NDR359, respectively as compared to 0.25% Zn+0% Fe. A percent increment of 0.8 and 14.1 in the protein content was observed in PD16 while 4.1 percent and 10.7 percent was noted in NDR359 at 0.5% Zn+0.5% Fe and 0.5% Zn+1.0% Fe, respectively in comparison to 0.5%Zn+0%Fe. The maximum (10.49%) protein content was recorded in PD16 at 0.5% Zn+1.0% Fe whereas, the minimum (5.85%) content was observed in NDR359 at 0% Zn+0% Fe levels of zinc and ferrous sulphate.

The results of the present study clearly indicated that the protein content in grains of both the rice varieties increased with the increasing levels of both zinc and iron. The possible reason for the observed effect is that zinc is an integral part of zinc finger motifs which bind to the specific locations of DNA and regulate the gene expression, ultimately leading to increased content of proteins in different plant parts (Roy *et al.*, 2012). Similarly the increased protein content due to increasing iron levels might be due to the increased expression of iron storage proteins in the grains. Between the varieties, PD16 showed relatively higher protein content as compared to NDR359 which is a zinc inefficient variety. Morshedi and Farahbakhshb, 2010, reported maximum grain protein content in wheat under 40 kg Zn ha^{-1} as compared to no application of zinc.

Conclusion

Regarding enzyme activities both the varieties showed mixed response against various combinations of Zn and Fe. With respect to grain protein content variety PD16 expressed more protein than ND359, thus exhibited better translocation for both Zn and Fe. Therefore it can be concluded that based on various physiological traits viz., uptake efficiency, translocation efficient etc superior varieties can be selected.

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