



Enhancing zinc availability in rice (*Oryza sativa*) grains by different zinc fertilization methods

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ABSTRACT

In an experiment to study the effect of basal and foliar application of zinc on antinutrient and pronutrient compounds in rice grains (*Oryza sativa*) the observed results showed that a combination of basal and foliar application resulted in an increase in the zinc content of rice plants by almost 58% during 2009 and up to 55% during 2010, which was confirmed by the measurement of acid phosphatase, grain and straw zinc content of the rice genotypes. Moreover, phytic acid content was found to be reduced and methionine content was enhanced. In general it was observed that rice genotypes respond to zinc application positively and zinc availability was found to increase. Hence, it can be concluded that proper zinc fertilization can enhance the availability of zinc in rice grains.

Key words: Antinutrients, Phytic acid, Pronutrients, Zn fertilization

Daily dietary requirements of zinc and other micronutrients is rarely met with the polished rice grains as they contain only about 20 % of the daily requirement of zinc and a small amount of iron. Hence when rice is a major part of diet Zn deficiencies prevail, which affect around three billion people worldwide (Welch and Graham 2004), most of this population residing in developing countries. Rice is an indispensable staple food for half of the world's population providing 50–85 % of daily energy source and is consumed in large amounts. Therefore, even a small increase in the nutritive value of rice can be highly significant for human nutrition (Sharma *et al.* 2013).

Cereals are inherently low in Zn contents with lesser bioavailability. Poor grain nutritive value of cereals is an important reason for widespread micronutrient malnutrition among populations eating rice (*Oryza sativa* L.) as staple food. Different strategies that can be used for biofortification of rice grains include genetic modification involving various metal transporters, zinc fertilization, scanning efficient genotypes and use of plant growth promoting rhizobacteria (Sharma *et al.* 2013). Plant foods contain substances that can either inhibit or enhance the absorption and/or utilization of micronutrients when eaten (Campen and Glahn 1999). An antinutrient is a substance occurring in the diet which acts

antagonistically towards one or multiple nutrients, reducing bioavailability. This is usually done through complex formation which reduces nutrient absorption (Graham *et al.* 2000). Staple plant foods can contain substances that inhibit the absorption and/or utilization (i.e. bioavailability) of trace elements (Graham *et al.* 2001). The absorption of mineral elements is affected by many factors, one such factor being formation of insoluble complexes by attachment of soluble minerals to the compounds like phosphates or organic acids (Ekholm *et al.* 2003).

Therefore in order to evaluate a group of rice cultivars commonly being used in northern parts of India for modulation of their nutrient and antinutrient compounds, an experiment was undertaken during the rainy seasons of 2009 and 2010 with an objective to enhance the pronutrients in rice grains by different regimens of zinc fertilization during the growing period of rice.

MATERIALS AND METHODS

The seeds of 10 rice genotypes, namely, IR 24, Jaya, Kalanamak, NDR-359, Pusa Basmati-1, Pant Dhan-12, Pant Dhan-16, Pant Dhan-19, Tarori Basmati and Type-3 were obtained from Department of Genetics and Plant Breeding, Govind Ballabh Pant University of Agriculture and Technology (GBPUAT), Pantnagar, India. The experiment was conducted at Norman E. Bourlaug Crop Research Center (CRC) of G B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India, during rainy seasons of 2009 and 2010. The nursery of all the 10 rice genotypes was raised by the dry seed bed method and 21 days old seedlings were uprooted and transplanted in the field in a split plot design with a plant density of 50

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plants/m². Geographically, the site lies in the tarai plains about 30 km southwards of foothills of Shivalik range of Himalayas at 29°N latitude, 79°29'E longitude and at an altitude 243.8 m. The nitrogen content of the soil was 31.8 mg/kg and phosphorus content was 10.12 mg/kg. DTPA extractable Zn was 0.47 mg/kg, Fe was 25.3 mg/kg, Cu 1.24 mg/kg and Mn 5.97 mg/kg. Fertilizer doses of nitrogen (100 kg/ha), phosphorus (45 kg/ha), potassium (60 kg/ha) and irrigation (flooded conditions) were maintained as per recommendations for cultivation of rice.

Zinc application: Zinc application was done as a basal dose of 20 kg/ha and basal dose accompanied by foliar spray of 0.5% (5 g/L) Zn to the rice plants at active tillering and flowering. Control did not receive any zinc application. The field experiment was carried out with three treatments and three replications of each in a split plot design. Proper spacing and water channels were maintained between the plots.

Zinc estimation by AAS. 1 g dried and powdered plant parts were treated with conc. HNO₃ overnight. Then after evaporating HNO₃, 10 ml mixture of nitric acid-sulfuric acid-perchloric acid (10: 1: 4 v/v/v) was added in each sample. The samples were then digested and residue was dissolved in 5 ml 6N HCl and volume made up to 50 ml with distilled water, filtered and stored. Zinc content was determined by double beam atomic absorption spectrophotometer at 213.9 nm.

Methionine. Methionine content was measured by according to Horn *et al* (1946). The liberated methionine gives a yellow color with nitro-prusside solution under alkaline condition and turn red on acidification. Glycine is added to the reaction mixture in order to inhibit color formation with other amino acids.

Acid phosphatase. Acid phosphatase activity was measured by the method described by Hooley (1984). Enzyme activity was demonstrated in *p*-nitrophenol phosphate substrate solution by recording absorbance at 405 nm against a NaOH blank. Specific activity of the enzyme is expressed as μ mol *p*-nitrophenol released/min/mg/protein.

Phytic acid. Phytic acid content was measured by the method followed by Latta and Eskin (1980). The phytate

content was measured by using the wade reagent with an absorbance maximum at 500 nm.

The data of the various parameters was analyzed in triplicates, subjected to ANOVA (Analysis of variance) in accordance with the experimental design (split plot design) using SPSS-16 statistical package to quantify and evaluate the source of variation.

RESULTS AND DISCUSSION

Micronutrient status: Distribution of zinc in rice grain (Fig 1) and straw was analyzed in order to access the uptake and localization of zinc. It was observed that increased availability of zinc in soil increases the amount taken up by the plants. When foliar zinc application was made alongside the basal application, a higher concentration of zinc in rice grains as well as straw was found. Basal zinc application alone resulted in around 14 to 57% increase in rice grain zinc content during 2009, whereas during 2010 increase in grain zinc content ranged between 6.7 to almost 50% in different rice genotypes in comparison to unfertilized rice plants. However for foliar plus basal zinc application, zinc accumulation was even more pronounced, the increase in grain zinc content ranged between almost 40 to 58% during 2009, while during 2010 the increase in the grain zinc content ranged between 17 to almost 55% in different rice genotypes in comparison to the unfertilized rice plants. In case of straw zinc content, again foliar along with basal zinc application was much more effective in increasing zinc content. Increase was almost around 17 to 54% in different rice genotypes during 2009, while during 2010 increase in the straw zinc content ranged between 34 to 80%.

Acid phosphatase activity: Acid phosphatases are zinc metallo-proteins involved in wall formation in plants. Hence, the data on enzyme activity of acid phosphatase is presented in the Fig 2. The data obtained showed a clear increase in the enzyme activity following zinc fertilization in rice plants indicating that greater zinc availability in the soil led to an enhancement in the zinc uptake and utilization by the plants. For different genotypes, acid phosphatase activity ranged between 1–41% during 2009 and 1.8–37.5% during 2010 for basal zinc application. However, for basal

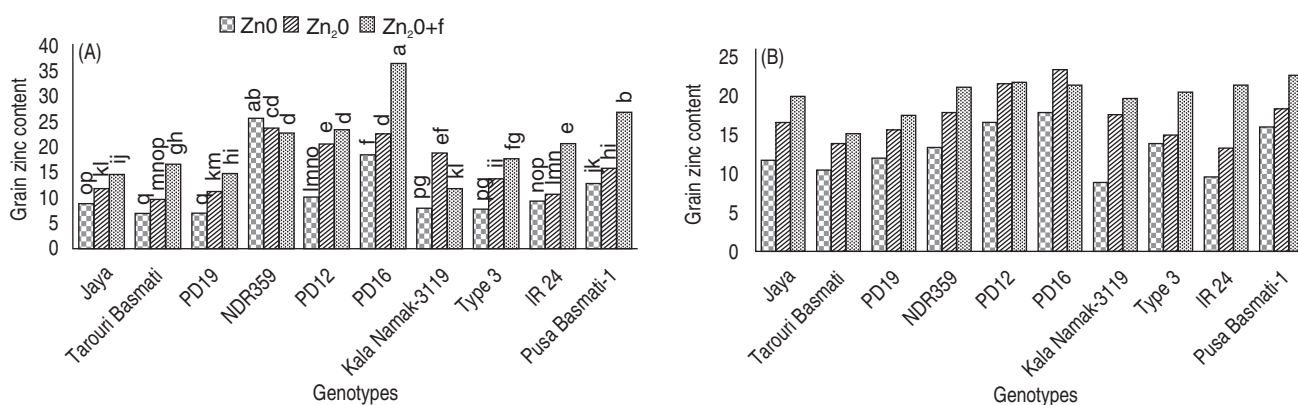


Fig 1 Effect of different modes of zinc application on grain zinc content (mg/g) during 2009 (A) and 2010 (B) in different genotypes of rice. Means followed by a common letter above bars are not significantly different.

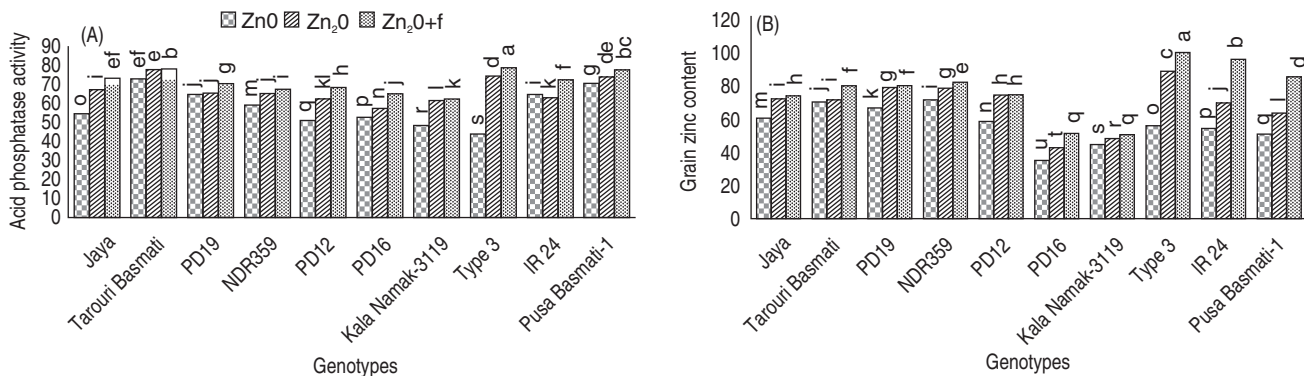


Fig 2 Effect of different modes of zinc application on acid phosphatase activity during 2009 (A) and 2010 (B) in different genotypes of rice. Means followed by a common letter above bars are not significantly different

dose accompanied with foliar application the increase in the activity of the enzyme ranged between 7.3–45.1% during 2009 and 12.1–44.6% during 2010. This data revealed that enzyme activity was affected to a greater extent when foliar spray of zinc was made alongside the basal dose, thereby indicating that foliar application is more effective in increasing the rice plant zinc content.

Phytic acid: Rice seeds contain phytic acid that can bind zinc in alkaline pH of human intestines and make it unavailable. Therefore, in the present investigation, phytic acid estimated (Fig 4) to identify the availability of zinc in the rice grains. Data on phytic acid showed that foliar combined with basal application of zinc resulted in significant reduction in the amount of phytic acid in comparison to the untreated rice plants. During 2009 due to basal application of zinc reduction in PA ranged from 3.9 to 31%, while during 2010 reduction in PA content ranged from 7.1 to 31% in comparison to rice plants that did not receive any zinc fertilization. This reduction was further enhanced when basal dose was combined with the foliar application of zinc, due to this treatment reduction in the PA content ranged between 23 to 41% during 2009 and between 20.3 to 40.4% in 2010 in comparison to the rice plants that did not receive any zinc fertilization.

Methionine: Antagonistic to phytic acid, methionine is an essential amino acid which increases the uptake of

zinc in intestines. Hence, in the present investigation effect of zinc on methionine content accumulation (Fig 3) was studied to identify the zinc availability potential in the rice genotypes. In case of basal application methionine content increased in a range of 7.2 to 32% during 2009, while during 2010 increase in the methionine content ranged between 3 to 17.3%. However, in case where basal zinc application was combined with foliar spray increase in the methionine content ranged between 13.9 to 50% during 2009 and 11 to 28% during 2010 in comparison to the rice plant which did not receive any zinc in the form of fertilizer.

Higher grain zinc content is indicative of the fact that zinc fertilizer application increased the amount of available zinc in the soil which was absorbed by the plants. When basal combined with foliar application of zinc was made the increase in the zinc content was still higher indicative and confirming the fact that foliar application of nutrients improves their uptake in the plants. Fertilization is necessary for those trace elements whose accumulation in the edible parts of plants is limited by the low availability in soil, such as Zn (Zhao and Shewry 2010). Our findings revealed increase in the grain zinc content in response to the zinc fertilization, were in accordance with previous studies, in which foliar Zn application significantly increased average grain Zn concentrations in the lowland plot. It is suggested that foliar Zn applications around flowering may be one way

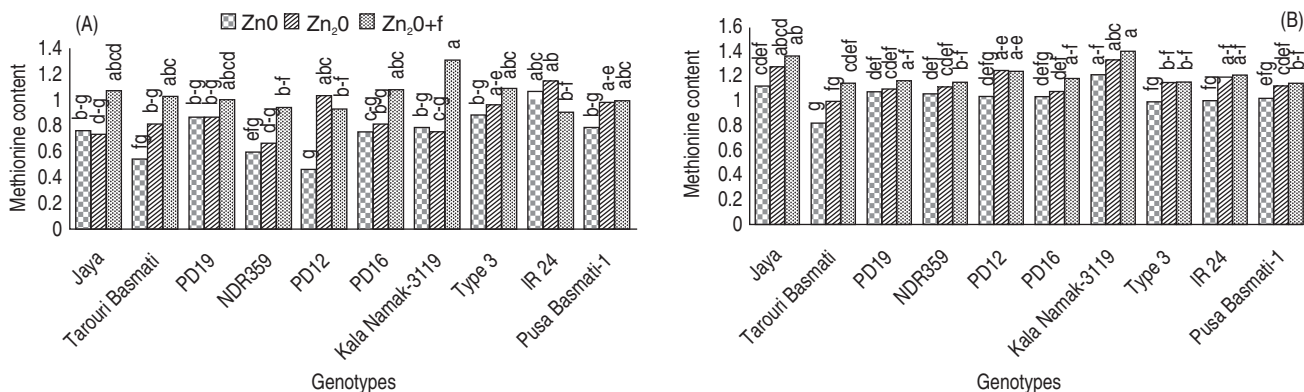


Fig 3 Effect of different modes of zinc application on methionine content (mg/g DW) in rice seeds during 2009 (A) and 2010 (B) in different genotypes of rice. Means followed by a common letter above bars are not significantly different.

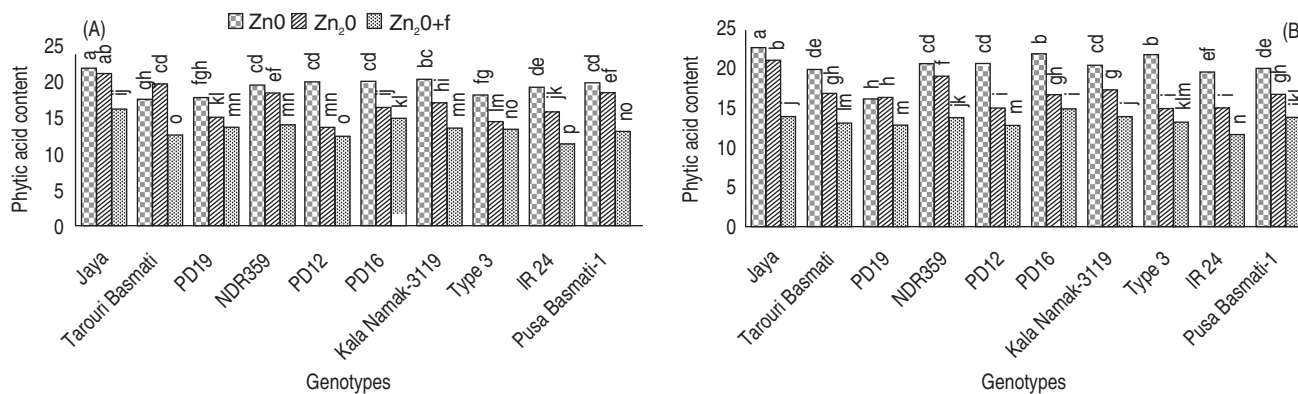


Fig 4 Effect of different modes of zinc application on phytic acid content in rice seeds during 2009 (A) and 2010 (B) in different genotypes of rice. Means followed by a common letter above bars are not significantly different.

to overcome such limitation as uppermost leaves should be readily exposed to foliar sprays (Wissuwa *et al.* 2008). Similar results were also found in case of wheat, where Zn application increased grain-Zn approximately up to three fold comparison with control (from 18.7 to 50.9 mg/kg) (Habib 2009).

Increase in the straw zinc content of the rice plants further confirmed our assumption that zinc fertilization enhances the plant's zinc status. In case of straw also foliar combined with basal zinc application was more responsive in stimulating zinc accumulation in the rice plants. In our experiment the application of zinc fertilizer has positive effect on straw zinc content, in accordance to the data observed for wheat (Cakmak *et al.* 1997), chickpea (Khan *et al.* 1998), common bean (Hacisalihoglu *et al.* 2004) and rice (Gao *et al.* 2005).

Another proof for the enhancement of zinc content of the rice plants was the increased activity of acid phosphatase enzyme. Acid phosphatases are ubiquitously distributed in plants, and occur in numerous tissues. Several classes of acid phosphatases from plant sources have been reported. An intensely studied plant enzyme is purple acid phosphatase (PAP) from the dry seeds of kidney beans (*Phaseolus vulgaris* L.). PAP from kidney beans is a homodimeric Fe(III)-Zn(II) metalloenzyme with a molecular mass of 111 kD PAP catalyses the hydrolysis of activated phosphoric acid esters and anhydrides like ATP at a pH range from 4 to 7 (Zhang *et al.* 2011). Therefore, increased acid phosphatase activity is a clear indicative of the fact that zinc fertilization not only enhanced the rice plant zinc content but also improved its assimilation in the plants. Similar observation was reported in tomato, where increasing Zn supply increased acid phosphatase activity (Kaya *et al.* 2000).

Reduction in phytic acid in response to the zinc fertilization is an indication of negative relationship between PA concentration and Zn absorption, i.e. when the amount of zinc absorbed increases it results in a reduction in the synthesis of PA and vice-versa. Phytate accumulates in the aleurone and embryo cells of cereal and legume seeds, and is a potent inhibitor of the absorption of micronutrients

from plant sources. Decreasing the phytate content in food can increase the bioavailability of mineral micronutrients (Zhao and Shewry 2010). Our results indicated that soil Zn and foliar Zn application both decreased grain phytic acid concentrations effectively. Similar results were obtained in wheat, where foliar Zn application decreased the phytic acid concentration and the phytic acid to Zn molar ratio in wheat grain (Yang *et al.* 2011).

From the data obtained for methionine, it can be deduced that foliar combined with basal zinc application not only induced higher amount of zinc accumulation but also enhances the amount of compounds like methionine, which improves the availability of the zinc present in the grains. The primary reason for the accumulation of larger concentrations of methionine in response to Zn uptake is the enzyme methionine synthase which catalyzes the final step in synthesis of methionine in plants, viz. conversion of homocysteine to methionine. This enzyme uses zinc as an essential cofactor; therefore, higher zinc concentration improves the amount of enzyme present in the cells which ultimately result in the synthesis of higher amount of methionine in plants as well as in grains. The inability of humans and many farm animals to synthesize certain amino acids has long triggered tremendous interest in increasing the levels of these so called essential amino acids in crop plants (Tan and Mai 2001). Methionine is a nutritionally essential sulfur-containing amino acid (Amir 2008). Zinc plays also an important role in protein biosynthesis and regulation of DNA-transcription, RNA processing, and translation (Hansch and Mendel 2009).

In the present investigation we recorded that the mode of zinc application changes the availability of zinc in the rice plant by enhancing the zinc concentration and modulating the concentration of anti-nutrient and pronutrient compounds. Amongst the parameters studied the content of methionine was found to increase in the rice grains in response to zinc fertilization and the amount of phytic acid was found decreased. Zinc fertilization improves rice plant zinc status has been shown in the earlier researches also. We also provide some positive insights like foliar zinc application reduces the phytic acid content of the

rice grains a potent anti-nutrient and improves the content of methionine a potent pronutrient. Therefore, from our research findings we conclude that proper zinc fertilization reduces the content of zinc inhibiting compounds like PA and enhances the zinc promoting compounds like methionine thereby improving the bioavailability of zinc. Therefore we recommend the use of foliar zinc application in order to improve the micronutrient status of the crop as well as of the populations consuming it.

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