

Effects of Phosphorus and Zinc Application and their Interaction on Yield and Zinc Nutrition of Rice (*Oryza sativa*)

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Abstract

A field experiment was conducted in an Inceptisol to study the effect of added phosphorus (P) and zinc (Zn) application on dry matter yield, P and Zn availability in soil, their uptake in plant and Zn recovery efficiency of *kharif* rice. The treatments included four doses of each P (0, 40, 60 and 80 kg ha⁻¹) and Zn (0, 5 kg ha⁻¹, 5 kg ha⁻¹ + one foliar spray and 5 kg ha⁻¹ + two foliar sprays). The application of P as well as Zn significantly increased the dry matter yield of rice. P application up to 80 kg ha⁻¹ caused significant increase in soil P availability, P:Zn ratio and P uptake by the crop with a simultaneous decrease in availability of applied soil Zn, concentration and uptake of Zn by rice and Zn use efficiency. However, Zn application causes significant increase in Zn availability in soil, Zn content and uptake by crop. Phytic acid/Zn molar ratio in grain decreased to the tune of 24.04 and 34.34% upon Zn application through soil + one foliar and soil + two foliar, respectively, over the control. Zn application further caused an increase in Zn-use efficiency by 1.5 and 2 folds upon soil + one foliar and soil + two foliar applications, respectively than only soil application. Zn application through soil + foliar over basal application could be a useful strategy for Zn enrichment in grain and reduction of phytate/Zn molar ratios, thus enhancing their bioavailability when higher P doses were applied.

Introduction

Zinc deficiencies, the most widespread micronutrient deficiencies in humans affecting one-third of the world's population (approximately two billion people), particularly in developing countries where cereal grains are the staple food [1, 2] with low concentration of bio-available Zn [3]. An estimated 50% of the world soils for cereal production have low available Zn which not only hampers crop yield but also produces Zn-deficient food products derived from the grains [4], leading to Zn deficiency in the population [5]. Intensification of paddy cultivation and less application of Zn fertilizers are the major reasons for high prevalence of Zn deficiency in soil, which has been reported to cover 49% cultivated area of India and about 30% in West Bengal [6].

Although the application of phosphorus (P) is required to achieve higher yield of rice [7], excessive P fertilization can lead to P surplus in soil [8] and affects the Zn nutrition of crops. Some research has indicated that high P application can inhibit Zn translocation from roots to shoots, especially to leaves, and lead to Zn deficiency in most of the crops [5, 9]. There is also evidence that soil P has a negative effect on grain Zn concentration [10] and bioavailability. The interaction of phosphorus and zinc, called P-induced Zn deficiency, is more prevalent due to the common practice of the farmers to apply higher amounts of P fertilizer. P-Zn interaction is generally represented by the magnitude of P and Zn concentration and P:Zn ratio in soils and plant tissues [11].

With this above background, the present investigation was undertaken to evaluate the effects of P and Zn along with their interaction on soil P and Zn availability, uptake of P and Zn, bio-availability of Zn, use efficiency of Zn and yield of rice plants.

Materials and Methods

Experimental site

The field experiment was conducted during *kharif* season (summer) for two consecutive years (2019 and 2020) in new alluvial zone of Nadia district, West Bengal, India at Central Research Farm of Bidhan Chandra Krishi

Viswavidyalaya, Gayeshpur (22°58.139' N latitude, 88°29.526' E longitude and 9.75m above from mean sea level) located under hot and humid climate with annual average rainfall ~ 1350 mm, maximum and minimum temperature of 37.5°C and 26.8°C and relative humidity of 95% during the crop growth period (June to September). The surface soil (0–15 cm) of the experimental site is characterized by sandy loam texture (*Aeric Haplaquept*) with neutral in reaction (pH 7.06), medium in organic carbon (5.8 g kg⁻¹), low in available N and K (242.4 kg ha⁻¹ and 138.2 kg ha⁻¹, respectively), high in available P (32.4 kg ha⁻¹) and low in available Zn (0.80 mg kg⁻¹).

Experimental design and treatments

The experiment was laid out in a factorial randomized block design with plot size of 20 sq.m (5 m × 4 m) by growing rice (var. MTU 7029) with three replications and sixteen treatment combinations i.e., four treatments both of P viz., no P (P₀), 40 kg P ha⁻¹ (P₄₀), 60 kg P ha⁻¹ (P₆₀) and 80 kg P ha⁻¹ (P₈₀) in the form of single superphosphate through basal and Zn, viz., no Zn (Zn₀), 5 kg Zn ha⁻¹ (Zn₅) in the form of zinc sulphate heptahydrate through basal, Zn₅ + one foliar spray at maximum tillering stage (Zn_{5+1F}) and Zn₅ + two foliar spray at maximum tillering and panicle initiation stage (Zn_{5+2F}) in the form of zinc sulphate heptahydrate (0.5% aq. solution). Twenty-five-day-old seedlings of farm-saved seeds of same location were transplanted at 20 cm row to row spacing and were fertilized with recommended doses of N and K (100 and 60 kg ha⁻¹ through urea and muriate of potash, respectively). One half of the N and entire amount of K were applied as basal during transplanting and the remaining half of N at maximum tillering stage (~ 21 DAT). All the recommended packages of practices such as irrigation and weeding were followed for raising the crop.

Collection and analysis of soil samples

Soil samples (0–15 cm) were collected before initiation of the experiment and after harvesting of rice, air dried, ground, passed through a 2.0 mm sieve, and analyzed for pH, oxidizable organic carbon [12], sand, silt and clay contents by hydrometer method [13], available N [14], P [15], K [16] and diethylene triamine pentaacetic acid (DTPA)-extractable Zn [17].

Collection and analysis of plant samples

At maturity, the crop was harvested plot-wise and the yield was recorded separately for grain and straw. Representative samples of grains, straw and roots were collected, washed thoroughly in tap water followed by distilled water and 1 M dilute HCl solution and dried through winnowing. The dried samples were digested in a diacid mixture (perchloric and nitric acid in 3:10 ratio) and were analyzed for P by vanado-molybdo-phosphoric yellow colour method [18] using spectrophotometer and Zn in an atomic absorption spectrophotometer (GBC Avanta, Model 912).

Phytic acid content of the grains was analyzed by extracting phytins (Ca or Mg phytates) from the samples with trichloroacetic acid (TCA) with subsequent precipitation of Fe-phytate on addition of FeCl₃ and measuring Fe concentration in the form of Fe(NO₃)₃ using atomic absorption spectrophotometer assuming a Fe:P ratio of 4:6 [19].

Calculations

The phytic acid:Zn (PA:Zn) molar ratio, an indicator for Zn bioavailability in edible food [20, 21] was calculated as follow:

$$\text{PA : Zn molar ratio} = \frac{\text{Phytic acid content in mg/kg} / 660}{\text{Zn content in mg/kg} / 65}$$

(Using 660 and 65 as molecular weight of phytic acid and Zn, respectively)

The zinc-use efficiency (ZnUE) or apparent Zn recovery was calculated by subtracting Zn uptake in control plot from Zn treated plot per unit amount of Zn applied.

$$\text{ZnUE (\%)} = \frac{[\text{Zn uptake in Zn treated plot (g/ha)} - \text{Zn uptake in control plot (g/ha)}] \times 100}{\text{Amount of Zn fertilizer applied (g/ha)}}$$

Statistical Analysis

Analyzed data of the experimental trial were pooled for both the years (2019 and 2020). All the data obtained were statistically analyzed using the F test for factorial randomized block design [22] and standard error of means (SEM $\pm$) and critical difference (CD) at 5% level of significance were calculated for determination of significance of difference between treatment means.

Result and Discussion

Availability of P and Zn in post-harvest soil of rice

Application of Zn, regardless of the method, the availability of Zn in post-harvest soil was significantly increased by 30.53 % over the control (no Zn) (Table 1). Whereas, application of P at higher dose caused a significant decrease in the availability of applied soil Zn but not in native soil Zn [23], the decrease could be due to the formation of insoluble $\text{Zn}_3(\text{PO}_4)_2$ compound in soil solution [24, 25]. Addition of P fertilizers may increase negative charges leading to increased sorption of Zn [26]. However, P application at all levels caused a significant increase in the availability of soil P.

Dry matter yield of rice

Phosphorus and Zn applications at all levels causes significant increase in dry matter yield as compared to the control (Table 2). The maximum dry matter yield in the study was obtained when P was applied at 80 kg ha⁻¹ in combination with soil application of Zn at 5 kg ha⁻¹ plus two foliar spray and the increase in grain and shoot yield was in the tune of 17.11 and 16.04 % respectively over control (P₀Zn₀). The beneficial effects of P and Zn may lead to higher yield due to their combined application [27]. Similar results were reported in rice [28, 29] and corn [30], indicating that combined P and Zn applications increased dry matter yield.

Concentration of Zn in rice

Results (Table 3) showed that application of Zn caused a significant increase in concentration of Zn in grain and shoot of rice and the enrichment was highest with soil plus two foliar application (15.03 and 17.49 %) followed by soil plus one foliar application (10.78 and 12.23 %) and only soil application of Zn (5.27 and 6.45 %). The results comply with [1] who reported about 3.5-fold increase in grain Zn content of rice upon soil + foliar application of Zn. Application of Zn regardless of doses, also increased Zn concentration in root to the tune of 47.24 % over control.

Results further showed that application of P caused a significant decrease in Zn concentration both in grain, shoot and root over the control. Such depletion in Zn was highest when P was applied at 80 kg ha⁻¹ (20.46, 21.18 and 21.72 % respectively compared to control). The previous findings also showed a significant decrease in Zn both in grain, shoot and root of rice [28], shoot and root of durum wheat [3] and wheat grain [32, 33] upon application of phosphate which may be due to the antagonistic effect of high phosphorus level on zinc solubility as well as the dilution effect of P [34]. Decline in root Zn concentration due to excessive use of P might be due to the formation of insoluble complex with Zn. Korkmaz *et al.* [35] stated that Zn gets tied up within the root cells and couldn't get transported to the leaves due to high P concentration in roots. Statistical analysis revealed that P and Zn interaction had a significant effect on the concentration of P and Zn in the root.

Uptake of Zn

Zinc applications resulted in a significant increase in Zn uptake by grain and shoot (Table 4) and the increase was highest with soil + two foliar applications of Zn (24.65 and 23.86 % over control) followed by soil + one foliar (17.84 and 17.10 %) and only soil application (10.52 and 10.14 %). Such enhancement in root was 55.78 % over control with Zn application regardless of doses. The more favorable conditions either due to an increase in solubility in soil solution or possible stimulation of root absorption may attribute to increased uptake [36]. Cakmak [1] also opined that foliar application of Zn or combined soil plus foliar application of Zn fertilizer is highly effective way to maximize uptake and accumulation of Zn in whole cereal grains.

On the other hand, application of P caused a significant decrease in Zn uptake by plant which was more prominent in case of grain and shoot, may be due to retardation of translocation. Such depletion was highest with the application of 80 kg P ha⁻¹, which was to the tune of 14.30, 13.38 and 10.95 % in grain, shoot and root respectively, compared to control. A stronger influence of P on Zn uptake was reported earlier by [37]. Application of P decreases the mycorrhizal colonization in the rhizosphere, which may explain the decreased uptake of Zn [38].

Relative transfer of Zn from root to shoot and from shoot to grain

Relative transfer of Zn from root to shoot and from shoot to grain were estimated as uptake of Zn in shoot divided by uptake of Zn in root and uptake of Zn in grain divided by uptake of Zn in shoot, respectively. Application of Zn resulted in a significant decrease in the transfer coefficient of Zn from root to shoot (Table 5) due to higher accumulation of Zn in roots, indicating its lower mobility from root to shoot and possibly after attaining optimum Zn concentration in tissues, shoots are unable to uptake more Zn from roots [39].

Uptake of P

Application of P significantly increased the uptake of P and the increase was to the tune of 27.16, 34.21 and 39.16 % in grain, shoot and root respectively over control at 80 kg P ha⁻¹ (Table 6), which is corroborated by the previous findings of [7]. On the contrary, P uptake by grain was significantly decreased to the tune of 2.08, 2.37 and 2.67 % with the application of Zn through soil, soil plus one foliar and soil plus two foliar applications respectively, compared to the control. It may be due to the inhibition of P translocation from roots to the tops owing to antagonistic effect of P and Zn [40]. The competition generating between the elements for the same absorption site in root may also reduce P uptake under Zn nutrition [41].

P/Zn ratio

The P:Zn ratio, an important factor of P-induced Zn deficiency, is reported as a bioavailability trait for Zn in cereals [42]. Application of Zn significantly reduced P:Zn ratio at all levels of P by 1.1 to 1.3 folds in grain and straw and 1.5 to 1.7 folds in root while P application significantly increased P:Zn ratio at various levels of Zn by 1.1 to 1.5 folds in grain and 1.1 to 1.6 folds in straw and root (Table 7). Similar increase in P/Zn ratios with P application in the shoot and root tissue of durum wheat was reported by [31] while in grain and straw of wheat by [33], the effect being greater with increasing the fertilization rate. Korkmaz et al. [35] also reported a decrease in P:Zn ratio by 1.2 to 1.9 folds with application of Zn while an increase in the ratio by 1.2 to 7.6 folds with the application of P in chia. The interaction effect on P:Zn concentration ratio in grain was also found significant. The decrease in the Zn concentration in the straw and grain due to P fertilization has been attributed to the physiological inhibition of Zn uptake by root and translocation of Zn from root to straw [33].

Phytic acid (PA) content and phytic acid-Zn molar ratio (PA:Zn) in grain

Phytic acid (myo-inositol hexaphosphate), the major anti-nutritional component in cereal grains, strongly chelates with dietary essential minerals (such as iron, zinc, calcium and magnesium) to form phytate or phytin (insoluble salt) and reduces their bioavailability in edible foods [43]. Application of Zn caused a significant decrease in PA content and such a decrease was maximized when Zn was applied through soil plus two foliar spray (24.25 % over the control) (Table 8), which is in accordance with the findings of [44] and [45]. The inverse relationship between P and Zn uptake [46] with consequent grain Zn enrichment may attribute to such reduction. This, in turn, reduced the PA/Zn molar ratio with Zn fertilization. Zinc application through soil plus two foliar spray caused the highest reduction (34.34 % over the control) followed by soil plus one foliar spray (24.04 %) and only soil application (17.60 %). This corroborates the findings of [47] and [48]. Since, PA commonly forms 70 % of the total P reserves in seeds; a greater P uptake might be a reason for an antagonistic relationship between PA and Zn nutrition [49, 44].

In contrast, P application results a significant increase in PA content and PA/Zn molar ratio and the magnitude of increase were 11.73 and 40.89 % respectively over control (no P) upon addition of 80 kg P ha⁻¹. The increased root uptake and shoot accumulation of P is accompanied by corresponding increases of P in grain due to the high phloem mobility of P [50]. When the inorganic P is transferred into grain, most of it is converted into phytic acid (phytate). The statistical analysis indicated that the interaction of P and Zn application had a significant effect on PA/Zn molar ratio in grain.

In this study, although P increased phytic acid content in grain, addition of Zn increased Zn concentration and decreased phytic acid concentration in grain. The overall effect of Zn was to decrease the molar ratio of phytic acid to Zn. Therefore, it is feasible to increase the Zn nutritive quality of wheat grain through agronomic

approaches. The supplemental Zn not only increased the Zn concentration in wheat grain but also improved its bioavailability.

Use efficiency of Zn (ZnUE)

The per cent use of applied Zn by different plant parts was estimated to get an insight view about Zn recovery by plant for judicious management or efficient utilization of applied Zn. Percent utilization of Zn within the plant followed the order: root> straw> grain (Table 9). Results revealed that Zn application through soil one foliar spray increased the ZnUE to the tune of 54.42 per cent (1.5 fold) than only soil application whereas the increase was 97.87 per cent (approx. 2 fold) with soil plus two foliar applications. Saha *et al.* [51] also reported that foliar application of Zn @ 0.5 % $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ twice with basal application of 20 kg Zn ha⁻¹ increased the per cent use of applied Zn to the tune of 2-fold in both grain and straw than only basal application of Zn fertilizer. This enormous increase in ZnUE could be due to modification in re-translocation process of Zn *via* phloem [51]. So, from the results, it can be opined that soil plus foliar application could be a useful strategy to increase the use efficiency of applied Zn. Results further showed that the use efficiency of soil applied Zn in rice was very low (less than 2 %), which was further lowered with the application of higher doses of P. Critical analysis of the results showed that the depressing effect of P application was more pronounced at lower level of applied Zn as compared to that at higher level which might be due to the fact that at higher level of Zn application, sufficient amount of Zn was left in the soil after interaction with added P to meet the requirement of the plants.

Conclusion

Results of this study revealed that combined application of both P and Zn was a prerequisite for achieving high dry matter yield. Application of P showed a significant effect on soil P availability, P/Zn ratio, P uptake by plant and phytate content in grain, however, markedly decreased concentration and uptake of Zn. Higher doses of P application also caused a significant decrease in the availability of applied soil and use efficiency of Zn. Undoubtedly, application of Zn was beneficial for availability of Zn in soil, enrichment of Zn in plant, nutritional status or bioavailability in grain and recovery of applied Zn but soil + foliar applications at maximum tillering and panicle initiation stage outperformed other Zn treatments. Moreover, the effect of P and Zn interaction was significant on root Zn concentration, P/Zn ratio and PA/Zn molar ratio in grain. Thus, Zn fertilization should be done at higher dose (preferably soil + foliar) for its nutrition at high level of P addition, otherwise Zn nutrition will be hampered.

Statements and Declarations

Competing Interests: The authors have no competing interests to declare that are relevant to the content of this article.

Significance Statement

The present investigation provides a good knowledge about the effect of phosphorus and zinc and their interaction on soil P and Zn availability, dry matter yield of rice, their uptake and P/Zn ratio in plant. The paper also evaluated the influence of P and Zn application on phytic acid/Zn molar ratio in grain and use efficiency of Zn in rice.

Data availability

The datasets would be available on making substantial request to the corresponding author (Email: mondal.sudeshna2010@gmail.com)

Conflict of interest

No conflict of interest exists among the authors.

Consent to participate declaration

Not applicable

Consent to publish declaration

Not applicable

Ethics approval and consent to participate

We have conducted the field experiment at Central Research Farm of Bidhan Chandra Krishi Viswavidyalaya, Gayespur, Nadia, West Bengal, India (University farm) by growing rice (*Oryza sativa* L.) (Variety MTU 7029). The plant samples viz. grain and straw were collected from the individual plots during maturity stage of rice for the purpose of further analysis, complies with national guidelines. Necessary permission for sampling was accorded.

Clinical trial number

Not applicable

Authors' contributions

All the authors contributed to the research study. The overall plan of the work was made by G.C.H. Material preparation, data collection, and analysis were performed by S.M. under the supervision and sincere guidance of P.K.M. The draft of the manuscript was written by S.M., which was reviewed and approved by all authors.

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Tables

Tables 1 to 9 are available in the supplementary files section

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