Grain Phytic Acid Accumulation of Domestic and Exotic Rice Genotypes in Zinc-Deficient Soil

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Abstract: Micronutrient malnutrition in humans living in rice growing areas is increasing rapidly due to less absorption of mineral nutrients chelated by phytic acid (anti-nutrients) present in rice grains. A field study was conducted to evaluate the grain phytic acid and zinc (Zn) accumulation of 10 field grown rice (*Oryza sativa* L.) genotypes on a Zn deficient soil. Both the Zn- efficient (Shua-92, IR-9, Shandar, IR-36, and IR-6) and Zn-inefficient (Sarshar,. UPL-48, Khushboo-95 and RG-120) rice genotypes were included in the study. The two Zn treatments (0 and 15 kg ha^{-1}) were arranged in a two factor randomized complete block design with three replications. Nitrogen (N) and phosphorus (P_2O_5) were applied at the rate of 120 and 80 kg ha^{-1}. The rice genotypes IR-36, UPL-79, Shandar and Shua-92 were the most Zn accumulators whereas; Sarshar, IR-9 and Khushboo-95 the least accumulator in Zn deficiency. Zinc in-efficient genotype Sarshar was the highest Zn accumulator in response to Zn application. Phytic acid content of rice genotypes was significantly influenced ($p < 0.05$) by the application of Zn fertilizer. Phosphorus concentration in rice grains decreased with Zn application. Zinc in-efficient genotypes accumulated more phytic acid in their food reserves than Zn-efficient genotypes. Phytic acid: zinc ratio decreased significantly more in Zn-inefficient genotypes as compared to Zn efficient genotypes, with application of Zn fertilizer. Zinc efficient genotype Shua-92 accumulated low concentration of phytic acid. The rice genotypes Shua-92, IR-9, Shandar and IR-36 low accumulators of phytic acid performed successfully and contained higher concentrations of Zn than other genotypes.

Keywords: Micronutrient, Rice, Genotypes, Zinc, phytic acid.

INTRODUCTION

Micronutrient malnutrition has affected more than 3 billion people due to utilizing cereal based diet poor in vitamins and minerals. This global nutritional and health issue is the lacking of functional food systems which do not always provide nutrient rich foods to meet the nutritional needs of high-risk people [1]. Food systems that nourish the world must be changed in a way that ensures that the supply of balanced nutrients is readily available to all people in adequate and affordable amounts [2]. A sustainable agriculture approach to reduce micronutrient malnutrition among those most at risk (i.e. resource-poor women, infants and children) is the enrichment of main staple crops with micronutrients through plant breeding strategies. The other approach is enhancing substances ascorbic acid (e.g, S-containing amino acids, etc.) that promote micronutrient bioavailability or decreasing anti-nutrient substances (eg phytate, polyphenols, etc.) that inhibit bioavailability of micronutrients are the two options that could be considered in breeding programs [3-5].

Phytic acid is an essential food component that has crucial negative impact on the absorption of Zn and rice proteins [6]. Phytic acid is billed as both an antioxidant and anti-nutrients, clouding the issue from the get-go. It is technically called as hexaphosphoinositol and is a powerful chelator. It forms complexes with divalent and trivalent metal ions, such as Zn^{2+}, Fe^{2+}, Ca^{2+} and Cu^{2+}, which are not absorbed in the gastrointestinal tract and reduce the bioavailability of essential elements leading to a deficiency or diseases [7]. Said wahab [8] found significant variation in different varieties which showed the lowest phytic acid may be included in the breeding program.

Zinc bioavailability is significantly reduced due to a high intake of phytate which significantly affect the absorption of Zn in the body [9]. Phytate: zinc ratio of <5:1 5-15:1> and 15:1 are considered an index of bioavailability high, medium and low Zn [10]. If phytate: zinc ratio exceeds 15:1 the absorption is low. Most agricultural crops such as wheat, legumes, leafy vegetables etc are sustainable and inexpensive source of micronutrients for rural people who are not able to use fruits, rich in trace minerals in their daily diet. Rice is most important cereal grain after wheat in Pakistan, supplying consumers with more calories. Breeding of rice genotypes that contain lower concentrations of phytic acid are crossed with high yielding genotypes or altering plant genes in ways that reduce or even eliminate anti-nutrient from grains is very much desired for increasing Zn availability [11].

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Keeping this in view, present field study was designed to assess response of different rice genotypes to Zinc application for enhancing Zinc concentrations and lowering the phytic acid accumulation.

MATERIALS AND METHODS

Collection of Samples

The grains of 10 rice genotypes were collected after harvesting of field experiment conducted at experimental farm of Nuclear Institute of Agriculture (NIA), Tandojam. The paddy was cleaned from unwanted foreign materials, like stones, dust, weeds seeds, etc. and stored at 4 °C in a refrigerator for further analysis. Prior to chemical analysis, the paddy seeds of each genotype were dried in forced draft oven at 80 ^{0}C and then milled, polished and ground in IKA FM-10 grinding mill to pass through 0.5 mesh sieve. Each genotype received fertilizers at the rate of 120 kg nitrogen, 80 kg phosphorus and 15 kg Zn ha^{-1} under field conditions.

Samples of ground material (0.5 ± 0.01 g) were transferred into an acid washed 100 ml Kjeldahl digestion tube. A 10 ml of concentrated Analar nitric acid (69%) was added to each tube and thoroughly mixed. The samples were left overnight in the fume hood. On the next day, the tubes were placed in digestion block and heated continuously for 1 hour at 60 ^{0}C. The temperature was gradually increased and the samples were digested for further 6 hours at 110 ^{0}C. The tubes were removed from the block, allowed to cool and filtered in acid washed 100 ml volumetric flasks through a filter paper (Whatman 40). Successive rinsing of tubes was ensured with deionised water and thus the volume of the flask was made up to mark. The concentration of Zn was determined through Atomic Absorption spectrophotometer, (Novaa-400, Germany). The P concentration of samples was determined by PC SPECTRO, LOVIBOND, UK, following the method of single acid digestion as described by Westerman [12].

The sensitive method of Haug and Lantzsch [13] was adapted for the assessment of phytic acid in rice flour samples. The sample extract (with 0.2N HCl) was heated with an acidic Iron III solution of known iron content. The phytate-P was measured as decrease in iron content (determined calorimetrically with 2, 2-bipyridine) in the supernatant. The defatted and finely ground flour samples (0.5 g) were extracted with 10 ml of 0.2N HCl for 1 hour. From this extract, 0.5 ml was taken into a stopper test tube. Ferric solution (1.0 ml) was added to this and covered with the stopper. These tubes were heated in a boiling water bath for 30 minutes and allowed to cool to room temperature. A 2, 2, Bipyradine solution (2.0 ml) was added to this and mixed. The absorbance was measured within 1.0 minute. A standard calibration curve was prepared using the same procedure with the standard solutions of known concentration of 0.0, 5.0 through 30 ppm sodium phytate. Using the standard curve the concentration of phytic acid in the sample was calculated by the formula:

$$\text{Phytic acid} = \text{P-phytate} \times 4.97$$

Statistical Analysis

The co-efficient of variation (CV) and Tukey's honestly significant difference (HSD) were computed by using STATISTIX® ver. 8.1 [Analytical Software, Inc., Tallahassee, FL, USA].

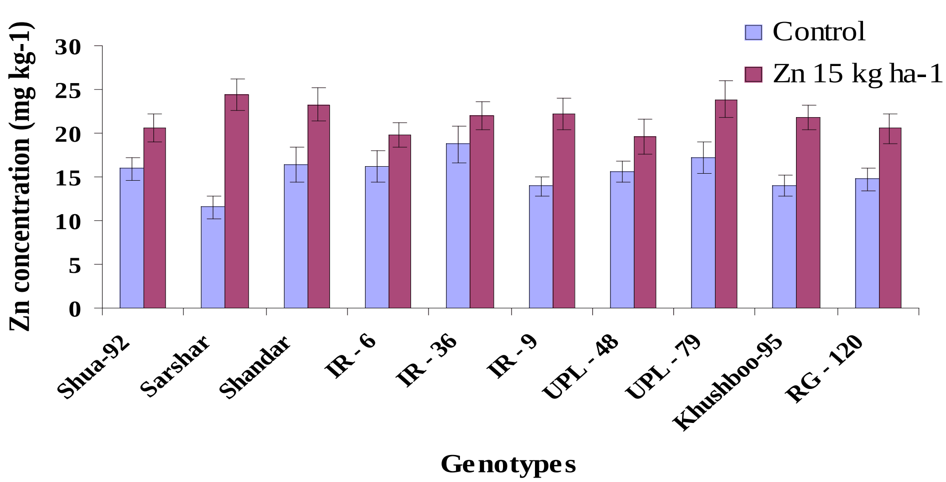

Figure 1: Zinc concentration in rice grains as affected by zinc application.

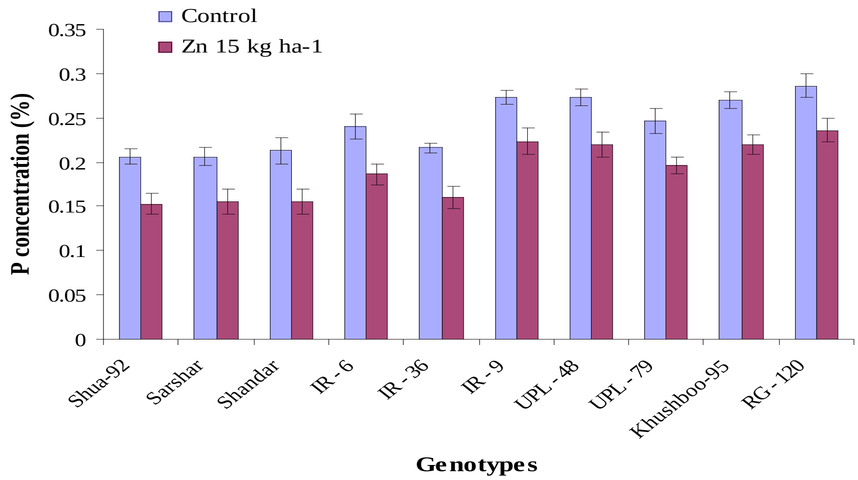

Figure 2: Phosphorus concentration in rice grain as affected by zinc application.

RESULTS

Zinc Concentration

Zinc concentration in rice grains increased linearly with increasing Zn application rate in the soil. The rice plants grown in control plots had lower Zn concentrations in grains than Zn fertilized plots, which ranged between 15.4 to 21.9 µg g^{-1} (Figure **1**). The genotype "Sarshar" accumulated significantly higher grain Zn concentration (50%) more than unfertilized plots, followed by IR-9 (35%) and Khushboo-95 (34%), respectively, with Zn application. As anticipated, the concentrations of Zn were much higher in Zn-sensitive rice genotypes supplied with 15 kg Zn ha^{-1}, as compared to unfertilized plots.

Phosphorus Concentration

The concentration of P decreased directly with increased Zn levels (Figure **2**). The effect of Zn application on P concentrations was more pronounced on Zn sensitive genotypes. As Zn concentration increased in the substrate, P concentrations decreased in the plants. The plants which received no Zn accumulated the highest levels of P and vice versa. The genotypes RG-120, IR-9 and UPL-48 contained higher concentration of P in Zn deficiency. The genotype RG-120 also contained higher P concentration in Zn fertilized plots than rest of the genotypes.

Phosphorus Zinc (P:Zn) Ratio

A nutrient balance exists in the plants under normal growth conditions and any imbalance in the concentration of nutrients may change their ratios. The data regarding P/Zn ratios are shown in Figure **3**. Zn deficient plants had higher P/Zn ratios than the healthier plants. P: Zn ratio in Zn deficient plants was always found to be greater than 100.

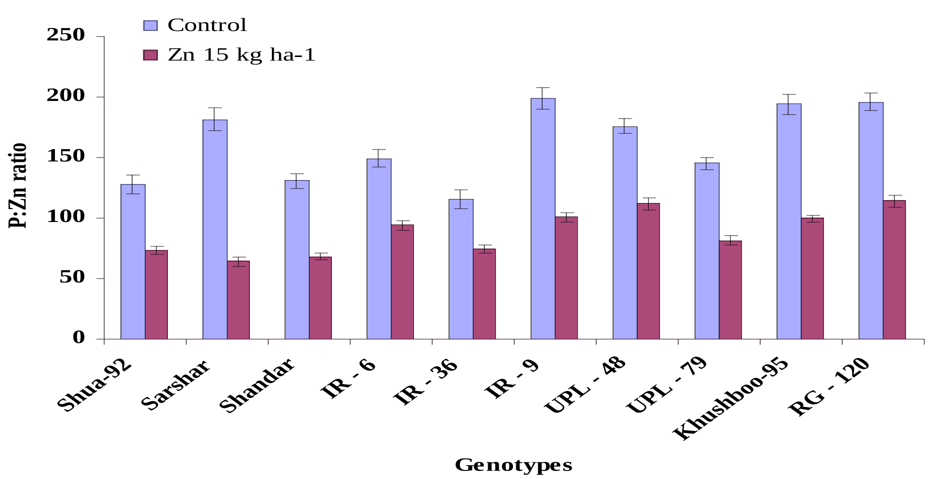

Figure 3: Phosphorus concentration in rice grain as affected by zinc application.

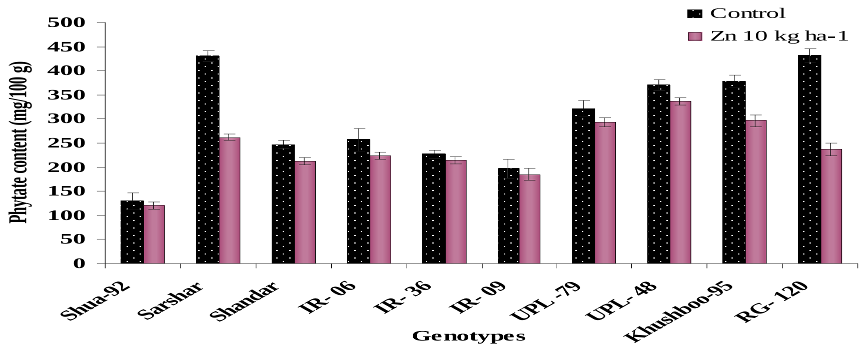

Figure 4: Phytic acid concentration of rice genotypes as affected by zinc application.

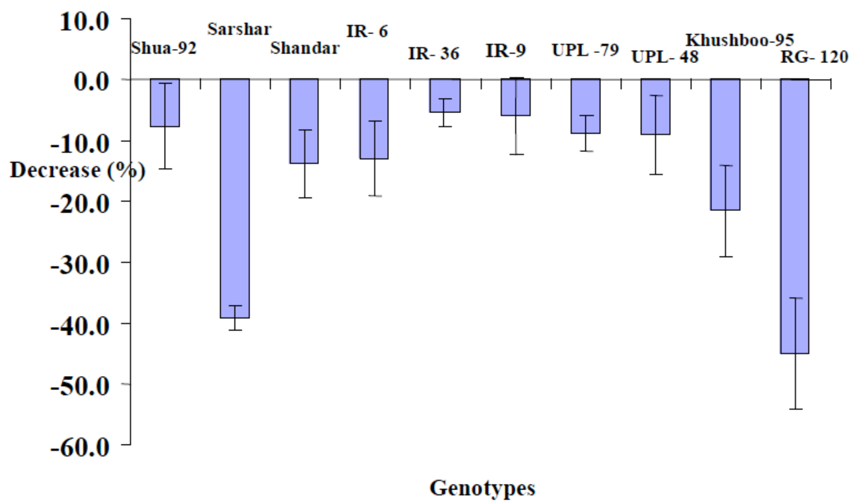

Figure 5: Phytic acid concentrations of different rice genotypes increased over control.

Phytic Acid Concentration

Zinc treatments significantly varied in phytic acid concentration of Zn in-efficient genotypes; however, Zn efficient rice genotypes did not demonstrate the significant variation (Figure **4**). In control plots, Zn in-efficient genotypes, Sarshar, RG-120, Khushboo-95 and UPL-79 contained maximum concentrations of phytic acid, i.e. 430, 432, 378 and 322 mg $100g^{-1}$, respectively, while Zn efficient genotypes viz., Shua-92, IR-36 and IR-9 proved as less accumulator of phytic acid. Under salt stress conditions, Zn application showed significant reduction in phytic acid content. Zn in-efficient rice genotypes particularly showed reduction in phytic acid content, i.e. RG-120 (45%), Sarshar (39%), Khushboo (22%) and Shandar (14%) (Figure **5**).

DISCUSSION

As expected, the Zn concentration was higher in the plants supplied with higher Zn fertilizer in Zn deficient soils, than in the plants without Zn fertilizer. Singh *et al*. [14] have suggested that Zn may be important for the structural and functional integrity of the root cell plasma. These results indicated that plants affected by Zn fertilizer, at lower Zn concentration, can tolerate stress for longer before significant reduction of seedling growth occurred [15].

There have been diverse reports in literature as to whether or not a “critical” P:Zn ratio exists. In this study, Zn in-efficient rice genotypes showed the higher ratios of P:Zn as compared to Zn efficient genotypes. Yield decreases occur when the P:Zn ratio in tissue exceed a “critical” level. This critical level may vary between 100 to 350 depending upon soil type and other conditions. In the present study, the P:Zn ratios were well above this critical ratio. These results also showed that the P:Zn ratio for plants growing normally was always less than 150 which was the ratio suggested for normal plant growth and a ratio greater 300 was indication of Zn deficiency. The ratios of P and Zn have been reported to be closely associated with the presence and severity of Zn deficiencies [16].

The present study further revealed that Zn efficient rice genotypes showed lower concentration of phytic acid in rice grains under Zn deficient conditions, while

Zn efficient genotypes illustrated high phytic acid content. The Zn in-efficient rice genotypes, such as Sarshar, RG-120, Khushboo-95 and UPL 48 showed high values of phytic acid than Zn efficient genotypes Shu-92, IR-9, IR-36 and IR6 (Figures **4** and **5**). Increasing Zn concentration in edible plant foods is the very first step in making these foods richer sources of micronutrients for humans. This is because not all of the micronutrients in plant foods are bio-available to humans that eat these foods. Plant foods can contain substances that interfere with the absorption or utilization of these nutrients in humans [17]. These results are identical with the findings of Ihsan *et al*. [18]) who reported that fermentation time had significant effect on the reduction of phytic acid content. They further stated that genotype Ghaznavi and Fakhr-e-Sarhad fermented for 45 minutes contained 280.3 and 280.4mg/100g phytic acid content respectively.

CONCLUSION

The rice genotypes Shua-92, IR-9, Shandar and IR-36 low accumulators of phytic acid and performed successfully than other genotypes. The Zn in-efficient genotypes Sarshar and RG-120 required supplementation of Zn for balancing the Zn concentration and absorption. Zinc fertilizeration improved Zn status of rice grains.

REFERENCES

[1] Welch RM. Biotechnology, biofortification, and global health. Food Nutr Bull 2005; 26: 419-21.

[2] Zuzana S, Gregováb E, Sturdík E. Chemical composition and nutritional quality of wheat grain. Acta Chimica Slovaca 2009; 2(1): 115-38.

[3] Welch RM. Breeding strategies for biofortified staple plant foods to reduce micronutrient malnutrition globally. J Nutr 2002; 132: 495S-99S

[4] Welch RM, Graham RD. A new paradigm for world agriculture: meeting human needs - Productive, sustainable, nutritious. Field Crops Res 1999; 60: 1-10. http://dx.doi.org/10.1016/S0378-4290(98)00129-4

[5] Welch RM. Biotechnology, biofortification, and global health. Food Nutr Bull 2005; 26: 419-21.

[6] Oberleas D, Muhrer ME, Dell OBL, Kinter LD. Effects of phytic acid on Zn availability in rats and swine. J Anim Sci 1961; 20: 1945.

[7] Wahab S, Anjum FM, Butt MS, Sarwar M, Zeb A. Phytic acid content of bread prepared from wheat varieties grown in KPK. Sarhad J Agric 2004; 20(1): 157-62.

[8] Levender OA. Selenium In: Trace Elements in Human and Animal Nutrition (Ed. W. Mertz), Academic Press Oriando, Florida 1987; pp. 209-279.

[9] Alloway BJ. Micronutrient deficiencies in global crop production, Springer Science + Business Media B. V. 2008.

[10] Graham RD. Breeding for nutritional characteristics in cereals. Adv Plant Nutr 1984; 1: 57-102.

[11] Westerman RL. Soil Testing and Plant Analysis. Soil Science Society of America: Madison, Wisconsin 1990.

[12] Haug W, Lantzch. Sensitive method for the rapid determination of phytate in cereals products. J Sci Food Agric 1983; 34: 1423-24. http://dx.doi.org/10.1002/jsfa.2740341217

[13] Singh B, Kumar S, Natesan A, Singh BK, Usha K. Improving zinc efficiency of cereals under zinc deficiency. Curr Sci 2005; 88(1): 1-9.

[14] Zeng L, Shannon MC. Salinity effects on seedling growth and yield components of rice. Crop Sci 2000; 40: 996-1003. http://dx.doi.org/10.2135/cropsci2000.404996x

[15] Graham RD. Breeding for nutritional characteristics in cereals. Adv Plant Nutr 1984; 1: 57-102.

[16] Welch RM, Graham RD. A new paradigm for world agriculture: meeting human needs - Productive, sustainable, nutritious. Field Crops Res 1999; 60: 1-10. http://dx.doi.org/10.1016/S0378-4290(98)00129-4

[17] Ihsan MQ, Wahab S, Shad AA, Zeb A, Ayub M. Effect of different fermentation time and baking on phytic acid content of whole wheat flour bread. Asian J Pl Sci 2003; 2(8): 597-601. http://dx.doi.org/10.3923/ajps.2003.597.601

[18] Lira PI, *et al.* Effect of zinc supplementation on the morbidity, immune function and growth of low birth weight, full term infants in northeast Brazil. Am J Clin Nutr 1998; 68: 418.

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