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Determination of Effect of Different Levels of Polishing on Zinc Content in Rice Grains

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ABSTRACT

Concern over the food chain transfer of zinc (Zn) is increasing because of its importance in human health. Increasing the micronutrient content of staple crops or bio-fortification will greatly improve human nutrition on a global scale. In this study, five elite lines and four checks differing for both grain Zn and Fe content were used in this study. The effect of polishing on loss of micronutrient in rice grains were quantified using different rice accessions. Polishing was done at two levels i.e. 5 % and 10 % in this samples Zn content was estimated using XRF in both brown rice and white rice. The results showed that per cent loss of Zn was more in 10 % polishing than in 5 % polishing. Hence, Zn content was reduced after polishing because Zn content is more in aleurone layer than in the endosperm. Among the nine genotypes AM-65 had highest Zn even after polishing, suggesting minimum loss of Zn content in these elite lines while AM-72 and ARB6 manifested moderate Zn content among the chosen genotypes.

Key words: *Biofortification, Micronutrients, Oryza sativa L., Zinc deficiency*

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important cereal crop for more than half of the world's population and cultivated over an area of 150 million hectares[17]. It is estimated that the per capita consumption of rice ranges from 62 to 190 kg/year in countries where it is used as staple food[7]. Rice provides approximately 20 per cent of the per capita energy and 13 per cent of the protein for human consumption worldwide. In many developing countries the dietary contribution of rice is 29.3 and 29.1 per cent for energy and protein, respectively[19].

The poorest families in developing countries spend 50–80 per cent of their income on food, depend heavily on low cost, high energy, starchy staples like rice which contains insufficient amounts of micronutrients such as Zn, Fe and vitamin A to provide recommended daily intake for human beings[6,8]. Southeast Asia is the major rice producer in the world where 71 per cent of population suffers from zinc deficiency.

Zinc, along with other metal elements, is required for human beings. In spite of its importance in overall health, it is not considered essential as deficiency symptoms are not so prominent.

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It is identified that more than 300 enzymes require zinc for their catalytic function. It participates in all major biochemical pathways and plays vital role in the DNA replication, transcription, protein synthesis and cell division[23]. The reliance on cereal based diets may induce Zn deficiency related health problems in humans, such as impairments in physical development, immune system, brain function, pneumonia, weight loss, growth retardation and delayed puberty in adolescents, poor appetite, delayed wound healing[5]. To solve the problem Zn food fortification and supplementation approaches are recommended but these methods are expensive and not easily accessible for people living in developing countries[3,17,20].

It is also reported that one more reason for deficiency of these mineral nutrients in rice is polishing of rice grains. Polishing of rice grains remove mineral nutrients to an extent of 70 percent. Aleuron layer which is loaded with many nutrients is lost during polishing. Martínez *et al*[13]., evaluated 11,400 samples of brown and milled rice for iron and zinc content. They found that, on an average, Brown rice was containing 10-11ppm iron and 20-25ppm zinc while milled rice was containing only 2-3 ppm of iron and 16-17 ppm of zinc.

Rice is commonly used as milled (white) rice produced by removing the hull and bran layer of the rough rice kernel (paddy)[16]. Brown rice (hulled rice) is composed of surface bran (6–7 % by weight), endosperm (90 %) and embryo (2–3 %). White rice is referred to as milled, polished or whitened rice when 8–10 % of mass (mainly bran) has been removed from brown rice[10]. During milling, brown rice is subjected to abrasive or friction pressure to remove bran layers resulting in high, medium or low degrees of milling depending on the amount of bran removed.

Milling and polishing are important operations during the production of white rice. The degree of milling and polishing has a significant effect on the nutritional aspects of white rice, especially on minerals, due to a non-uniform distribution of nutrients in the kernel. Information on the distribution of nutrients in rice will greatly help in understanding the effect of milling and aid in designing procedures that improve technological and sensory properties of rice while retaining its essential nutrients as much as possible[11].

Virk *et al*[21]., found that polished rice grains preferred by consumers lose on an average 24 per cent of Zn during milling. Its concentration in rice bran (aleuron and embryo tissue) is 50.3 ppm compared to 8.6 ppm in the endosperm[14]. Differential depositions of mineral in different compartments of developing grains such as bran, embryo and endosperm has been known to play important role in generating genotypic variation in grain Fe/Zn content in cereals[18].

Biofortification is one of the best methods to alleviate malnutrition and development of new cultivars with elevated concentrations of micronutrients using conventional breeding and biotechnological approaches[7,25]. The main advantages of biofortification compared to other strategies are (i) it can reach relatively to remote poor rural populations relied on rice as staple diet, (ii) cost-effective and (iii) sustainable strategy[3,17].

As rice is consumed primarily in the polished state, it would be more appropriate to study the genetic variation for iron and Zinc content in the polished rice grains. Outer layers of brown rice (pericarp, tegmen and aleurone etc.) are removed during milling. In this process most of the minerals that are concentrated in the outer layers of rice grains are lost. On an average 70 per cent of iron is lost during milling while loss of zinc during milling is around 24 per cent[21].

Thus, improving micronutrient content including Zn content in grains of rice shall ameliorate health problems induced by its deficiency[7,12]. The objective of this study was therefore to evaluate the Zn concentration in grains of selected elite rice genotypes.

MATERIAL AND METHODS

The present experiment was carried out in the rice biotechnology laboratory of the Department of Plant Biotechnology, UAS, GKVK, Bangalore.

Plant Materials:

Five elite lines with four checks differing for grain Zn content were selected based on earlier studies of Bekele[1] using more than 1200 rice accessions. These studies were based on X-ray fluorescence (XRF) readings for Zn and Fe estimation. The list of the selected genotypes is given in Table 1.

Experimental conditions:

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. To meet the nutrient demand of the plants, fertilizers were provided. As the experiment was done in aerobic condition, irrigation was done once in three days for all the plants. All necessary measures were taken to control pest and disease infestation. The soil at the experimental site had 0.74ppm for Zn (estimated using DTPA extractable method), a pH 6.1 and 7.4g kg[1] of organic carbon (Table 2).

Table 1: List of selected elite lines with checks differing for grain Zinc content

Sl. No.	Variety name/ Cross	Pedigree	Grain type
1	AM-72	Azucena X Moromutant	Long bold
2	AM-143	Azucena X Moromutant	Medium slender
3	AM-65	Azucena X Moromutant	Long bold
4	AM-1	Azucena X Moromutant	Long bold
5	AM-180	Azucena X Moromutant	Medium bold
6	Vandana	Local accession	Medium bold
7	Sebati	Local accession	Long slender
8	Jeerigesanna	Local accession	Short bold
9	ARB6	Budda X IR-64	Medium bold

Table 2: Physico-Chemical properties of soil recorded in the experimental site

Sl. No.	Parameters	Procedure/ Methodology	Value recorded
1	Soil pH (1: 2.5)	Soil: water suspension (1: 2.5) was measured for pH using potentiometer after standardizing with appropriate buffers.	6.1
2	Organic carbon (g kg^{-1})	Soil was digested with $K_2Cr_2O_7$ and Conc. H_2SO_4, the unutilized $K_2Cr_2O_7$ was back titrated against ferrous ammonium sulphate using diphenyl amine indicator.	7.4
3	Available Zn (ppm)	Diethylenetriamine penta acetic acid extractable methods (DTPA-$CaCl_2$, pH 7.3)	0.82
4	Available Fe (ppm)	Diethylenetriamine penta acetic acid extractable methods (DTPA-$CaCl_2$, pH 7.3)	16.5

Estimation of Grain zinc content (ppm):

Zinc content was estimated in the brown rice (Dehusked unpolished grain) and polished rice (5 % and 10 % polished) from the genotypes grown in the field. The zinc content in these grains was calculated from X-ray fluorescence (XRF) at MSSRF, Chennai, Tamil Nadu. The instrument was switched on 24 hours before the time when observations were to be recorded. Initially, five grams of grains of IR64 variety was subject to XRF as a standard to check the calibration of the equipment. Then the samples of the nine genotypes were subjected to XRF and the concentration was recorded in ppm. Three replications were maintained and their average was considered.

Statistical analyses

Mean values of three plants used for recording the observations were computed for different plant characters for each of the genotypes. The phenotypic data for all the genotypes for each character was subjected to statistical analysis.

The analysis of variance for different characters was used to partition the variance due to different sources following the method given by Panse and Sukhatme[15]. The significance was tested by comparing with the table values as given by Yates[24]. Standard error of means (SE_M) and Critical difference (CD) were worked out using appropriate formula for comparing individual line means.

RESULTS

Performance of genotypes

The genetic variability parameters viz., minimum, maximum, mean, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (h2) and genetic advance as per cent mean (GAM) for grain Zn (Brown rice) and polished grain Zn content in all genotypes are presented in Tables 3.

Table 3: Estimates of genetic parameters for different quantitative traits among selected rice accessions for Zinc content

Sl. No	Zinc content in	Mean ± SE	Min	Max	PCV (%)	GCV (%)	H^2 broad sense (%)	GAM (%)
1.	**Brown rice**	27.24±0.77	23.40	34.10	11.99	10.94	72	18.9
2.	**5 % polished grain**	19.26±0.28	12.00	25.70	20.56	20.41	98	41.72
3.	**10 % polished grain**	18.07±0.28	11.50	24.50	22.70	22.53	98	46.08

PCV = Phenotypic Coefficient of variation GCV= Genotypic Coefficient of variation

H^2 % = Heritability percentage in broad sense GAM= Genetic Advance as per Mean

Zinc content in selected genotypes

Zinc content in brown rice, 5 % polished grain and 10 % polished grain of selected genotypes was estimated using XRF at MSSRF, Chennai, is presented in Fig 1, Fig 2, Fig 3 and Fig 4. Highest grain Zn in brown rice was observed in AM-65 (33.90ppm), lowest in Sebati (23.85ppm). Highest Zn in 5% polished grain was observed in AM-65 (24.63ppm), lowest in Jeerigesanna (12.3ppm). Highest Zn in 10% polished grain was observed in AM-65 (24.27ppm), lowest in Jeerigesanna (11.63ppm).

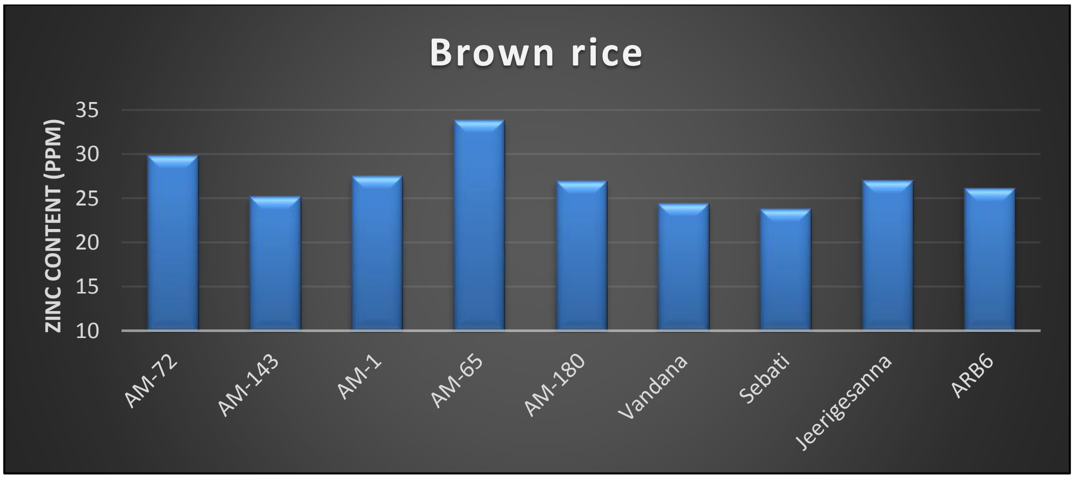

Fig. 1: Average Zinc content (ppm) in Brown rice (Dehusked unpolished grain)

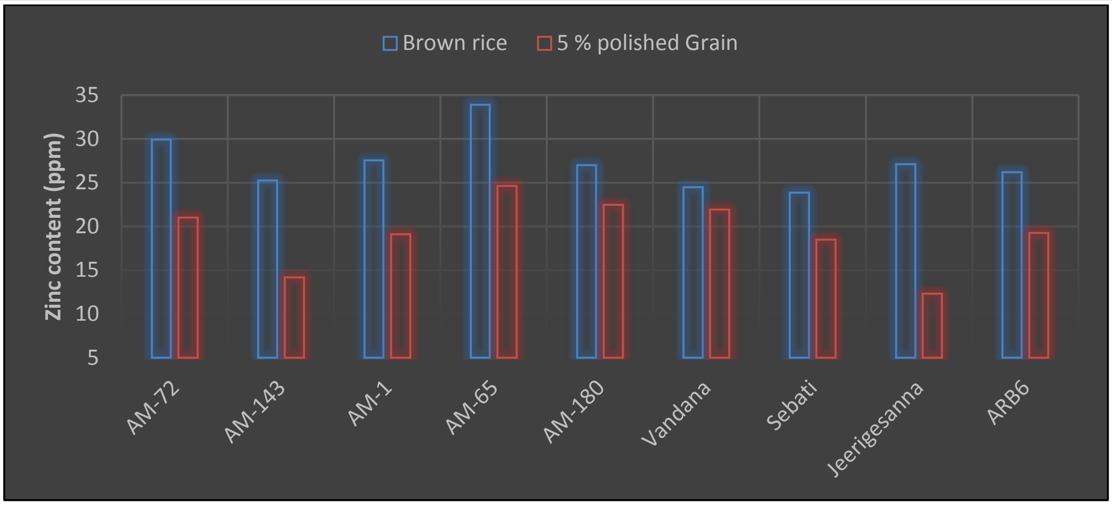

Fig. 2: Average Zinc content (ppm) in Brown rice and 5 % polished rice

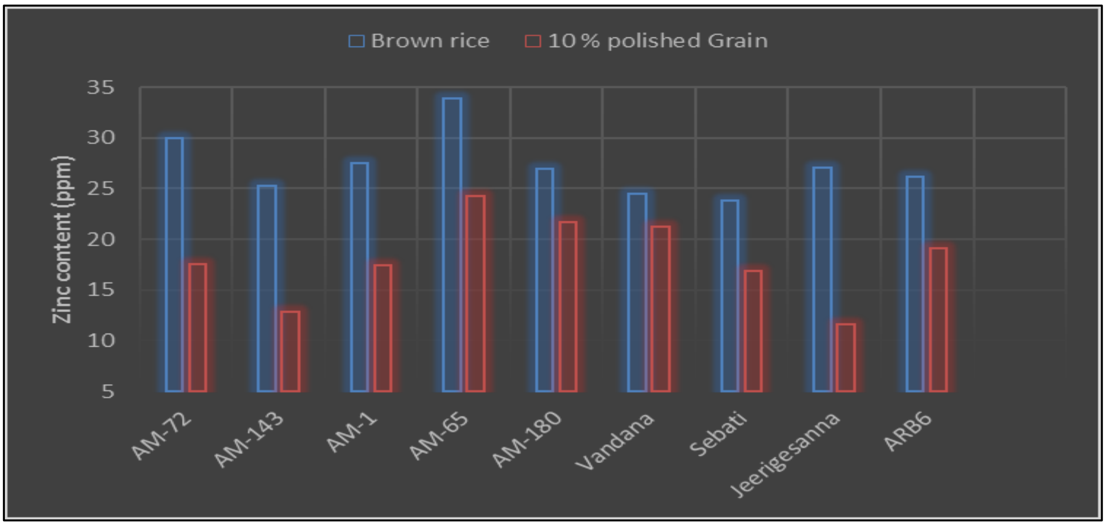

Fig. 4: Average Zinc content (ppm) in Brown rice and 10 % polished rice

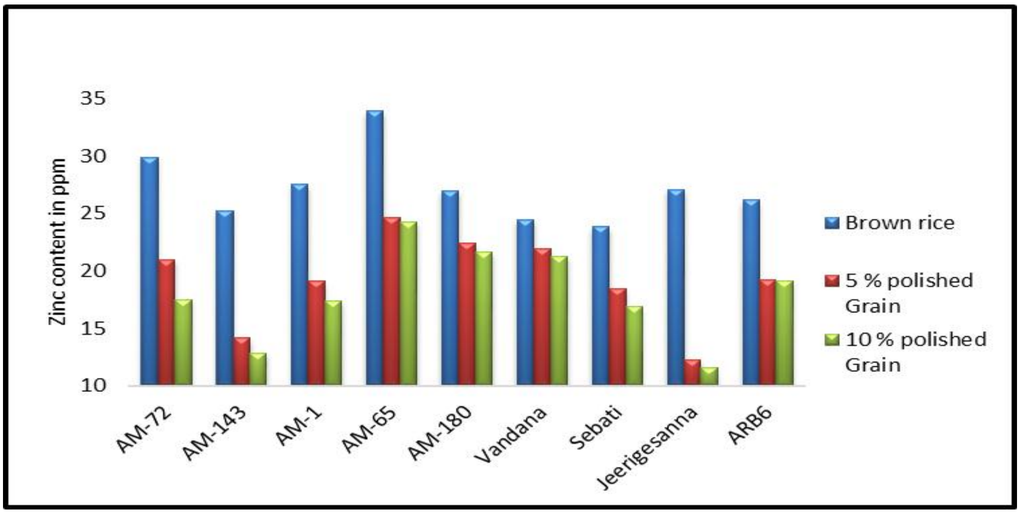

Fig. 3: Average Zinc content (ppm) in the selected genotypes of rice

Analysis of variance

The mean sums of squares due to various sources of variation for Zinc content of nine genotypes are represented in Table 4. Highly significant differences among the genotypes were observed for all the characters indicating wide variability for all the characters among the genotypes studied.

Table 4: Analysis of variance for Zinc content in selected rice accessions

Source of Variation	df	Mean Sum of Squares		
		Brown rice Zinc content	5% polished Grain Zinc	10% polished Grain Zinc
Genotype	**8**	28.46**	46.61**	50.02**
Error	**16**	1.78	0.23	0.24
CD at 5%		2.31	0.84	0.86
CD at 1%		3.18	1.16	1.19
CV		4.89	2.53	2.76

** Significant at 1%

DISCUSSION

Micronutrient deficiency or hidden hunger affects more than 3.7 billion people worldwide, according to WHO figures from 2012, predominantly women and children because of their physiological needs. Zinc (Zn) deficiency is a well-documented global health problem, affecting nearly half of the world population, particularly in developing countries, where high proportion of cereal crops such as rice and wheat, consumed as a staple food. The reliance on cereal based food induce Zn deficiency related health problem, such as impairments in physical growth, immune system and brain function, complications in pregnancy and child birth, poor child growth and learning ability. Low dietary Zn intake is considered to be the major reason for widespread occurrence of Zn deficiency in human populations, especially in developing countries[22].

Among the cereals, Rice (*Oryza sativa* L.), being one of the leading staple crops for half of the world's population and hence, is the main source of Zn to human beings. Rice, however unfortunately, is a poor source of metabolizable Zn, due to inherently low in Zn content and the bio-available Zn. Enrichment of Rice with high bio-available Zn is, therefore, suggested as a way to generate major health benefits for a large number of susceptible people.

In cereals, the two minerals are almost exclusively stored in the husk, the aleurone and the embryo and large proportions, therefore, are lost during milling and polishing. This implies that the full potential of the genotype-determined increments in Fe and Zn content is not realized for improving human nutrition.

Milling and polishing are important operations during the production of white rice. The degree of milling and polishing has a significant effect on the nutritional aspects of white rice, especially on minerals, due to a non-uniform distribution of nutrients in the kernel.

The polishing of rice grain, however, is an essential process which is carried out by all rice industries and commercial farmers to remove the oil rich aleurone layer that would otherwise make the rice seed rancid during long storage. The present study of micronutrient distribution in polished and unpolished rice grain indicates that normal polishing process done at two levels 5 % and 10 % removes almost all the aleurone and embryo, which is the main storehouse for major micronutrients.

Although there have been many reports on grain Zn content, there is a huge gap between concentration of Zn reported in rice grains and the contribution of rice towards Zn supply to human body. This difference arises mainly because, unlike other cereals people consume polished rice which loses about 35-50 % Zn present in aleurone layer[13]. So it is really necessary to develop rice varieties with higher Zn content particularly in endosperm rather than considering the varieties with higher whole grain Zn content.

Improvement of any trait depends on the existence of the genetic variability and also the heritability. Any attempt to improve traits without the knowledge of heritability would of no use. Thus, information on phenotypic and genotypic co-efficient of variation and heritability would be advantageous. Hence, PCV and GCV are used to compare the variability observed among the studied characters. The heritability estimates aid in determining the relative amount of heritable portion of variation. However, heritability values itself provides no indication of the amount of genetic progress that would result from selecting the best individuals. Heritability estimates in broad sense would be reliable when accompanied by high genetic advance[4,9].

High heritability coupled with moderate genetic advance as percent mean was recorded for days to maturity. This result is in accordance with Bisne *et al*[2]. In case of grain Zn, 5 % polished Zn and 10 % polished Zn high heritability coupled with moderate genetic advance as percent mean was recorded.

CONCLUSION

Polishing of rice grain, however, is an essential process which is carried out by all rice industries and commercial farmers to remove the oil-rich aleurone layer that would otherwise make the rice seed rancid during long storage. The present study of micronutrient distribution in polished and unpolished rice grain indicates that normal polishing process, done at two levels (5 % and 10 %) removes almost all the aleurone and embryo, which is the main storehouse for major micronutrients. Per cent loss of Zn was more in 10 % polishing than in 5 % polishing. Hence Zn content was reduced after polishing because Zn content is more in aleurone layer than in the endosperm. Among the nine genotypes AM-65 had highest Zn content even after polishing. Therefore percent loss of micronutrient was less in this elite line even after polishing. Though the loss of micronutrient is more with greater polishing it is interesting to note that varietal differences for extent of loss depends on other traits like grain size, shape and density.

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