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Assessment of Iron Content and Distribution in Different Parts of Rice Grain

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ABSTRACT

Micronutrient deficiency (hidden hunger) is a major health problem which affects more than half of the world's population and contributing to high rate of children and women's mortality. Iron deficiency is probably the most widespread micronutrient deficiency in humans. Since rice is the main staple food for more than half of the global population, estimation of the iron content in different parts of rice grain and identification of iron rich genotypes is a perspective and an effective way to solve this problem. The present paper evaluates the iron content in different parts of rice grain (paddy, brown rice and white rice). The data show that the iron content in Jeerigesanna is higher than in the other rice types, and in polished rice iron content in AM-180 is high.

Key words: *Micronutrients, Polishing, Oryza sativa L, Biofortification, Rice Grains.*

INTRODUCTION

Rice (*Oryza sativa* L.) is the dominant cereal staple food of almost of half the world's population[8]. It provides 23% of all the calories consumed by the world's population which is more than wheat and corn, and even provides 50-80% of the energy intake of the people in the developing countries (IRRI, 2006). Rice, however, is a poor source of most of many essential micronutrients, especially iron (Fe) and zinc (Zn), for human nutrition[30].

The polished rice contains an average of only 2 mg kg-1 Fe and 12 mg kg-1 Zn, whereas the recommended dietary intake of Fe and Zn for humans is 10-15 and 12-15 mg, respectively[24]. Up to date malnutrition of Fe and Zn which may weaken immune function and impair growth and development of human[26] afflict more than 50 % of the world's population[23,25]. In China, more than 20 % of the people are suffering from Fe and Zn deficiency[28,29]. Heavy and monotonous consumption of rice with low concentrations of Fe and Zn has been considered a major reason for Fe and Zn malnutrition[12]. Therefore, a slight increase in its nutritive value would be highly beneficial for alleviation of Fe and Zn malnutrition and for human health[11].

In rice grains, the highest concentrations of Fe and Zn co-localize with protein and free amino acids in the embryo and with lower concentrations in endosperm.

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This is problematic from a human nutrition perspective, since processing of rice, wheat, or other cereals in ways that remove these parts results in greatly reduced nutritional value. Seeds or grains supply nutrients which include Iron (Fe) and Zinc (Zn) which are supplied by parental plant thus being under influence of genes and environment. Biofortification will involve production of such genotypes with increased concentration of micronutrients by understanding control over supply of nutrients. A major challenge of biofortification is an incomplete understanding of the pathways and the rate limiting steps involved in translocating minerals to the seeds.

Rice contains less Fe levels, most of which is lost during processing. Populations with monotonous diets consisting mainly of cereals are especially prone to Fe deficiency. Fe biofortification in rice is done by generating cultivars that efficiently mobilize, uptake and translocate Fe to the edible parts through conventional and transformation processes[20]. Most research in plant breeding was focussed on breeding for high yields, resistance to biotic factors and tolerance to abiotic stresses. Recently the trend has changed to incorporate desired quality parameters to the grains from health and economic prospective[3].

It has been reported that rice is poor in some of the micronutrients such as Fe, Zn, Ca, Mg, Cu, I and Se etc., which are important for human beings for their proper growth and development[2] especially after polishing. Interestingly, malnutrition is more prevalent in countries where rice is the major dietary component. Whereas, the polished grain, also known as white rice, contains nutritionally insufficient concentrations of iron (Fe), zinc (Zn) and pro-vitamin A to meet daily requirements in diets based on this staple[14].

Iron (Fe) is essential for virtually all living organisms. Fe deficiency is the most widespread human nutritional problem in the world. There are two billion anaemic people worldwide, and 50 % of all anaemia cases can be attributed to iron deficiency. In plants, iron plays a key role in electron transfer in both photosynthetic and respiratory reactions in chloroplasts and mitochondria. Although abundant in mineral soils, iron is sparingly soluble under aerobic conditions at high soil pH. Consequently, plants grown on calcareous soils often exhibit severe chlorosis because of iron[16]. Various components of Fe uptake and transport have been elucidated in much detail, including metal transporters, synthesis of Fe chelators, and transcription factors regulating Fe uptake.

The poorest families in developing countries spend 50 – 80 percent of their income on food; depend heavily on low cost, high energy, starchy staples like rice which contains insufficient amounts of micronutrients such as Zinc (Zn), Iron (Fe) and vitamin A to provide recommended daily intake for human beings[10,13]. Southeast Asia is the major rice producer in the world where 71 percent of population suffers from Zinc deficiency.

Deficiencies in bioavailable Fe, Zn and other essential cation minerals in human food, causing mineral malnutrition, affect a large proportion of the world population. Of all micronutrients which are essential for sustaining human health, Fe, Zn, iodine and vitamin-A have been reported to be most at risk of malnutrition[24]. Key reasons for human mineral malnutrition are the relatively low content of cation minerals in plant-based foods in combination with the abundance of antinutrient compounds that severely reduce their bioavailability. It is estimated that more than 2 billion people in the world are deficient in Vitamin A, Iodine, Iron, Zinc and more than 2.5 billion people in the developing world are zinc deficient.

Micronutrient enrichment biofortification is cost effective, stable and can meet requirements of economically weaken community. The genetic basis of accumulation of micronutrients in the grains and mapping of the quantitative trait loci (QTL) will provide the basis for preparing the strategies and improving grain micronutrient content through marker-assisted selection[9].

Though the symptoms of zinc and iron deficiency could be alleviated through traditional medications, but it is difficult to be cured entirely. Cost input is also main barrier through artificial means. In addition, the side effects have also been concerned. Therefore, the biofortification technologies for improvement of Zn & Fe content in foodstuff through breeding methods are highly regarded in recent years[24].

Recent advances in the field of molecular biology have led to evolution of Marker Assisted Selection (MAS) which has greatly increased the efficiency of the breeding programs. Molecular markers are currently used as great tools in selecting agronomically important traits. Advantage of molecular markers is that they help in selection of the traits even before traits are expressed[7].

Considering all these, the present study was undertaken to find the Iron rich lines among the selected genotypes and the effect of levels of polishing on grain iron content.

MATERIAL AND METHODS

The present investigation was carried out in the aerobic rice biotechnology laboratory of the Department of Plant Biotechnology, UAS, GKVK, Bengaluru.

Plant Materials and sampling:

Based on earlier studies of Narayanrao[17] using more than 1200 rice accessions, five elite lines with four checks differing for grain Fe content were selected for present study. These studies were based on X-ray fluorescence (XRF) readings for 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 four days for all the plants. All necessary measures were taken to control pest and disease infestation.

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

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

Estimation of iron content (ppm):

Iron content was estimated in the paddy (grain with husk), brown rice (Dehusked unpolished grain) and polished rice (5 % and 10 % polished) collected from the genotypes grown in the field. Grains of individual lines were harvested manually and hand threshed to avoid any contamination. Unbroken, uniform grains were taken for readings. No dehusking and hydrochloric acid treatment was followed in this particular method.

a) In paddy:

Iron content in paddy 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. The content was recorded in ppm. Three replications were maintained and their average was considered.

b) In Brown Rice:

Iron content was estimated in the brown rice collected from the genotypes grown in the field. Grains of individual lines were harvested manually and hand threshed to avoid any contamination. The grains were then manually dehusked. Unbroken, uniform grains were then washed in diluted hydrochloric acid and then with double distilled water to remove any surface contaminants and dried in hot air oven at 70°C for 72 hours. The washed samples of the nine genotypes were subjected to XRF and the content was recorded in ppm. Three replications were maintained and their average was considered.

c) In Polished grains:

Iron content was estimated from the 5 % and 10 % level polished rice grains using commercial top miller installed at PHT scheme GKVK, UAS-Bangalore. Machine was cleaned with brush internally and externally after every turn of polishing.

Statistical analyses

Mean values of five 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 were 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[18].

RESULTS

Iron content

Iron content in different parts of rice grain i.e., paddy (grain with husk), brown rice (after removing husk), 5 % and 10 % polished rice grain of elite genotypes was estimated using XRF at MSSRF, Chennai.

Among the selected genotypes, highest iron content in paddy was found in AM-65 (17.27ppm) and lowest in Sebati (11.10ppm). Iron content in different parts of rice grain was varied between genotypes. Highest iron content in brown rice was observed in Jeerigesanna (11.75ppm), lowest in Vandhana (7.40ppm). Iron content of selected genotypes are depicted in Fig. 1-3. Brown rice is subjected to different levels (5 % and 10 %) of polishing which leads to loss of iron. Maximum loss in iron was found in AM-143 at both the levels of polishing (Fig.4).

Among the nine genotypes AM-180 had highest Fe even after polishing, suggesting minimum loss of Fe content in these elite lines while Vandana and Sebati manifested moderate Fe content among the chosen genotypes (Fig.5).

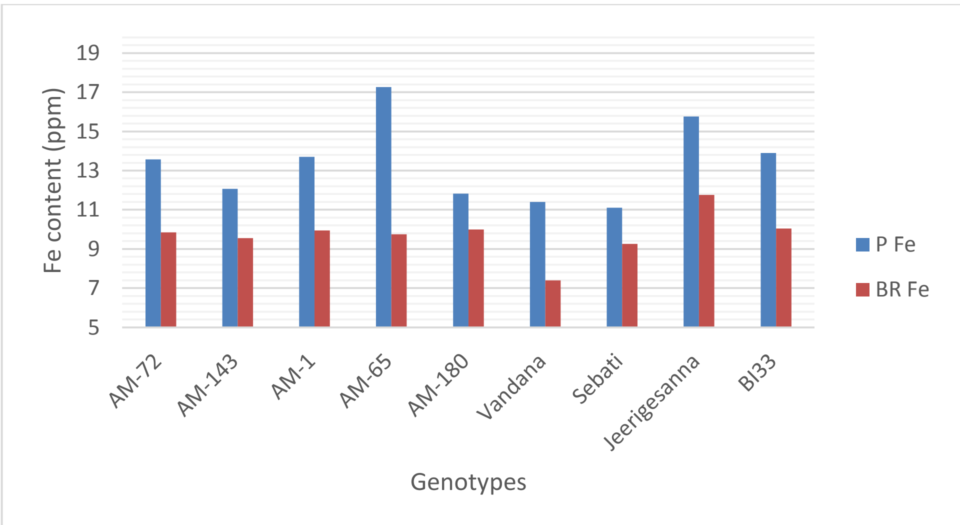

Fig. 1: Average Iron content (ppm) in Paddy and Brown rice

Note: P- Paddy (grain with husk)
BR- brown rice (after removing husk)
5 % Fe Iron content in 5 % polished rice grain
10 % Fe- Iron content in polished rice grain

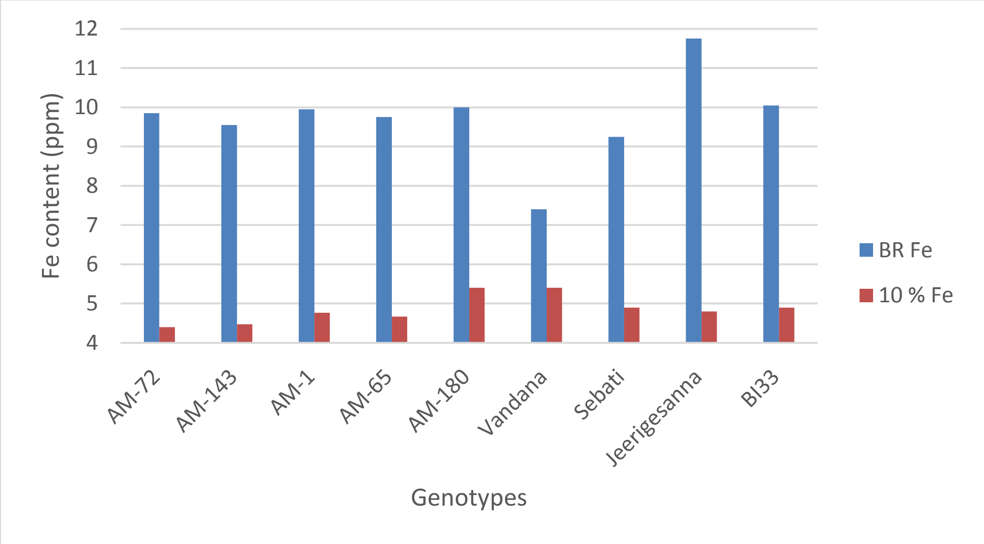

Fig. 2: Average Iron content (ppm) in Brown rice and 5 % polished rice

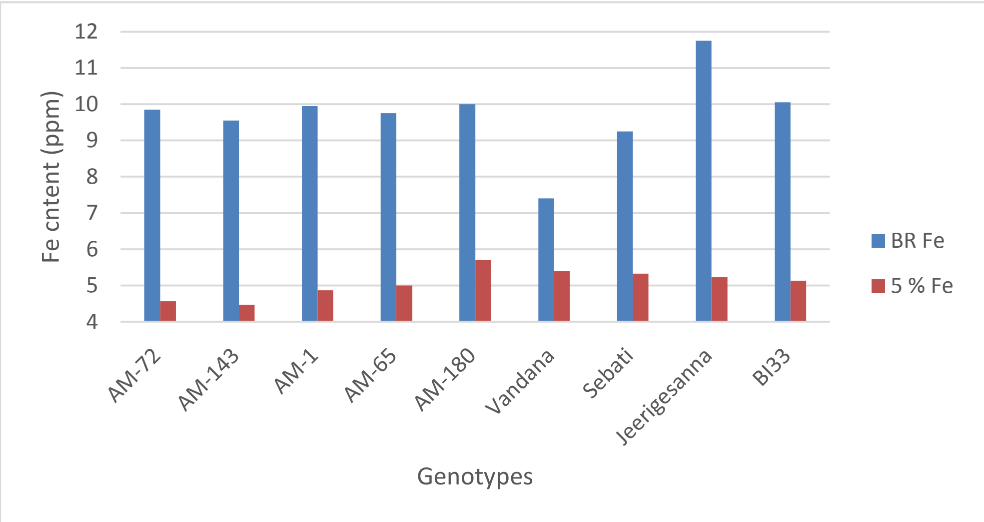

Fig. 3: Average Iron content (ppm) in Brown rice and 10 % polished rice

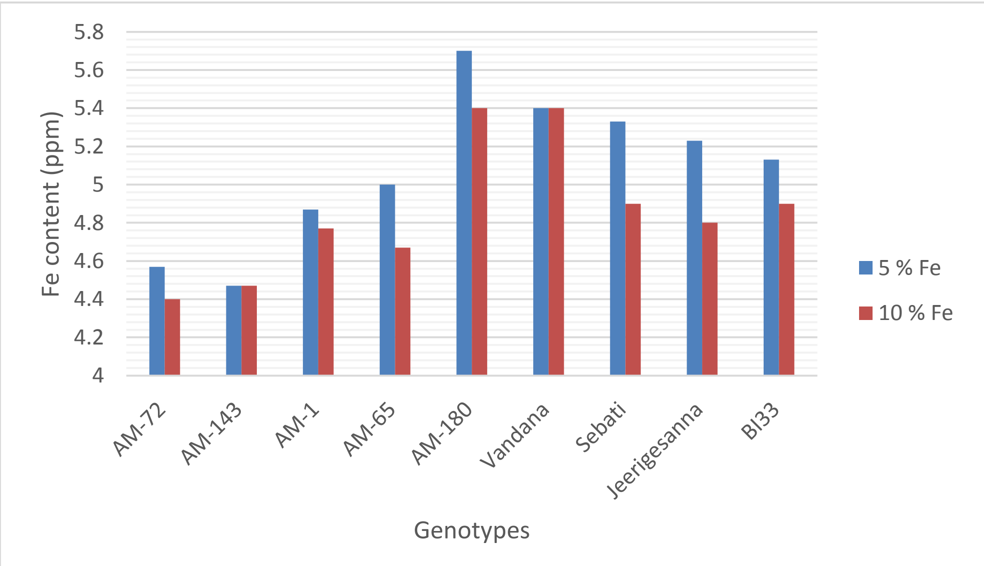

Fig. 4: Average Iron content (ppm) in different levels of polished rice grains

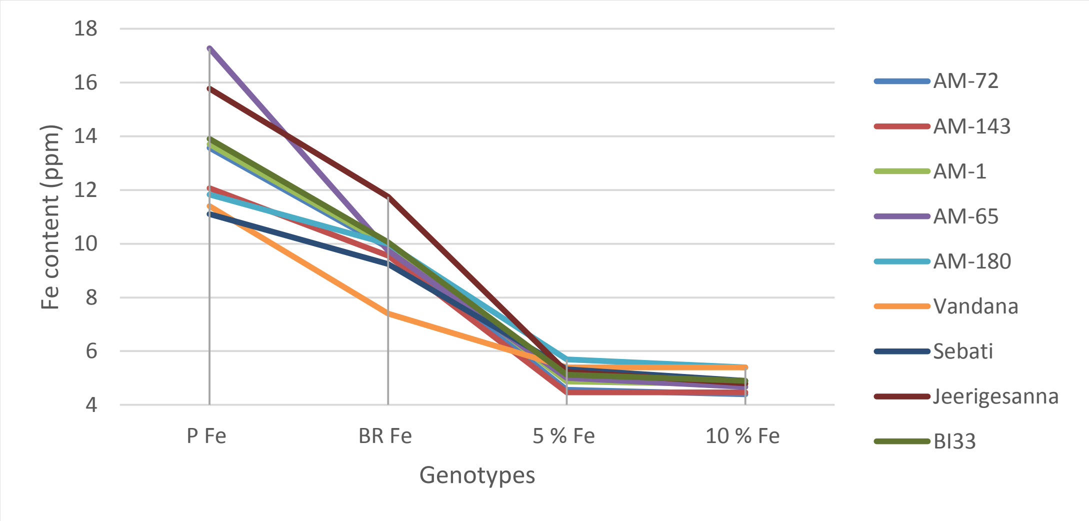

Fig. 5: Average Iron content (ppm) in Different parts of rice grain

Analysis of variance

The mean sums of squares due to various sources of variation for grain iron content of nine genotypes are represented in Table 2. Highly significant differences among the genotypes were observed for all the characters indicating wide variability for all the characters among the genotypes studied.

Table 2: Analysis of variance for Iron content in selected rice accessions

Source of Variation	df	Mean Sum of Squares		
		Brown rice Iron content	5 % polished Grain Iron	10 % polished Grain Iron
Genotype	**8**	3.75**	0.47**	0.37**
Error	**16**	0.42	0.01	0.003
CD at 5%		1.12	0.17	0.10
CD at 1%		1.55	0.24	0.14
CV		6.69	1.99	1.26

** Significant at 1%

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 iron content in all genotypes grown in *kharif-2013* are presented in Table 3.

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

Sl. No	Iron content in	Mean ± SE	Min	Max	PCV (%)	GCV (%)	H^2 broad sense (%)	GAM (%)
1.	**Brown rice**	9.72 ± 0.37	7.30	12.30	12.73	10.83	72	18.97
2.	**5 % polished grain**	5.07 ± 0.05	4.40	5.90	8.01	7.75	93	15.47
3.	**10 % polished grain**	4.85 ± 0.03	4.30	5.50	7.36	7.25	97	14.72

PCV = Phenotypic Coefficient of variation GCV= Genotypic Coefficient of variation
H^2 % = Heritability percentage in broad sense GAM= Genetic Advance as per Mean

DISCUSSION

Hunger seems to be the burning topic of this decade particularly in Africa which represents extreme case of this situation. Even if we increase the quantity to feed every mouth we will be lagging with quality which will effect large population of the world, as most often quality and quantity are negatively associated. If the same trend continues we will be having people with full stomach but hidden hunger of micronutrients[19].

Zinc and Iron form the important part of micronutrients used for essential physiological processes of human beings. Zn is involved in numerous aspects of cellular metabolism. It is required for the catalysis activity of approximately 300 enzymes. Moreover, many transcriptional factors need Zn for their activity[21]. As such, humans do not synthesis or store Zn in their system hence, its intake is essential. Zn deficiency causes poor and impaired growth and effects immune system[27].

On the other hand Iron is important for people of all age groups particularly children and expecting mothers need it in more quantity. Iron is required in less quantity by human beings, 8 mg/day is recommended for adults whereas, 10-12mg of Zn is recommended per day for adults[1]. Vividly, Zn and Fe forms important part in the physiology of human beings. Looking to the present scenario, where half of the world's population is rice eating, particularly Indian, rice doesn't give optimum amount of micronutrients required daily. Rice for a common man means white rice and technically polished rice grain.

Earlier it was thought that people will be motivated to eat brown rice but it seems to be a futile exercise because it is difficult to change the habits of people inculcated over a long time, but now as education is improving availability and consumption of brown rice has increased. One of the major reasons for consuming white rice is that, brown rice contains oil and causes rancidity during storage[6]. In spite of this, scientists intend to solve the problem of hidden hunger with biofortification of the rice. Biofortification wouldn't have come to picture if people would have consumed brown rice.

In present investigation, brown rice also contained high Fe, but only very poor and highly health conscious people prefer to eat it. Majority of people prefer white rice though it is significantly less nutritious as almost 70 % of micronutrients are lost during polishing[6,22]. The highest iron content is found in *Jeerigesanna*, followed by *ARB6* in brown rice. Polished rice also contains the highest relative amount of iron, *AM-180* and *Vandana* had highest iron content at both the levels of polishing. Therefore, iron nutrition may be improved by increasing the supply of these elite lines. However, it is also important to study the mechanism of iron absorption and utilization in these elite lines and the related genes, for enhancement of iron nutrition by using different approaches.

The information on phenotypic and genotypic co-efficient of variation and heritability would be more advantageous because any attempt to improve traits without the knowledge of heritability would of no use. 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[5,15].

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*[4]. In case of grain Fe, 5 % polished Fe, 10 % polished Fe high heritability coupled with moderate genetic advance as percent mean was recorded. There are no reports of paddy and polished grain Fe content. The present investigation revealed high heritability coupled with high genetic advance as per cent of mean for most of the characters indicating the presence of considerable variation and additive gene effects. Hence, improvement of these characters could be effective through phenotypic selection.

CONCLUSION

Rice is the staple food for half of the world's population, and improving the iron content in rice is one important approach to solve iron malnutrition. 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 research mainly focused on the micronutrient (Fe) 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 Fe was more in 10 % polishing than in 5 % polishing. Among the nine genotypes, AM-180 and Vandana had highest Fe content even after polishing. Therefore per cent 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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