Mysore J. Agric. Sci., 52 (2) : 345-348, 2018

Quantifying Micronutrient (Fe) Content in Super Elite Accession at Varying Level of Polishing by Using X-Ray Fluorescence in Rice Grain Grown under Aerobic Condition

D. B. SANTHOSH, H. E. SHASHIDHAR AND D. DAYAL DOSS
Department of Plant Biotechnology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
E-mail: santhuroyal99@gmail.com

ABSTRACT

The research aimed to compare different genotypes of aerobic rice for their iron content and percentage of losses among different genotypes, from brown to white rice. Quantification of Fe was estimated by using X-Ray fluorescence spectroscopy, in brown rice, white rice, 5 per cent bran and 10 per cent bran. A significant difference was observed in loss of micronutrients from brown to white rice. Jeerigesanna had highest Fe content in brown rice and ARB-6 had highest Fe content in polished rice. Azucena had lowest Fe content in brown and white rice, respectively. White rice was deficient in Fe. During the polishing process almost entire aleurone and most part of the embryo is removed which are the main store house for major micronutrients. It is estimated that more than 50 per cent of micronutrients are lost during polishing process. There was a greater quantitative and cumulative loss in Fe content, suggesting that Fe is present comparatively more in the outer aleurone layer.

Keywords : Aerobic rice, brown rice, white rice, iron content

RICE (*Oryza sativa* L.) is one of the most important crop plants and feeds more people than any other single plant species on earth. Asia is considered as a 'Rice Bowl' of the world and more than 90 per cent of rice is produced and consumed in Asia. In India rice is cultivated over an area of 49.78 million hectares and with a production of 146.21 million tonnes (FAO, 2017). Andhra Pradesh, Bihar, West Bengal, Tamil Nadu, Punjab Uttar Pradesh and Karnataka are important rice growing states in India.

Micronutrient malnutrition has reached to frightening situation with billions of people suffering from Iron, Zinc and Vitamin-A related disorders. It is more common among the population having cereal based diets especially rice, as it is rich in carbohydrates but poor source of minerals and nutrition. Micronutrient supplementation, food fortification and biofortification are the three basic approaches used to alleviate micronutrient deficiencies.

The density of minerals and vitamins in food staples eaten widely by the poor may be increased either through conventional plant breeding or through use of transgenic techniques, a process known as *biofortification*. In broad terms, three things must happen for biofortification to be successful. First, the breeding must be successful – high nutrient density must be combined with high yields and high profitability. Second, efficacy must be demonstrated – the micronutrient status of human subjects must be shown to improve when consuming the biofortified varieties as normally eaten. Thus, sufficient nutrients must be retained in processing and cooking and these nutrients must be sufficiently bioavailable. Third, the biofortified crops must be adopted by farmers and consumed by those suffering from micronutrient malnutrition in significant numbers (Saltzman *et al.*, 2013).

Iron plays a determining role for proper functioning of various enzymes and proteins by acting as a cofactor for such proteins and enzymes like cytochrome b6f, FAD, Fe-S complex in photosynthesis and also components of blood. So Iron deficiency is the most common nutritional deficiency in the world (Soetan *et al.*, 2010).

MATERIAL AND METHODS

Iron content was estimated in the grain samples collected from the genotypes grown in the experimental plots. Grains of individual lines were harvested manually and hand threshed to avoid any contamination. The grains were then manually dehusked. Unbroken, uniform grains, of brown rice, polished rice, 5 per cent bran and 10 per cent bran 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 ^{o}C for 72 hours.

The Iron content in these grains was calculated from X-ray fluorescence (XRF) at M. S. Swaminathan Research Foundation, Chennai. The instrument was switched on 24 hours before the time when observations were to be recorded. Initially, five grams of grains of Jaya variety was subject to XRF as a standard to check the calibration of the equipment. The samples of the ten genotypes were subjected to XRF and the concentration was recorded in ppm. Three replications were maintained and their average was considered.

Results and Discussion

In the present study, ten genotypes with high grain Iron (Fe) content were selected based on earlier studies of Narayanrao (2012) and Bekele (2012) using more than 1200 rice accessions. These studies were based on estimation of Fe by using XRF based analysis. The list of the selected genotypes is given in Table I.

In the present study, Iron content in paddy and brown rice of rice grain is shown in Table II. Grain with husk (paddy) and brown rice (after removing husk) iron content of elite genotypes was estimated using X-Ray Fluorescence instrument at M. S. Swaminathan Research Foundation, Chennai. Among the varieties studied in brown rice maximum iron content was recorded in variety Jeerigesanna (11.75ppm) and minimum in variety Azucena (7.40

TABLE I

List of the elite genotypes for high Iron content in grain

Variety name/ Cross	Parents
AM-72	Azucena x Moromutant
AM-65	Azucena x Moromutant
ARB-6	Budda x IR 64
BJ-21	Budda x Jeerigesanna
AM-1	Azucena x Moromutant
AZUCENA	Local accession
JAYA	TN-1x T-141
JEERIGESANNA	Local accession
CHITTIMUTHYALU	Local accession
BLACK RICE	Local accession

TABLE II

Fe content in experiment rice varieties

Varieties	Brown Rice - Fe Content(mg kg-^{1})			White Rice - Fe Content(mg kg-^{1})		
	mean content	Standard Deviation	Standard Error	mean content	Standard Deviation	Standard Error
AM-72	9.85	0.42	0.30	3.20	0.14	0.10
AM-65	9.55	0.21	0.15	3.00	0.14	0.10
ARB-6	9.95	0.42	0.30	3.65	0.21	0.15
BJ-21	9.75	0.14	0.10	3.05	0.21	0.15
AM-1	10.00	0.14	0.10	2.95	0.07	0.05
AZUCENA	7.40	0.00	0.00	2.70	0.21	0.15
JAYA	9.25	0.07	0.05	3.40	0.14	0.10
JEERIGESANNA	11.75	0.07	0.05	3.35	0.14	0.10
CHITTIMUTHYALU	10.05	0.07	0.05	3.50	0.28	0.20
BLACK RICE	8.60	0.42	0.30	2.75	0.21	0.15

ppm). Among the varieties studied in white rice (polished rice) maximum iron content was recorded in variety ARB-6 (3.65 ppm) and minimum in variety Azucena (2.70 ppm). From Table II it is also observed that high iron content was observed in brown rice compared to white rice. In white rice more than 50 per cent of micronutrients were lost due to polishing. The results clearly indicate that a considerable loss of Fe occurred during the polishing process of brown rice to white rice.

Similar results were also reported by Anuradha *et al.* (2012) for high Fe concentration of 14.8 ppm in brown rice of improved cultivars. Prom-u-thai *et al.* (2007) analyzed grain Fe concentration in rice genotypes and found that it varies between 7–19 mg Fe kg-1 in brown rice. In another study, Prom-u-thai *et al.* (2007) obtained 10 to 20 mg kg-1 and 3–11 mg kg-1 of Fe in brown and white rice, respectively among different rice cultivars of diverse genetic backgrounds. Chandel *et al*. (2010) indicated that grain Fe concentration generally varied much more with genotype than with environment and genotype x environment interactions. Several studies suggests that variation in grain Fe concentration of same or different accessions may depends on sample lots harvested, position of grain on the panicle, presence or absence of embryo in grains or time of harvest (Anuradha *et al.*, 2012).

The results dipicted in Table III revealed that the Iron in brown rice ranged from 7.40 to 11.75 mg kg-1 (Fe), while on the other hand, ranged from 11.10 - 17.27 mg kg-1 in paddy. In case of 5 per cent bran, it is ranging from 12.23 to 21 mg kg-1 and in10 per cent bran it is ranging from 12.35 to 30.55 mg kg-1. The results showed that iron content in 5 per cent bran and 10 per cent bran was very high compared to iron content in paddy and brown rice. This is because during polishing aleurone layer and part of embryo were removed from the rice grains. Aleurone layer and embryo are the main store house of the micronutrients. 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 but one should look for other alternatives like tissue specific promoters for expression and transporters which are responsible accumulation of micronutrients into the endosperm of rice grain.

TABLE III

Average Iron content (ppm) in 5 per cent and 10 per cent Bran, Paddy and Brown rice

Variety name	5 per cent Bran	10 per cent Bran	Paddy	Brown Rice
AM-72	19.26	19.90	13.57	9.85
AM-65	12.23	12.35	12.07	9.55
ARB-6	14.25	15.55	13.70	9.95
BJ-21	15.85	18.00	17.27	9.75
AM-1	20.24	21.05	11.83	10.00
AZUCENA	21.00	30.55	11.40	7.40
JAYA	19.71	22.75	11.10	9.25
JEERIGESANNA	18.85	25.20	15.77	11.75
CHITTIMUTHYALU	18.42	22.05	13.90	10.05
BLACK	16.10	17.50	10.75	8.60
Mean	20.49	17.58	13.14	9.61
Standard deviation	5.14	2.86	2.13	1.12
Standard error	1.63	0.91	0.68	0.37
Minimum	12.35	12.2	10.75	7.4
Maximum	30.55	21	17.27	11.75

All selected genotypes restrained high Fe in brown rice contrast to white rice. Attempt was made to understand the reason for loss from brown to white rice and the possible reason found was that polishing of brown rice causes leaching of nutrient and leads to decrease in content of Fe. The order of high Fe and Zn includes bran (embryo + aleurone layer) > hull > whole grain > brown rice > polished rice (endosperm). The similar results are obtained by Lu *et al.* (2013). The nutrients are transported to the endosperm through single ovular vascular trace present on the ventral side of the ovary. In early stage of development of rice caryopsis, solutes enter through the chalaza into the nucellar projection and then into the endosperm. At later stages, transport occurs through the nucellar epidermis, centripetally towards endosperm, a unique way in which the nutritional components are distributed in the rice caryopsis. In mature rice grain, aleurone and embryo are the major store-house for lipids. It also stores phytin granules which contain abundant calcium, potassium and iron.

Fig. 1 indicates more than 50 per cent loss of micronutrients in the grains of aerobic rice genotypes selected for the study. This loss could be because of the expression of transporter responsible for moving nutrient inside the endosperm is less as compared to aleuron layer and the endosperm of rice is starchy and hence less content of minerals like Iron. Similar results were obtained by Wirth *et al.* (2009) and Johnson *et al.* (2011).

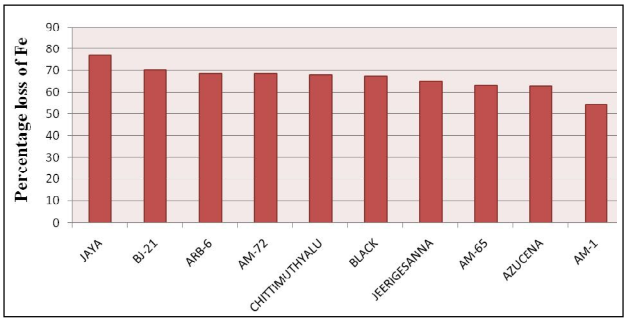

Fig. 1: Extent of loss in Fe content from brown to polished rice

Hence, the study indicated that during the polishing process almost entire aleurone and most part of the embryo is removed which are the main storehouse for major micronutrients. It is estimated that more than 50 per cent of micronutrients are lost during polishing process.

References

ANURADHA, K., AGARWAL, S., RAO, Y. V., RAO, K. V., VIRAKTAMATH, B. C. AND SARLA, N., 2012, Mapping QTLs and candidate genes for iron and zinc concentrations in unpolished rice of Madhukar × Swarna RILs. *Gene.*, **508** : 233 - 240.

BEKELE, B D., 2012, Identification and validation of molecular markers for zinc content in grains of rice (*Oryza sativa*) grown under aerobic condition. *Ph.D. Thesis*, Univ. Agril. Sci., Bengaluru.

CHANDEL, G., BANERJEE, S., VASCONCELOS, M. AND GRUSAK, M. A., 2010, Characterization of the root transcriptome for iron and zinc homeostasis-related genes in *indica* rice (*Oryza sativa* L.). *Pl. Bioche. Biotech.*, **19** : 1 - 8.

FAOSTAT, 2017, http://faostat.external.fao.org/faostat.

JOHNSON, A. A., KYRIACOU, B., CALLAHAN, D. L., CARRUTHERS, L., STANGOULIS, J., LOMBI, E. AND TESTER, M., 2011, Constitutive overexpression of the OsNAS gene family reveals single-gene strategies for effective iron- and zinc-biofortification of rice endosperm. *PLoS One*, **6** (9) : 244 - 256.

JORHEM, L., ÅSTRAND, C., SUNDSTRÖM, B., BAXTER, M., STOKES, P., LEWIS, J. AND GRAWÉ, K. P., 2008, Elements in rice on the Swedish market : Part 2. Chromium, copper, iron, manganese, platinum, rubidium, selenium and zinc. *Food Additives and Contaminants*, **25** (7) : 841 - 850.

LU, L., TIAN, S., LIAO, H., ZHANG, J., YANG, X., LABAVITCH, J. M. AND CHEN, W., 2013, Analysis of metal element distributions in rice (*Oryza sativa* L.) seeds and relocation during germination based on X-ray fluorescence imaging of Zn, Fe, K, Ca, and Mn. *PLoS One*, **8** (2) : 573 - 580.

NARAYANRAO, G. D., 2012, Molecular and genetic evaluation for drought resistance and productivity in high iron and zinc accessions of rice (*Oryza sativa*) grown under aerobic condition. *M.Sc. Thesis*, Univ. Agril. Sci., Bengaluru.

PROM-U-THAIC, FUKAIS, GODWINI, D. AND HUANG, L., 2007, Genotypic variation of iron partitioning in rice grain. *J. Sci. Food Agric.*, **87** : 2049 - 2054.

SALTZMAN, A., BIROL, E., BOUIS, H. E., BOY, E., DE MOURA, F. F., ISLAM, Y. AND PFEIFFER, W. H., 2013, Biofortification : progress toward a more nourishing future. *Global Food Security*, **2** (1) : 9 - 17.

SOETAN, K. O., OLAIYA, C. O. AND OYEWOLE, O. E., 2010, The importance of mineral elements for humans, domestic animals and plants-A review. *Afr. J. Food Sci.*, **4** (5) : 200 - 222.

WIRTH, J., POLETTI, S., AESCHLIMANN, B., YAKANDAWALA, N., DROSSE, B., OSORIO, S. AND SAUTTER, C., 2009, Rice endosperm iron biofortification by targeted and synergistic action of nicotianamine synthase and ferritin. *Pl. Biotechnol. J.*, **7** (7) : 631 - 644.

(*Received :* May, 2018 *Accepted :* June, 2018)