RESEARCH ARTICLE **https://doi.org/10.58297/RMCN2142**

Characterization of African Rice (*Oryza glaberrima* Steud.) Germplasm for Grain Iron and Zinc Content

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Received : 2nd April, 2023; Accepted : 4th June, 2023

Abstract

Micronutrient deficiency is one of the major challenges for food security in developing nations. There is a need for the identification of micronutrient-rich genotypes for their direct use in the genetic enhancement of staple food crops using plant breeding strategies. In the present study, grain iron (Fe) and zinc (Zn) contents of 29 accessions of *Oryza glaberrima* along with check varieties were analyzed for three seasons. Grain Fe ranged from 6.40 ppm to 12.10 ppm with a mean of 8.57 ppm, while Zn content exhibited manifold variation by ranging from 7.30 ppm to 34.40 ppm in the brown rice. There was a two-fold variation in Fe and Zn concentrations between accessions indicating the potential to boost these micronutrients in rice grain. Fifteen African rice accessions were better than the checks for grain Fe content, while four accessions outperformed checks with higher Zn content. Altogether, one *O. glaberrima* accession, CG 239 was found to be having high Zn content (34.7 ppm) in the brown rice making it a valuable source for biofortification of popular rice varieties using conventional and molecular approaches.

Keywords: Iron, zinc, biofortification, *Oryza glaberrima*, brown rice

Introduction

Rice (*Oryza sativa* L.) is the primary source of nourishment for more than half of the world's burgeoning population. Nearly 20% of the daily calories consumed by 3.5 billion people across the globe come from rice, with Asia accounting for 90% of world rice consumption (Muthayya *et al.*, 2014). Nevertheless, the levels of the key micronutrients such as iron (Fe) and zinc (Zn) are insufficient in order to meet the daily dietary requirements (Bouis and Welch, 2010). The deficiency is also inherently difficult to apprehend inflicting economic mayhem of several countries globally (Maganti *et al.,* 2019). Annually, micronutrient deficiencies tend to account for seven percentage of the global disease burden annually (Muthayya *et al.,* 2014) with over three billion people suffering predominantly from Fe and Zn malnutrition in developing nations (Sperotto *et al.,* 2010). Iron (Fe) is a key player in hemoglobin synthesis and erythrocyte production (Lynch, 2003), while zinc (Zn) acts as a stabilizer of the structures of membranes, cellular components and for detoxification of highly aggressive free radicles (Cakamn, 2000). Almost 40 per cent of the zinc binding proteins are needed for gene regulation and 60 per cent enzymes and proteins are involved in ion transport (Andreini *et al.,* 2006). Therefore, insufficient levels of Fe and Zn in the diet tend to have adverse health consequences making it imperative to enhance their concentration and bioavailability in rice grain (Solomon, 2003).

In this regard, biofortification with nutrient enrichment of staple crops through plant breeding has been considered as a sustainable approach which is technically feasible without compromising agronomic productivity (Nestel *et al.,* 2006). The approach has been game-changing for alleviating hidden hunger as indicated by recent successes in rice, maize and wheat crops with the combined efforts of researchers and policymakers. Recently, the breeding target is approximately fixed at 40 ppm for iron and 30 ppm for zinc biofortification (Tripathy, 2020). This could be achieved with the identification of germplasm with high efficiency of Fe and Zn accumulation in the endosperm with their bioavailability from existing germplasm collection. Selection of such micronutrient-rich cultivars could be either within the existing germplasm or generation of new material *de novo* through genetic modification and utilizing them as donors pose to be a potent and reliable way to provide trace elements nutrition benefits to the native farmers and local population (Prom-u-thai *et al.,* 2006).

Accordingly, exploitation of large genetic variation existing in wild species and landraces is an important approach to increase micronutrients concentration. Several studies reported high Fe and Zn contents in the brown rice of some wild rice species and their derivatives (Gregorio *et al.,* 2000; Anandan *et al.,* 2011; Norton *et al.,* 2014). African rice, *Oryza*

Table 1: List of *O. glaberrima* accessions and checks used in the present investigation

S. No.	Entry	Accession No.	Origin	Biological status
1	CG 208	EC 861784	Guinea	Traditional cultivar/ Landrace
2	CG 209	EC 861785	Guinea	Traditional cultivar/ Landrace
3	CG 211	EC 861787	Guinea	Traditional cultivar/ Landrace
4	CG 214	EC 861790	Guinea	Traditional cultivar/ Landrace
5	CG 215	EC 861791	Guinea	Traditional cultivar/ Landrace
6	CG 216	EC 861792	Guinea	Traditional cultivar/ Landrace
7	CG 217	EC 861793	Guinea	Traditional cultivar/ Landrace
8	CG 218	EC 861794	Guinea	Traditional cultivar/ Landrace
9	CG 219	EC 861795	Guinea	Traditional cultivar/ Landrace
10	CG 220	EC 861796	Guinea	Traditional cultivar/ Landrace
11	CG 221	EC 861797	Guinea	Traditional cultivar/ Landrace
12	CG 223	EC 861799	Guinea	Traditional cultivar/ Landrace
13	CG 225	EC 861801	Guinea	Traditional cultivar/ Landrace
14	CG 226	EC 861802	Guinea	Traditional cultivar/ Landrace
15	CG 227	EC 861803	Guinea	Traditional cultivar/ Landrace
16	CG 228	EC 861804	Guinea	Traditional cultivar/ Landrace
17	CG 229	EC 861805	Guinea	Traditional cultivar/ Landrace
18	CG 230	EC 861807	Guinea	Traditional cultivar/ Landrace
19	CG 231	EC 861808	Guinea	Traditional cultivar/ Landrace
20	CG 232	EC 861809	Guinea	Traditional cultivar/ Landrace
21	CG 233	EC 861810	Guinea	Traditional cultivar/ Landrace
22	CG 234	EC 861811	Guinea	Traditional cultivar/ Landrace
23	CG 236	EC 861813	Guinea	Traditional cultivar/ Landrace
24	CG 237	EC 861814	Guinea	Traditional cultivar/ Landrace
25	CG 239	EC 861816	Guinea	Traditional cultivar/ Landrace
26	CG 240	EC 861817	Guinea	Traditional cultivar/ Landrace
27	CG 241	EC 861818	Guinea	Traditional cultivar/ Landrace
28	CG 242	EC 861819	Guinea	Traditional cultivar/ Landrace
29	CG 243	EC 861820	Guinea	Traditional cultivar/ Landrace
30	IR 64	-	Philippines	Variety
31	BPT 5204	-	India	Variety
32	Chittimutyalu	-	India	Traditional variety/ Landrace

glaberrima is known to possess ample genetic diversity for essential micronutrients like Fe and Zn apart from its well-known resistance to biotic and abiotic stress (Kennedy and Burlingame, 2003; Oko and Ugwu, 2011; Oko *et al.,* 2012; Amoatey *et al.,* 2015; Lakshmi *et al.,* 2019). With this view, the present investigation was aimed to identify superior accessions of *Oryza glaberrima* to develop biofortified varieties for combating micronutrient malnutrition.

Materials and Methods

Plant material

The experimental material comprised of 29 accessions of *Oryza glaberrima* obtained from IRRI along with three *O. sativa* checks (IR64, BPT5204 and Chittimutyalu) **(Table 1).** The accessions and checks were grown for three seasons at ICAR-Indian Institute of Rice Research, Hyderabad during *rabi* 2020-21, *kharif 2021 and rabi* 2021-22.

Grain Fe and Zn estimation

For the estimation of grain Fe and Zn in brown rice, the seed samples were dehusked and 5 g of each sample in two replications was analyzed using energy dispersive X-ray fluorescent spectrophotometer (ED-XRF) as per the standardized protocols (Rao *et al.,* 2014). The high Fe and Zn rich genotypes identified along with check varieties were re-evaluated in *kharif* 2021 and *rabi* 2021-22 for the estimation of grain Fe and Zn contents.

Results and Discussion

The popular high yielding rice varieties are usually deficient in grain Fe and Zn trace elements (Anuradha *et al.,* 2012; Pradhan *et al.,* 2020). Enhancing the Fe and Zn contents in rice grain through the utilization of elite promising lines with enormous genetic potential is highly recommended in biofortification programmes (Chandel *et al.,* 2010). Few land races and wild species of rice such as *O. nivara*, *O. glaberrima*, *O. rufipogon*, *O. latifolia*, *O. officinalis*, and *O. granulata* retain high amounts of Fe and Zn, 2-3 folds higher than the cultivated rice that make them to be good sources for biofortification (Garcia-Oliveira *et al.,* 2008, Sarla *et al.,* 2012, Roy and Sharma 2014, Swamy *et al.,* 2016; Mishra *et al.,* 2020). In the present investigation, grain Fe content showed a good amount of variation in the *O. glaberrima* accessions ranging from 6.40 ppm (CG 236) to 12.10 ppm (CG 227) with a mean of 8.57 ppm **(Table 2, Figure 1)**. Brown rice accessions were categorized as low with <12 ppm Fe content, moderate with 12.1-15 ppm Fe and >15.1 ppm as considered high as suggested by. Accordingly, all the accessions except CG 227 were designated as low Fe bearers including checks. Fifteen accessions recorded higher Fe content compared to the checks *viz*., IR64 (7.60 ppm), BPT5204 (8.60 ppm) and Chittimutyalu (8.40 ppm). Of these fifteen accessions, CG 227 (12.10 ppm) followed by CG 239 (11 ppm) recorded the highest Fe content.

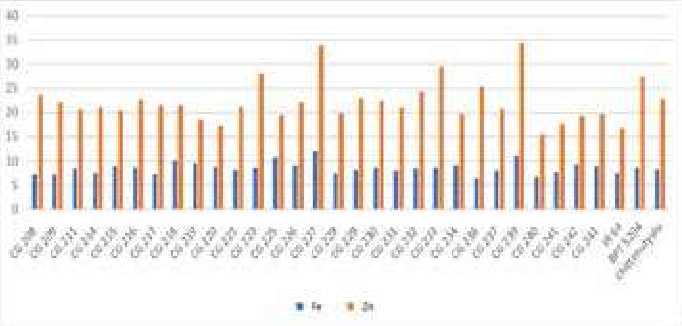

Figure 1: Histogram depicting the iron and zinc contents in rice accessions

Likewise, grain Zn content showed a good amount of variation ranging from 7.30 ppm (CG 220) to 34.40 ppm (CG 239) in brown rice **(Table 2, Figure 1).** Genotypes were classified as low (<20 ppm), moderate (20.1-29 ppm) and high (>29 ppm) Zn containing accessions as suggested by Maganti *et al.,* (2019). Of the 29 accessions along with checks, 11 accessions were categorized as low, 19 as moderate, while three (CG 227, CG 239, CG 233) were high Zn containing accessions. Compared to the check varieties IR64 (16.70 ppm), BPT5204 (27.40 ppm) and Chittimutyalu (22.80 ppm), *O. glaberrima* accessions namely, CG 223 (28.1 ppm), CG 227 (33.9 ppm), CG 233 (29.5 ppm) and CG239 (34.40 ppm) recorded higher zinc content. Of these, two accessions namely, CG 239 (34.40 ppm) and CG 227 (33.9 ppm) recorded the highest Zn content, while CG 240 (15.50 ppm) recorded the lowest. Interestingly, both the accessions CG 227 and CG 239 revealed a good amount of grain Fe content addition to high Zn content. Altogether, the accessions exhibited two-fold variations in Fe and Zn concentrations suggesting the genetic potential to enhance these micronutrients **(Figure 2)**. Similar research for Fe and Zn biofortification was carried out by Tripathy (2020), who reported manifold variations in grain Fe and Zn content in 92 genotypes of brown rice local land races, improved breeding lines and released varieties. Correspondingly, studies on biofortification related to wild species and landraces of rice were taken up by Brar *et al.,* (2011); Anuradha *et al*., (2012); Maganti *et al.,* (2019) who reported the immense potential of landraces and wild rice accessions in the biofortification of popular varieties through conventional and non-transgenic methods.

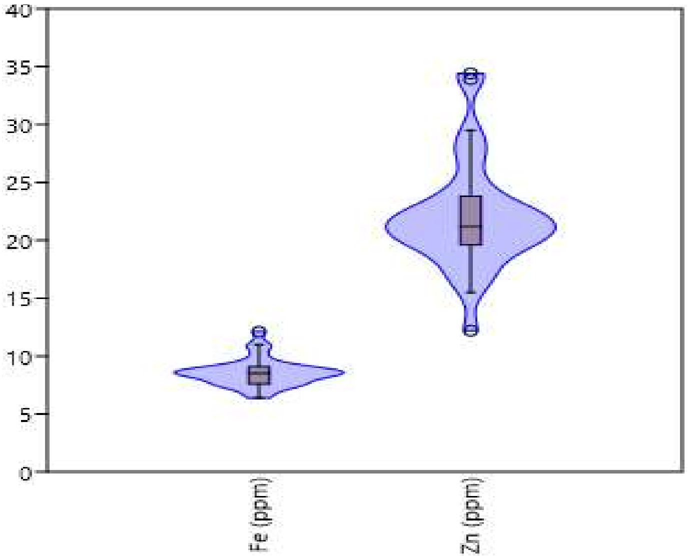

Figure 2: Box plot comparing the amount of variation in grain Fe and Zn contents

Table 2: Performance of *O. glaberrima* accessions and checks (*O. sativa*) for grain iron and zinc content during *rabi* 2020-21

S.No.	Accession	Fe (ppm)	Zn (ppm)
1	CG 208	7.20	23.80
2	CG 209	7.30	22.10
3	CG 211	8.50	20.70
4	CG 214	7.60	21.10
5	CG 215	8.90	20.50
6	CG 216	8.60	22.70
7	CG 217	7.40	21.50
8	CG 218	10.10	21.50
9	CG 219	9.50	18.60
10	CG 220	8.80	17.30
11	CG 221	8.20	21.20
12	CG 223	8.60	28.10
13	CG 225	10.80	19.60
14	CG 226	9.10	22.10
15	CG 227	12.10	33.90
16	CG 228	7.60	19.80
17	CG 229	8.20	23.00
18	CG 230	8.70	22.40
19	CG 231	8.00	21.10
20	CG 232	8.50	24.30
21	CG 233	8.60	29.50
22	CG 234	9.10	19.70
23	CG 236	6.40	25.50
24	CG 237	8.10	20.90
25	CG 239	11.00	34.40
26	CG 240	6.70	15.50
27	CG 241	7.80	17.70
28	CG 242	9.30	19.30
29	CG 243	8.90	19.70
30	IR 64	7.60	16.70
31	BPT 5204	8.60	27.40
32	Chittimutyalu	8.40	22.80

In this context, the identified accessions (CG 227 and CG 239) with high grain Zn and Fe content along with checks were again subjected to micronutrient analysis for two seasons. In the first season, CG 227 recorded an Fe content of 9.7 ppm and Zn content of 29.8 ppm, while CG 239 posed to be a dense micronutrient accession with 10.0 ppm and 34.1 ppm of Fe and Zn contents, respectively **(Table 3).** Both the accessions performed better than the check varieties *viz*., IR64 with 6.9 ppm Fe and 17.1 ppm Zn; BPT5204 with 8.9 ppm Fe, 25.6 ppm Zn and Chittimutyalu with an Fe content of 7 ppm and Zn content of 22.2 ppm. Likewise, the results were similar in second season with CG 227 (Fe-6.9 ppm and Zn-18.7 ppm) exhibiting comparatively lower Zn content and CG 239 (Fe-10.8 ppm and Zn-34.7 ppm) out-performing the check varieties by recording high grain Fe and Zn contents. The results for Fe and Zn estimation were quite consistent after all the seasons of evaluation for the two accessions. In this regard, the high zinc donor (CG 239) unearthed in the current study makes it a prospective donor for biofortification in marker-assisted breeding program.

Table 3: Mean performance of the identified promising accessions with checks evaluated in all the seasons

	***Rabi* 2020-21**		***Kharif* 2021**		***Rabi* 2021-22**	
Accession	**Fe (ppm)**	**Zn (ppm)**	**Fe (ppm)**	**Zn (ppm)**	**Fe (ppm)**	**Zn (ppm)**
CG 227	12.10	33.90	9.7	29.8	6.9	18.7
CG 239	11.00	34.40	10.0	34.1	10.8	34.7
IR-64	7.60	16.70	6.9	17.1	6.8	17.5
BPT 5204	8.60	27.40	8.9	25.6	8.3	25.3
Chittimutyalu	8.40	22.80	7.0	22.2	6.4	21.1

Conclusion

African rice is a rich source of natural allelic variations for grain micronutrient traits. In order to combat micronutrient deficiencies, biofortification of rice varieties with higher densities of trace elements is addressed to be effective. Our present investigation was useful in the identification of one *O. glaberrima* accession *viz.,* CG 239 with high Zn concentration that could be utilized as a potential donor in crop improvement programmes targeting biofortification.

Acknowledgments

The authors greatly acknowledge the support provided by the ICAR-Indian Institute of Rice Research, Hyderabad for providing the resources for conducting the experiment.

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