Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 23, No. (1) : 2022 : 153-157
© Global Science Publications
ISSN-0972-3005

http://doi.org/10.53550/AJMBES.2022.v24i01.025

GENETIC DIVERSITY ANALYSIS THROUGH SSR MARKERS FOR GRAIN FE AND ZN CONTENT IN AEROBIC RICE

RAKESH KUMAR PRAJAPAT[1], SHASHIDHAR H.E.[2] AND VIJAYA KUMAR SWAMY[2]

[1]School of Agriculture, Suresh Gyan Vihar University, Jaipur 302 017, Rajasthan, India
[2]University of Agricultural Sciences, Bangalore 560 065, Karnataka, India

(Received 10 December, 2021; Accepted 2 January, 2022)

***Key words**: Rice, Biofortification, Nutritional security, SSR marker*

Abstract–Rice is important for sustainable food and nutritional security; however, nominal micronutrient quantities in grains aggravate malnutrition in rice-eating poor populations. In this study, we evaluated genetic diversity in grain iron (Fe) and zinc (Zn) contents using trait-linked simple sequence repeat (SSR) markers in the representative subset of a large collection of local and exotic rice germplasm. The objective of the study was to evaluate genetic variability parameter and discern correlation for Iron (Fe) and Zinc (Zn) content in grains of different RPHP rice accessions grown in *Kharif*-2012 under aerobic condition. Single molecular marker analysis using 19 SSR markers showed that RM1089 and RM144 were associated with grain Fe and Zn content respectively. The genetic information generated and molecular markers identified from this study may be used for future biofortification programme of rice.

INTRODUCTION

Rice is one of the most consumed food crops globally, especially in developing countries. The world population is increasing at an exponential rate; however, current annual genetic gain in rice production is insufficient to meet future food requirements, demanding continuous efforts to breed high yielding and more nutritious rice cultivars. Thus, there is an urgent need to boost global rice production, along with nutritional quality, for sustainable food and nutritional security (Tripathy *et al*., 2017).

Plant breeding based biofortification is the cheaper and sustainable approach to improve grain micronutrient contents and eradicates malnutrition from rice-eating poor populations. Enormous genetic potential of rice germplasms for grain Fe and Zn contents could be exploited through marker-assisted biofortification breeding for development of micronutrient dense rice cultivars (Buise *et al*., 2010).

Zn is a vital micronutrient for all organisms, which plays important role in many reactions of the cellular metabolism and biological processes like carbohydrate metabolism, antioxidative defense, auxin metabolism, protein synthesis, and stability of genetic materials (Broadley *et al*.,2007). To address these issues, Fe and Zn food fortification and supplementation approaches are beneficial, but these practices are not easy to implement in developing countries due to its higher cost (Howarth and Ross, 2010; Pfeiffer and McClafferty, 2007).

Among various molecular markers, simple sequence repeat (SSR) or microsatellite markers are most widely and preferentially employed for genetic diversity studies. SSRs are cost-effective, easy to score, rapid, reliable and require minimal quantities of DNA [24]. They can efficiently establish genetic relationships due to their extensive distribution across genome, relative polymorphic abundance and codominant nature (Mondini *et al*., 2009). Although, recent studies have evaluated genetic diversity in diverse rice germplasm using several random and trait linked SSR markers (Singh *et al*., 2019); however, none of these have used grain Fe and Zn contents linked SSR markers. The present study was conducted to assess genetic diversity in the representative subset of a large local and exotic rice germplasm collection using grain Fe and Zn contents linked SSR markers. Furthermore, possible population structure, genotypic relationships and marker-trait associations were also investigated, which could facilitate the conservation and utilization of studied germplasm resources.

Thus considering the importance of single marker analysis approach, the present study has been applied to 45 RPHP rice accessions with 19 SSR markers to identify tightly linked marker associated with Fe and Zn content in rice grains.

MATERIALS AND METHODS

Experimental Design

The experiment was carried out during *Kharif*- 2012 at aerobic rice research laboratory of department of Plant Biotechnology, UAS, GKVK, Bangalore. The study included samples collected from 10 locations over India by Directorate of Rice Research (DRR), Hyderabad, for high Fe and Zn Content, under the project name Rice Project Harvest Plus (RPHP). The experiment was laid out in augmented experimental designs which consist of 45 rice genotypes and two check varieties (Azzucena and Kalinga-3) **(Table 1)**. The data were collected and recorded from five plants at random from middle rows for Plant height at maturity, Number of tillers per plant, Culm Biomass per plant (g), Grain yield per plant (g) and Grain Fe and Zn content (PPM).

Estimation of Fe and Zn contents

Aerobic rice samples were prepared for all accessions using paddy dehusker (Satake, Japan). Fe & Zn contents were estimated by wet-digestion method (Estefan *et al*. 2013). For this, one gram of sample was chemically digested with 10 ml of nitric acid and perchloric acid mixture (2:1). The mixture was heated in a cold-digestion block until turned colourless or white precipitation occurred. Afterwards, double-distilled deionized water was added for dissolving the crystals and diluting the extract to 50 ml. Finally, extract was filtered through Whatman No. 41/42 filter paper before feeding to atomic absorption spectrophotometer (200 Series AA, Agilent Technologies, USA) for quantification of mineral contents. The analysis was repeated thrice for each accession during two cropping seasons separately and mean values were expressed in parts per million (ppm = µg/g). Pearson correlation between grain Fe and Zn contents was computed using Statistix 8.1 and scatter plot was drawn by setting an intercept value of '0' with Microsoft Excel 2016.

DNA isolation and SSR based genotyping

Genomic DNA was isolated by following modified cetyl tri-methyl ammonium bromide method (Doyle, 1987) from leaf tissues collected during cropping season 2018. DNA quantity and quality were assessed using Nanodrop Spectrophotometer (ND 2000, Thermo Scientific, USA) and 0.8% agarose gel electrophoresis with 1X Tris-borate-EDTA buffer and ethidium bromide staining, respectively. Twenty-four SSR marker primer pairs were selected for genotyping based on their inherent association with grain Fe and Zn contents (Raza *et al*., 2019). The detailed information of all SSR markers is given in S2 Table in S1 File. Series of polymerase chain reactions and 8% polyacrylamide gel electrophoresis were performed to detect SSR fingerprints by following our previously published protocol (Sabar *et al*., 2019). Molecular marker size of different alleles was determined using a 20 bp DNA ladder (Fermentas, USA). The amplified product was resolved electrophoretically on a 2.5% agarose gel for 2-3 hrs, visualized under UV trans-illuminator and documented. Single marker analysis (SMA) was performed with SPSS16.0 (SPSS Inc.).

RESULTS AND DISCUSSION

Fe and Zn content in grains

RPHP accessions were cultivated under aerobic condition, which is a novel approach developed by crossing of lowland rice varieties with upland varieties to grow in rainfed, under non-flooded conditions (Bouman *et al*., 2007; Shashidhar, 2007). Atomic absorption spectrometry showed wide variation in grain Fe and Zn content among RPHP rice accessions. The Fe content in grains varies from 14.8 PPM (RPHP156) to 36.1 PPM (RPHP33) with an average value 25.71 PPM. Whereas, the Zn content varies from 27.45 PPM (RPHP53) to 43.90 PPM (RPHP16) with an average value 35.80 PPM under aerobic condition. The range of variation for grain yield from of 0.2g (RPHP42) to 16.40g (RPHP36) with an average yield of 5.1g per plant.

The parental lines used by Anuradha *et al*. (2012) had grain Zn concentration of 53.7 ppm (Madhukar) and 27.2 ppm (Swarna) and the RIL population generated from this cross showed Fe concentration in the range of 0.2 to 224 ppm and Zn in the range of 0.4 to 104 ppm. The study conducted by Bekele *et al*. (2013) on the estimation of genetic variability and correlation studies for grain Zn concentration in 64

Table 1. Iron and Zinc content of selected 47 RPHP rice accession under aerobic condition

Sl. No.	DRR code	Variety name / Cross	Iron content in rice grains (ppm)	Zinc content in rice grains (ppm)
1	RPHP-7	AM-72	29.3	42.4
2	RPHP-9	BJ-23	33.4	41.15
3	RPHP-10	AM-27	29.8	40.95
4	RPHP-11	AM-143	24.3	43.75
5	RPHP-16	AM-65	33.8	43.9
6	RPHP-21	AM-94B	27.2	42.35
7	RPHP-27	AZUCENA	32	40.15
8	RPHP-29	BJ-5	32.3	36.05
9	RPHP-33	BJ-21	36.1	41.1
10	RPHP-36	TKM-9	21.5	37.25
11	RPHP-37	Mainpuri	31	32.35
12	RPHP-42	Shalimar Rice -1	25.7	30.9
13	RPHP-44	BR- 2655	19.3	28.15
14	RPHP-45	Panvel -3	24.1	37.95
15	RPHP-47	Pathara	22.5	31.15
16	RPHP-48	Bindli	28.2	35.6
17	RPHP-51	Vandana	25.4	42
18	RPHP-52	Sebati	26	39.95
19	RPHP-53	PB-164	24.7	27.45
20	RPHP-55	Kalinga -3	28.2	36.75
21	RPHP-56	IRRI-44	27.8	29.3
22	RPHP-68	Subhdra	20.9	34.05
23	RPHP-80	24(K)	29.6	41.55
24	RPHP-87	140(M)	25.5	31.3
25	RPHP-90	182(M)	25.1	36.85
26	RPHP-91	185(M)	30	36.95
27	RPHP-92	196(M)	26.3	34.95
28	RPHP-93	Type-3	32.4	34.55
29	RPHP-102	Kanchana	16.1	41
30	RPHP-104	Kasturi	24.4	33.5
31	RPHP-105	Moirangphou	24.3	28.85
32	RPHP-106	Akutphou	23.8	34.65
33	RPHP-107	Improved ChittiMutyalu	23.5	27.7
34	RPHP-108	High Iron Rice	28.9	31.25
35	RPHP-125	NDR 2026	20.1	40.5
36	RPHP-129	Kamad	28.1	29.4
37	RPHP-130	China 1007	25.7	35
38	RPHP-134	NJA VORA	17.3	35.3
39	RPHP-135	KADAMAKUDY POKKALI	17.5	32.3
40	RPHP-138	EDAVANKUDI POKKALI	22.2	41.6
41	RPHP-140	VYTILLA ANAKONDAN	19.3	34.45
42	RPHP-156(ABCDE)	BPT 5204 /ChittiMutyalu-SB	14.8	34.1
43	RPHP-157 (ABCDE)	BPT 5204 /ChittiMutyalu-MS	28	42.05
44	RPHP- 161	ChampaKhushi	31.5	31.8
45	RPHP-163	Seeta sail	24.7	38.45
46	RPHP -165	Tilakkachari	34.3	34.85
47	RPHP-166	NC 365	20.3	31.75

rice genotypes revealed the range of variation for grain Zn concentration as 18.90 ppm to 36.90 ppm with an average value of 26.74 ppm. Wide variation was observed in Fe concentration among the population than Zn at both locations and similar results were obtained earlier (Anuradha *et al*., 2012; Pandian *et al*., 2011). There could be several reasons for these variations that may include effect of environment, genotype and environment interactions, soil properties like pH, organic matter

Table 2. SMA of polymorphic SSR marker used in RPHP for iron and zinc content in rice grains in *Kharif*-2012

Sr. No.	Marker	Chr. No.	Trait	P value	$R^{2\,(\%)}$
1	RM513	1	Iron	0.068	0.072
			Zinc	0.391	0.016
2	RM148	3	Iron	0.304	0.053
			Zinc	0.409	0.040
3	RM131	4	Iron	0.097	0.101
			Zinc	0.403	0.043
4	RM1089	5	Iron	0.026*	0.153
			Zinc	0.243	0.063
5	RM204	6	Iron	0.860	0.007
			Zinc	0.897	0.005
6	RM264	8	Iron	0.532	0.028
			Zinc	0.941	0.003
7	RM144	11	Iron	0.953	0.002
			Zinc	0.004*	0.220
8	RM167	11	Iron	0.800	0.010
			Zinc	0.379	0.044
9	RM3331	12	Iron	0.435	0.037
			Zinc	0.130	0.089

* Significant at 5%

content, Fe and Zn levels in the soil etc. (Pandian *et al*., 2011; Chandel *et al*.,2010).

Molecular analysis of rice accession with SSR markers

Fe and Zn accumulation in rice grains is a complex trait which is governed by number of QTLs located on different chromosomes (Lu *et al*., 2008; Garcia-Olivera *et al*., 2009; Zhang *et al*., 2011). Out of 19 SSR markers, 9 SSR markers were found polymorphic for the RPHP lines (Table 2). The segregation pattern of all RPHP rice accession with a represented primer RM144 is depicted in Fig. 1. The range of phenotypic variance (R^2 %) for these 9 SSR markers was 0.2 to 22.0%. Among these 9 markers, RM1089 and RM144 werefound to be associated with Fe and Zn content in grains respectively. RM1089 which is associated with Fe content,is located on chromosome 5, with phenotyping variation of 15.3%. In earlier reports, Poli *et al*. (2013) reported that RM1089 was associated with number of tillers and yield/plant. Cho *et al*. (2012)identified RM1089 flanked a QTL for culm length and days to flowering. RM144 is located on chromosome 11, exhibits association with Zn content by showing 22% of phenotyping variation under aerobic condition. RM144 was previously reported to be associated with plant height and yield per plant (Brar *et al*., 2015). Thus, these markers can be used for marker-assisted selection or genomic assisted breeding for Fe and Zn biofortification programme for future scrutiny.

CONCLUSION

In the present study, 45 RPHP lines obtained from different location of India were evaluated for grain Fe, Zn content and other grain yield related traits, grown during *Kharif* 2012. Assessment of the relationship between grain Fe and Zn content and grain yield per plant in 45 rice accession using linear regression showed a linear relationship between Fe and Zn content with increase in grain yield. Using single-marker analysis in RPHP rice varieties, it was found that one of the SSR marker (RM144) showed significant association with grain Zn content while another SSR marker (RM1086) showed significant association with grain Fe content.

ACKNOWLEDGEMENT

The research was carried out in University of Agricultural Sciences, Bangalore, India. We are highly grateful to Department of Biotechnology, India for providing the financial assistance to carry out the research work.

Author contribution

Rakesh Kr. Prajapat had designed and executed the experimental procedures and recorded the biometric parameters. Shashidhar, H.E and Vijaya Kumar Swamy were the Principal Investigator and Co- Principal Investigator respectively, who helped in monitoring the work and conducting the experiments smoothly.

REFERENCES

Anuradha, K., Surekha, A., Venkateswara, R. Y., 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 x swarna RILs. *Gene*. 508 : 233-240.

Bekele, B. D., Rakhi, S., Naveen, G. K., Kundur, P. J. and Shashidhar, H. E. 2013. Estimation of genetic variability and correlation studies for grain zinc concentrations and yield related traits in selected rice (*Oryza sativa* L.) genotypes. *Asian j. Exp. Biol. Sci*. 4: 345-351.

Bouis, H. E. and Welch, R. M. 2013. Biofortification—a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Science*. 50 20-32.

Bouman, B. A. M., Lampayan, R. M. and Tuong, T. P. 2007. Water management in irrigated rice: coping with water scarcity. *Int. Rice res. Inst*., Los Banos, Philippines, isbn: 9789712202193, pages: 54.

Brar, B., Jain, R. K. and Jain, S. 2015. Correlation of molecular marker allele size with physio-morphological and micronutrient (Zn, Fe) traits among rice genotypes. *Int. J. Curr. Sci*. 15: e 42-50

Broadley, M., White, P. J., Hammond, J., I. Zelko, Lux, A. 2007. Zinc in plants. *New phytol*., 173: 677-702..

Chandel, G., Banerjee, S., See, S., Meena, R., Sharma, D. J. and B. Verulkar, S. 2010. Effects of different nitrogen fertilizer levels and native soil properties on rice grain Fe, Zn and protein contents. *Rice Sci*. 17 : 213-227.

Cho, J. H., Upreti, H. K., Song, Y. C., Ha, W. G., Khatiwada S. P., Lee, J. H., Lee, J. Y., Yeo, U. S. and Kang, H.W. 2012. QTLs analysis of agronomic traits based on cultivation of low and high altitude locations in rice (*oryzasativa* L.). *Kor J Breed Sci*. 44(2) : 110–120.

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bulletin*. 19 : 11–5.

Estefan, G., Sommer, R. and Ryan, J. 2013. Methods of soil, plant, and water analysis. A manual for the West Asia and North Africa region. 3-9.

Mondini, L., Noorani, A. and Pagnotta, M. A. 2009. Assessing plant genetic diversity by molecular tools. *Diversity* 1(1) : 19–35.

Pfeiffer, W. H. and McClafferty, B. 2007. Harvestplus: breeding crops for better nutrition. *Crop Science*. 47 : s88-s105.

Poli, Y., Basava, R. K., Panigrahy, M., Vinukonda, V. P., Dokula, N. R., Voleti, S. R. and Neelamraju, S. 2013. Characterization of a nagina22 rice mutant for heat tolerance and mapping of yield traits. *Rice*. 6 : 36.

Raza, Q., Riaz, A., Sabar, M., Atif, R. M. and Bashir, K. 2019. Meta-analysis of grain iron and zinc associated QTLs identified hotspot chromosomal regions and positional candidate genes for breeding biofortified rice. *Plant Science*. 288 : 110214.

Sabar, M., Akhter, M., Bibi, T., Riaz, A., Haider, Z. and Khan, A. R. 2019. Basmati rice lines development carrying multiple bacterial blight resistance genes pyramided using the marker-assisted backcross breeding approach. *Molecular Breeding*. 39(11) : 155.

Shashidhar, H. E. 2007, Aerobic rice: an efficient water management strategy for rice production. *Food and Water Security in Developing Countries.* Chapter 12. Pp. 131–139.

Singh, S. P. and Singh, Y. T. 2019. Rice of Northeast India harbor rich genetic diversity as measured by SSR markers and Zn/Fe content. *BMC Genetics*. 20(1): 79.

Tripathy, S., Dash, M. and Behera, S. 2017. Nutrient rich quality rice-a journey to healthy life. *Adv Plants Agric Res*. 7(5) : 364–7.