

Genetic evaluation of advanced breeding lines of rice (*Oryza sativa* L.) for grain yield and nutrient characters in upland condition

Abstract

The present study was carried out at Agricultural Research Station, Mugad, Karnataka during *kharif* 2023 using augmented block design in upland condition using sixty advanced breeding lines of rice (*Oryza sativa* L.) and six checks *viz.*, IR64, BPT 5204, DRR Dhan 45, Kagisali and Chittimutyalu to evaluate genetic variability for yield component traits and grain quality traits, to evaluate the association of yield with quality traits and to validate the genotypes for quality traits using SSR markers. Analysis of variance revealed the presence of significant differences among the genotypes for all the traits under study. Genotypic and phenotypic coefficients of variances were high for number of productive tillers per hill and grain yield depicting the wide range of variation for these traits. High heritability allied with high genetic advance over mean was seen for the traits like plant height, number of tillers per hill, number of productive tillers per hill, panicles per sqm, grain yield, grain iron content, zinc content and protein content indicating the preponderance of additive gene action and least influence of environment on these traits, thus, they possess better scope for improvement through selection. The correlation analysis revealed the negative significant association of grain yield with grain zinc content and negative non-significant association with all other quality traits. Validation of the putative markers is generally carried out to check the reproducibility of those markers. SSR markers RM211 and RM242 were validated for grain iron content, RM303 and RM234 for grain zinc content and RM23914, RM1235, RM219, RM206 and RM1337 for grain protein content. Therefore, these markers can be further used for effective biofortification of rice.

Keywords: Advanced breeding lines, upland, coefficients of variances, validation, SSR markers, biofortification

Introduction

Rice (*Oryza sativa* L.) is accloded as the Global Grain for a reason that it is one of the most staple food grains of the world, as it helps to sustain one-third of world's population. Globally, rice is being cultivated in an area of around 167.2 million ha, producing around 769.6 million tonnes per year with a productivity of 4600 kg/ha. In India, around 43.77 m hectares of area is under rice cultivation, with the production of 117.47 million tonnes and productivity of 2570 kg/ha (Anon, 2021). For the cultivation of rice, average annual rainfall required is around 1500 mm. On an average, 2500 litre of water is required for the production 1 kg of rough rice (Bouman, 2009). But many experts are stating that we would face a major shortage of water in the coming future. In Asia, 17 million hectares of irrigated rice might experience "physical water scarcity" by 2025, whereas 22 million hectares could face "economic water scarcity" (Tuong and Bouman, 2003). Thus, efficient water utilisation in rice farming is the need of the hour for achieving long-term rice production. Upland rice is one such strategy to reduce water requirements in rice production. The direct seeded rice (DSR) is gaining favours of the researchers as well as farmers due to its low input demand, rapidity and easiness in planting, shorter growing season of 7-10 days fitting well into the different intensive cropping system, lower methane emission and reduction of labour share on the total cost of cultivation. Upland rice is normally grown in rainfed, naturally well-drained soils in sloppy, undulated or terraced land, without surface water accumulation.

Rice provides 75 % of the calories and 55 % of the protein in the average daily diet of the people although it has an incomplete amino acid profile and comprises confined number of essential micronutrients. Across the globe more than two billion people suffer from micronutrient deficiency due to lack of vital vitamins and minerals in their diet (Moreno *et al.* 2016). The iron and zinc malnutrition are the most common types of malnutrition especially in the countries with substantial dietary reliance on staple food crops (Sands *et al.* 2009). Around two billion people are suffered by anaemia due to iron deficiency, The World Health Organization 2013 stated. Zinc deficiency is the fifth major cause of diseases and deaths in developing countries (Ravindra babu, 2013). Thus, other than yield and yield related traits, attention has also been given to increasing Zn and Fe and also protein content in rice grain which includes a selection of genotypes with high content of micronutrients and protein, breeding micronutrient and protein efficient crop that produces high yields and accumulation of more micronutrients from soil and increasing bio-available Zn and Fe (Ullah *et al.* 2023).

Genetic variation is the most crucial pre-requisite for the successful designing and deployment of breeding programme. And it also provides a great array of genotypes that could be selected to develop new varieties or breeding materials. Knowledge regarding the heritability helps breeders to know the nature and magnitude of variation and helps in making appropriate selection. Amidst all this yield being a complex character is influenced by several other component traits. If the nature and degree of association between yield and yield related traits could be deduced, it will aid in improving yield through indirect selection of its component traits which are highly correlated. And also the idea of association between yield and quality traits would assist in deciding whether or not simultaneous improvement of yield and quality traits could be performed in single breeding programme. With this regard, correlation study has been proven to be quintessential. Breeding rice crop for improved nutritional quality is a major requirement and has got wider scope. This process could be fastened with the use of molecular markers for the selection of genotypes with higher nutritional content in the seedling stage itself. This process demands the identification and validation of markers which have significant association with the nutritional traits.

Materials and methods

Plant materials and experimentation

The experiment was conducted at Agricultural Research Station, Mugad, Dharwad, Karnataka during *kharif* 2022.

The study encompassed sixty advanced breeding lines of rice along with five checks namingly, BPT5204, IR64, DRR Dhan 45, Chittimutyalu and Kagisali which were evaluated under upland condition in an augmented block design with four blocks, where each block contained 15 genotypes and checks were repeated in every block. The seeds were sown directly in the main field following a spacing of 20 cm X 10 cm.

Phenotypic observations

The advanced breeding lines were evaluated to study genetic variability for yield component traits and grain quality traits and association of yield with quality traits. Five plants from each genotype were picked at random to record the data on yield related traits *viz.*, plant height (PH), panicle length (PL), number of tillers per hill (TPH), number of productive tillers per hill (PTH), number of panicles per sqm (PSM), spikelet fertility percentage (SF) and grain yield (GY) and grain quality traits *viz.*, grain length (GL), grain breadth (GB) and grain L/B ratio (L/B).

Estimation of grain iron and zinc content

The rice grains from each genotype were dried to a moisture content of 13% and dehusked using a palm dehusker. One gram of dehusked rice sample from each genotype was used to estimate iron and zinc content through destructive method using atomic absorption spectroscopy (AAS) following a standard protocol given by Yankanchi *et al.* (2022).

Estimation of grain protein content

For the estimation of grain protein content, firstly, percentage nitrogen was measured by standard micro-Kjeldhal method following a standard protocol given by Arunima *et al.* (2022) which included three steps *viz.*, digestion process, distillation process and titration process. Then percentage nitrogen was multiplied with a conversion factor 5.95 to obtain per cent protein content.

Statistical analysis

For statistical analysis, the mean values of five selected plants were used for all the traits under investigation and were analysed using R studio software. Analysis of variation was done to examine the data collected for various traits. The genetic variability parameters such as genotypic and phenotypic coefficients of variances (Burton and Devane 1953), heritability in broad sense and genetic advance over mean (Johnson *et al.* 1955) were calculated. Phenotypic correlation coefficients were calculated in OPSTAT.

Genomic DNA extraction

Genomic DNA was extracted from fresh leaves of all the genotypes by Cetyl Tri Methyl Ammonium Bromide (CTAB) method (Doyle and Doyle 1987). Quantification was done using the nano drop method. DNA samples were amplified in 10 µl volume contained primers 0.5 µl

each, 3 μ l master mix and 5 μ l molecular grade water. The PCR product was loaded in 3 per cent agarose gel of 1x TAE buffer containing ethidium bromide. The gel was run at 90 volts. The gel was photographed using a gel documentation system.

Single marker analysis and validation

Ten trait specific markers out of which two were earlier reported to be linked to grain iron content, three to grain zinc content and five to grain protein content were used to carry out single marker analysis and validate among the ABLs using single marker regression analysis in Microsoft Excel. List of SSR markers used in the validation is presented in Table 1.

Results

Analysis of variance and estimates of genetic variability parameters

The analysis of variance was carried out for seven yield component traits and six grain quality traits and significant variation among the genotypes was seen for all the traits depicting the presence of adequate amount of variation among them. The mean, range and genetic variability parameters estimated for yield component traits and grain quality traits are presented in Table 2 and Table 3 respectively. The genotypic and phenotypic coefficients of variances were shown to be high for number of productive tillers per hill and grain yield indicating the presence of wide range of variation for these traits among the genotypes. Moderate values of GCV and PCV observed for plant height, number of tillers per hill, panicles per sqm and grain quality traits like, L/B ratio, grain iron content, zinc content and protein content suggested the presence of moderate amount of variation among the genotypes. For all the traits under study, PCV values were slightly higher than GCV values indicating the least influence of environmental factors on the expression of these trait. Heritability gives breeders an idea about the portion of variation present in the material which is actually heritable to further generations. However, heritability estimates alone fail to give an account on the amount of progress expected under selection (Yadav *et al.* 2010). Thus, heritability estimates accompanied with genetic advance would be more meaningful and accurate. High heritability coupled with high genetic advance over mean was recorded for yield component traits like plant height, number of tillers per hill, number of productive tillers per hill, panicles per sqm and grain yield and grain quality traits like grain iron content, zinc content and protein content suggesting the action of additive genes on the expression of these traits and enough scope for further improvement of these characters through selection.

Correlation and association of grain yield with grain quality parameters

When the association of grain yield with grain quality traits was assessed, grain yield was showing positive non-significant correlation with grain length (0.203) and L/B ratio (0.188). Wherein, it was showing negative significant association with grain zinc content (-0.280), negative non-significant association with grain breadth (-0.085), grain iron content (-0.113) and grain protein content (-0.127). Estimates of phenotypic correlation coefficients are presented in Table 4.

Correlation and association of yield contributing traits with grain yield

Among the set of genotypes used in this study, grain yield was shown to be having positive significant correlation with spikelet fertility percentage (0.733). Whereas, it showed positive non-significant association with most of the yield contributing traits like panicle length (0.177),

number of tillers per hill (0.115), number of productive tillers per hill (0.048) and panicles per square meter (0.113). And negative non-significant association trend of grain yield was seen only with one of its component traits *i.e.*, plant height (-0.037).

Molecular validation and trait association of ABLs for grain quality traits using SSR markers

Single marker analysis was done to see the association between marker and trait using single marker regression analysis in Microsoft Excel. Amplification of ten SSR markers was carried out in the advanced breeding lines. Genotypes were scored with markers and analysis of bands was carried out to establish a relationship between phenotypic and genotypic data. Genotypic data scoring was based on the presence or absence of band with trait specific marker, where markers were scored as 0 for the absence and 1 for the presence of band in the respective genotypes.

The significant associations between marker and trait were indicated by P-value <0.05 (5 % significance level) and <0.01 (1 % significance level) with corresponding R² value. R² value for each marker gives us an account of the total phenotypic variation for a trait that is accounted by that particular marker. Results of single marker analysis are presented in Table 5.

The analysis revealed that out of two markers used for the validation of ABLs for grain iron content, RM211 showed significant association with highest R² value of 11.36 % followed by RM242 with R² value of 7.55 %. Whereas, RM234 and RM303 showed significant association with grain zinc content with R² value of 8.32 % and 7.45 % respectively. But the other marker RM72 did not show significant association with zinc content. Out of 5 markers used for grain protein content, all of them showed significant association with protein. Among them highest R² value of 17.49 % was shown by RM206 followed by RM23914 (15.63 %), RM1337 (11.73 %), RM219 (11.32 %) and RM1235 (9.92 %).

Discussion

Estimates of phenotypic and genotypic coefficients of variances are of invaluable importance in knowing the amount of variability present in the material. Difference in the values of GCV and PCV accounts for the existence of environmental influence on the character. So, greater the difference between them more the influence of environment and vice-versa. Heritability estimates along with genetic advance provide an account of progress under selection.

High GCV and PCV values were reported by Ajmera *et al.* (2017) for number of productive tillers per hill and Islam *et al.* (2015) for grain yield. High heritability coupled with high genetic advance over mean was recorded for plant height by Uday *et al.* (2016), number of tillers per hill by Srujana *et al.* (2017), number of productive tillers per hill by Ajmera *et al.* (2017), panicles per sqm by Quadri *et al.* (2023) and grain yield by Islam *et al.* (2015) and for grain quality traits like grain iron content and protein content by Akshay *et al.* (2022) and zinc content by Babu *et al.* (2012).

Grain length and grain L/B ratio were found to be correlated with grain yield in a positively non-significant manner, whereas grain breadth had negative non-significant association with grain yield. These results were bolstered up by the findings of Sabesan *et al.* (2009) for grain length and grain breadth, Sarangi *et al.* (2022) for L/B ratio. Yield had its association with grain zinc content in a significantly negative way and with grain iron and protein content in a negatively non-significant way indicating that selection for yield and these

quality traits must be executed separately. The same outcomes were quoted by Namata (2022) for grain zinc content, Bhattacharjee *et al.* (2020) for grain iron content and Srihari *et al.* (2023) for grain protein content.

Sravan *et al.* (2012) reported positive significant association of grain yield with spikelet fertility percentage. Grain yield was seen to be in positive non-significant association with most of its other component traits like panicle length, number of tillers per hill, number of productive tillers per hill and panicles per sqm. Similar results were reported by Dhavaleshvar *et al.* (2019) for panicle length, Bhati *et al.* (2015) for number of tillers per hill and Abdul *et al.* (2011) for number of productive tillers per hill and Talekar *et al.* (2022) for panicles per sqm. Thus, yield can be improved by practicing indirect selection for these component traits. Whereas, plant height had negative non-significant association with grain yield suggesting that selection for dwarf plant type would be beneficial in improving yield. Same result was quoted by Adhikari *et al.* (2018).

Single marker analysis was carried out using trait specific markers to find out their association with grain iron, zinc and protein content. Two markers *viz.*, RM211 and RM242 showed significant association with grain iron content with phenotypic variations of 11.36 % and 7.55 % respectively, two out of three markers *viz.*, RM303 and RM234 showed significant association with grain zinc content with phenotypic variations of 7.45 % and 8.32 % respectively and RM72 didn't show significant association with zinc content. In unison with these, Wattoo *et al.* (2019) reported the association of RM211 with QTL associated to iron content *i.e.*, qFe-3 with phenotypic variance of 26.25 %, Raza *et al.* (2020) quoted significant association of RM234 with grain zinc content with R² value of 21.70 % and Yerva *et al.* (2018) studied parental polymorphism using 647 SSR markers for grain zinc content, where, 108 markers (including RM303 and RM72) revealed polymorphism between parents and concluded that these could be used in the fine mapping of zinc trait.

Whereas, all five markers *viz.*, RM206, RM23914, RM1337, RM219 and RM1235 used for grain protein content showed significant association with phenotypic variations of 17.49 %, 15.63 %, 11.73 %, 11.32 % and 9.92 % respectively. On par with these results, Yun *et al.* (2014) reported the presence of QTL qPro-9 in the marker interval RM219-RM23914 and association of RM1235 with QTL qPro-8, Zhang *et al.* (2019) reported the significant association ($p < 0.01$) of RM1337 with albumin protein and Ashwini *et al.* (2011) reported the significant association of RM206 with protein content with phenotypic variance (R² value) of 8.75 %. Thus, these validated markers can be used for rice grain iron, zinc and protein biofortification programmes with further full characterization.

Conclusion

It is concluded that most of the yield related traits showed high heritability twinned with high genetic advance as a percent of mean, which indicates the fixation of genes and presence of additive gene action in these traits. Thus, selection for these characters would be fruitful. Correlation analysis showed negative significant association of yield with grain zinc content and negative non-significant association with grain iron and protein content. This indicates that yield and quality improvement cannot be done simultaneously and selection must be executed separately. And those markers whose association have been validated could be used in marker assisted selection for biofortification of rice.

Authorship Contribution Statement:

Vilas G M Carried out research, processing and analysis of research data and manuscript preparation, Dr. Shashidhara N involved in finalization and implementation of research programme, technical guidance and preparation of manuscript during research work, Dr. Uday G involved in finalization of research topic, technical guidance and corrected the drafts, Dr. Nethra P involved in finalization of research topic, technical guidance and corrected the drafts. Sainath involved in preparation and finalization of manuscript.

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Table 1. List of SSR markers used along with their forward and reverse sequence, annealing temperature and expected product size.

Sl.no.	Marker	Forward primer	Reverse primer	Ann. temp. (°C)	Expected PCR product size	Reference
Iron (Fe) linked markers:						
1.	RM211	CCGATCTCATCAACCAACTG	CTTCACGAGGATCTCAAAGG	55	161	Chen-X <i>et al.</i> (1997)
2.	RM242	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG	55	225	Chen-X <i>et al.</i> (1997)
Zinc (Zn) linked markers:						
1.	RM303	GCATGGCCAAATATTAAAGG	GGTTGGAAATAGAAGTTCGGT	55	200	Temnykh-S <i>et al.</i> (2000)
2.	RM72	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG	55	166	Temnykh-S <i>et al.</i> (2000)
3.	RM234	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	55	156	Chen-X <i>et al.</i> (1997)
Protein linked markers:						
1.	RM23914	GAGGATCCTTACCATCAAACCTTCG	CCAAGAACCTGCATTCTTCAAGG	55	196	Nature (2005)
2.	RM206	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	55	147	Chen-X <i>et al.</i> (1997)
3.	RM1235	AGCAGAGGAGGAGATGATGG	GGACCAAACGAAGCTATCC	55	118	McCouch-S-R <i>et al.</i> (2002)
4.	RM219	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	55	202	Chen-X <i>et al.</i> (1997)
5.	RM1337	GTGCAATGCTGAGGAGTATC	CTGAGAATCTGGAGTGCTTG	55	210	McCouch-S-R <i>et al.</i> (2002)

Table 2. Estimates of mean, range and genetic variability parameters for yield and its component traits

Characters	Mean	Range		Coefficient of variation		Heritability (bs)	Genetic advance over mean
		Min.	Max.	PCV (%)	GCV (%)		
Plant height (cm)	61.98	42.03	93.75	15.82	14.98	89.66	29.25
Panicle length (cm)	20.73	15.04	25.36	8.25	6.78	67.48	11.48
No. of tillers per hill	14.68	10.34	22.13	16.37	14.97	83.56	28.23
No. of productive tillers per hill	12.59	5.20	21.98	29.63	25.32	73.02	44.63
Panicles per sqm	234.52	153.80	313.80	19.10	17.02	79.39	31.29
Spikelet fertility percentage	87.52	79.98	96.03	4.63	4.21	82.73	7.90
Grain yield (kg/ha)	3118.21	1089.47	5486.98	32.10	29.68	85.53	56.63

Table 3. Estimates of mean, range and genetic variability parameters for grain quality traits

Characters	Mean	Range		Coefficient of variation		Heritability (bs)	Genetic advance over mean
		Min.	Max.	PCV (%)	GCV (%)		
Grain length	8.88	7.05	10.77	7.81	7.62	95.31	15.36
Grain breadth	2.52	2.17	3.05	7.50	6.27	69.92	10.82
L/B ratio	3.54	2.65	4.45	10.84	10.09	86.60	19.37
Grain iron content	13.29	9.33	17.21	15.52	15.31	97.29	31.15
Grain zinc content	15.69	10.72	23.85	17.58	16.89	92.31	33.48
Grain protein content	7.08	5.22	8.40	10.51	10.15	93.26	20.21

Table 4. Estimates of phenotypic correlation coefficients for yield, yield related traits and grain quality traits

	PH	PL	TPH	PTH	PSM	SF	GL	GB	L/B	GFeC	GZnC	GPC	GY
PH	1												
PL	0.344**	1											
TPH	0.236	0.145	1										
PTH	-0.003	-0.127	0.336*	1									
PSM	-0.091	-0.150	0.161	0.084	1								
SF	-0.036	0.217	0.014	-0.042	-0.032	1							
GL	-0.035	-0.092	-0.012	-0.066	-0.147	0.109	1						
GB	0.212	0.187	0.024	-0.088	-0.148	-0.123	-0.049	1					
L/B	-0.132	-0.169	-0.023	-0.007	-0.035	0.165	0.759**	-0.680**	1				
GFeC	-0.051	-0.149	-0.226	0.088	-0.142	-0.190	0.030	0.138	-0.090	1			
GZnC	0.069	0.069	-0.084	-0.021	-0.004	-0.075	0.004	0.272*	-0.161	-0.028	1		
GPC	-0.023	-0.086	0.241	0.133	0.019	-0.193	0.022	-0.171	0.115	-0.030	-0.102	1	
GY	-0.037	0.177	0.115	0.048	0.113	0.733**	0.203	-0.085	0.188	-0.113	-0.280*	-0.127	1

(PH- Plant height, PL- Panicle length, TPH- Tillers per hill, PTH- Productive tillers per hill, SF- Spikelet fertility percentage, GL- Grain length, GB- Grain breadth, L/B- Grain length to breadth ratio, GFeC- Grain iron content, GZnC- Grain zinc content, GPC- Grain protein content and GY- Grain yield)

Table 5. Single marker analysis for SSR markers in advanced breeding lines of rice for grain iron, zinc and protein content.

Marker	Trait	p-Value	R ² (%)	Association
RM211	Fe	0.0084**	11.36	Significant
RM242	Fe	0.033*	7.55	Significant
RM303	Zn	0.034*	7.45	Significant
RM234	Zn	0.025*	8.32	Significant
RM72	Zn	0.207	2.72	Non-significant
RM23914	Protein	0.0017**	15.63	Significant
RM1235	Protein	0.014*	9.92	Significant
RM219	Protein	0.008**	11.32	Significant
RM206	Protein	0.0008**	17.49	Significant
RM1337	Protein	0.0073**	11.73	Significant

* - Significant at 5 % probability level

** - Significant at 1 % probability level