



RESEARCH ARTICLE

Genetic variability, character association and grain quality assessment in segregating rice (*Oryza sativa* L.) lines of TUNGA × KPR1

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Abstract

Micronutrient enrichment and desirable grain quality are crucial targets in rice improvement to address malnutrition and enhance consumer acceptance. The present study was conducted to evaluate the performance of segregating lines of cross, Tunga x KPR 1 in an augmented design during Kharif 2022 - 23 at the Zonal Agricultural and Horticultural Research Station (ZAHRS), Mudigere to investigate genetic variability parameters, correlation, direct and indirect effects and grain quality parameters. The analysis of variance (ANOVA) revealed significant phenotypic and genotypic differences for all the studied characters. High values of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were for the number of filled grains per panicle (NFG; 20.39 and 20.03 % respectively). Moderate PCV and GCV values were recorded for grain yield per plant (GY; 16.39 %, 11.29 %) and spikelets per panicle (SP; 15.28 %, 14.84 %). Most of the characters recorded minimal differences between PCV and GCV. The NFG showed high heritability values (96.59 %) and high values of genetic advance as a percentage of mean (GAM; 40.62 %). Filled grains per panicle, productive tillers per plant, spikelets per panicle, tillers per plant and panicle length (PL) showed a positive and significant association with grain yield. A positive direct effect on grain yield was observed for days to maturity (DM), productive tillers per plant, panicle length and filled grains per panicle. The top 10 superior lines from the cross were analyzed for quality parameters such as zinc (35.65 ppm), iron (13.92 ppm) and amylose content (28.20). All three traits, zinc (0.37), iron (0.31) and amylose (0.35) have a weak positive correlation and they were found to be promising and can be used in crop improvement programmes. Entry 44,41 and 12 have outperformed all other lines in zinc and iron content respectively.

Keywords: correlation; GCV; iron; PCV; zinc

Introduction

Rice is one of the important staple cereal crops belonging to the Poaceae family, with a chromosome number $2n = 24$ (1). Rice provides 20 % of the world's dietary energy and is a source of dietary energy for more than 17 Asian countries and also in countries belonging to region of South and North America as well as Africa (2). In India, rice is cultivated in 42.70 Mha constituting 30 % of the net cultivated area of 142.20 Mha. Out of this, 93 % is being cultivated during Kharif season (3).

Rice plays a significant and crucial role in advancing the UN Sustainable Development Goals (SDGs) in the world, especially SDG 1 (no poverty), SDG 2 (zero hunger) and SDG 3 (good health and well-being), as it serves as a vital source of calories (4). It is primarily consumed as polished white rice which has approximately 80 % starch and 6 - 8 % proteins, while lipids, dietary fibre, minerals and secondary metabolites are present in trace amounts (5).

The demand for rice keeps increasing to feed the increasing population with an estimated demand of about 137.30

million tonnes by 2050. In order to meet this target, there's a need for an increase in production per unit area from 2.20 t/ha to 3.3 t/ha. However, due to demand from urbanization, competition from other crops and industrialization, there may be a decline in rice area by 6 - 7 Mha by 2050 (6). Under such a scenario, the realistic productivity target would be around 3.90 t/ha. Hence, there is a need to increase productivity to feed the increasing population by identifying high-yielding rice genotypes in the segregating population generated from a breeding program between the best desirable parents (7).

Estimation of genetic variability parameters like phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability and genetic advance as a percentage of mean (GAM) from germplasm and new variability created by crossing the best available varieties provide information on selecting superior individual as parents for hybridization and getting good superior segregants (8). The ratio of genotypic variation to total phenotypic variation is called a broad sense of heritability, which is also known as the degree of genetic determination. Estimation of heritability for each character in all

the crosses will guide for opting selection for different traits. It is important to estimate the heritability component for all the traits (9). Genetic advance is commonly predicted as the product of the heritability and selection of differential components. High values of heritability coupled with high values of genetic advance as % of mean indicate additive gene action presence. Whereas high values of heritability coupled with low values of genetic advance as % of the mean show the presence of epistasis effect and dominance effect. Hence, it is important to estimate genetic advance to carry out effective selection (10).

Knowledge of character association study forms the basis for selecting desirable genotypes. Correlation is an important measure to analyze the association between traits and helps to identify the most crucial traits to be used for handling selection, mainly in segregating generation for increasing yield (11). Path coefficient is a statistical measure that partitions the values of correlation coefficients into direct and indirect effects towards the dependent trait, with this the contribution of each trait towards yield (dependent trait) can be measured (12). Both correlation and path analysis measures are used in plant breeding activities to determine the nature of the relationships between yield and yield-related components that are useful to increase the yield.

Moreover, in the present world, high yielding rice variety needs to be combined with micro-nutrient-rich quality with a preferred good texture. Micronutrients are important to reduce the malnutrition of the country and good texture is meant to increase the consumer acceptance (13). Hence, it is important to understand the genetic factors controlling these traits in a breeding programme. Tunga is a well-known rice cultivar in the hill zone of Karnataka, consumed for its superior grain quality and high yield (55 q/ha). However, over time, it has gradually become susceptible to leaf blast and yield decrease. With this background, the current study is being carried out to for further improvement of Tunga rice variety.

Materials and Methods

Experimental material and climatic conditions

The present study was conducted during Kharif 2022-23 at ZAHRS, Mudigere, which comes under the Western Ghats of typical hill zone (Region V and Zone-9) of Karnataka. It lies at 13° 7' North latitude and 74° 37' East longitude and an altitude of 982 m above mean sea level (AMSL). The experimental material of the present study is composed of F_4 lines from a cross between TUNGA \times KPR 1, which were developed, advanced and maintained at ZAHRS, Mudigere, with four checks mainly TUNGA, KHP 13, KPR 1 and KHP 11. The breeding scheme is as shown on Fig. 1. Single seedling per hill was planted with a spacing of 20 cm \times 10 cm in an augmented design. The details of the experiment are provided in supplementary Table S1 and the features of parents and checks are presented in supplementary Table S2.

Observation recorded

Recommended agronomic practices were practiced throughout the crop growth period. Data were recorded from random 10 plants for 12 traits respectively for days to 50 % flowering (DFF), days to maturity (DM), plant height (PH) (cm), number of tillers per plant (NT), number of productive tillers per plant (NPT), panicle length (PL), spikelets per panicle (SP), number of filled grains per

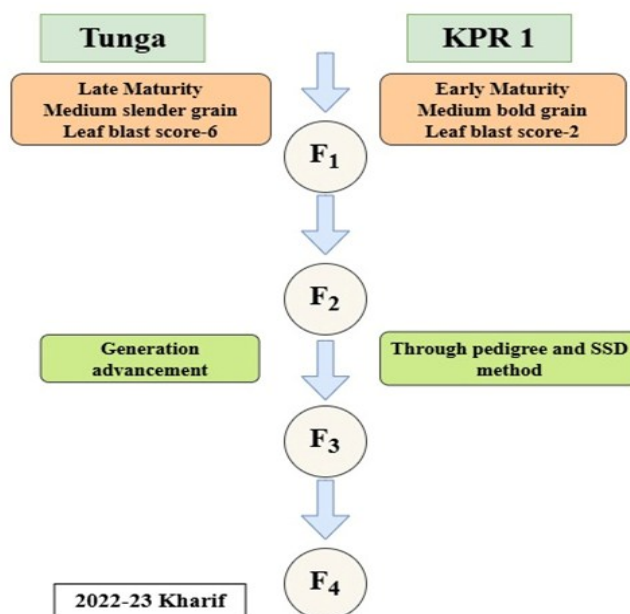


Fig. 1. Flowchart of the breeding procedure of cross Tunga \times KPR 1. panicle (NFG), panicle fertility (PF) (%), test weight (TW) (g), length and breadth ratio (LB) and grain yield per plant (GY) (g).

Grain quality parameters

The top-performing lines are subjected to estimation of rice quality parameters, mainly zinc, iron and amylose content. The zinc and iron content were estimated using the atomic absorption spectrophotometer (AAS) method by feeding the prepared mineral solution to the AAS, having appropriate hollow cathode lamps, after getting values for standard solutions and the concentrations of zinc and iron were expressed in ppm (14). Amylose content was estimated using the iodometric method. For amylose determination, 20 mg of rice flour was cooked at 100 °C for 15 min after treatment with 0.5 mL of ethanol and 4.5 mL of sodium hydroxide (NaOH). The reaction was then carried out by adding 100 μ L of glacial acetic acid and 200 μ L of potassium iodide, followed by incubation at room temperature for 20 min. The readings were taken at 620 nm using multimode reader (15).

Statistical analysis

The mean data for each trait was subjected to a normality test and statistical analysis was done using the RStudio software. The analysis of variance (ANOVA) test and variability parameters were analyzed by the Augmented randomized complete block design (RCBD) package. Correlation and path analysis were done using the metan and corplot packages.

Results and Discussion

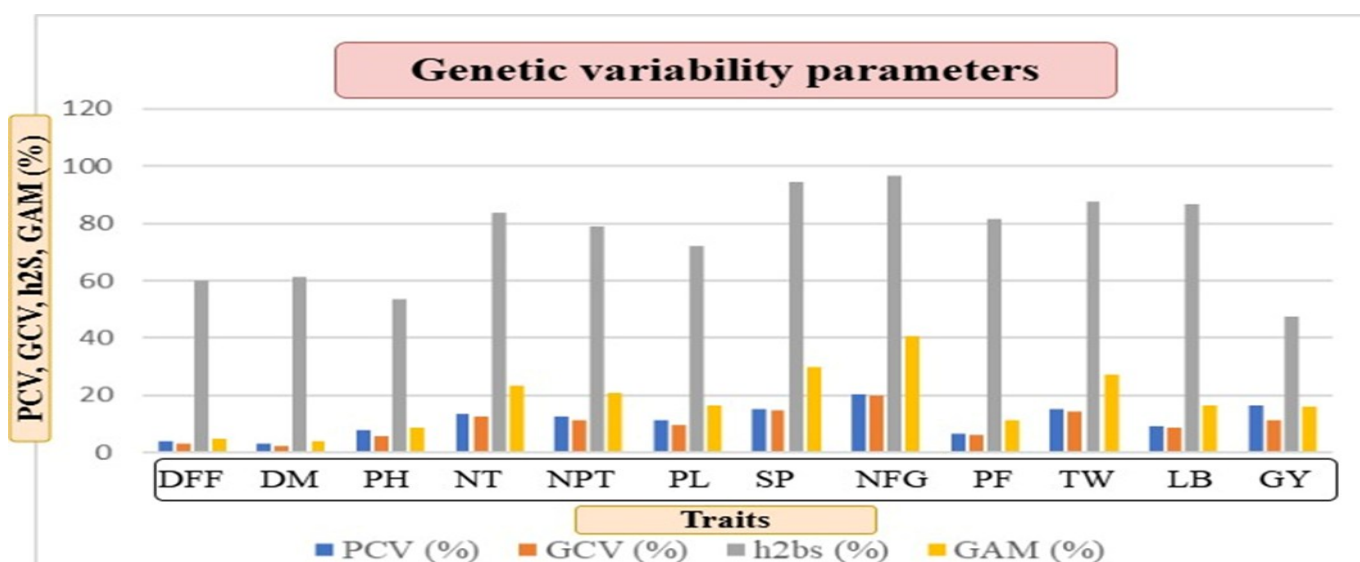
Analysis of variance of augmented block design

It is marked from the ANOVA that the lines are significantly varied for various studied traits. The present experiment documented the presence of wider variation for genetic variability parameters in and among the lines of the population of rice for DFF, DM, PH, NT, NPT, PL, SP, NFG, PF, TW, LB and GY (g). The ANOVA results are presented in Table 1. These findings are in accordance with other study (16).

Table 1. Analysis of variance for yield and yield contributing characters in F₄ population of cross Tunga x KPR 1

Source of variation	Degree of freedom	Mean sum of square											
		DFF	DM	PH	NT	NPT	PL	SP	NFG	PF	TW	L/B	GY
Block (eliminating treatments)	5	4.97	4.07	9.44	0.31	0.66	0.91	46.01	33.94	12.30	1.44	0.01	0.48
Genotypes + Checks (eliminating blocks)	63	27.36*	24.67*	56.23*	4.21**	3.63**	6.86**	804.04**	1048.92**	38.27**	15.70**	0.06**	28.18*
Genotypes	59	24.91*	23.02*	44.6	2.02**	1.67**	6.91**	501.73**	671.25**	33.97**	17.36**	0.07**	19.07
Checks	3	21.83	34.33*	180.95**	1.39*	0.76	10.28*	170.81**	118.58*	3.20	2.11	0.07**	2.21
Checks Vs. Genotypes	1	236.8**	159.47**	397.91**	174.59**	147.81**	0.46	21764.53**	27694.01**	496.55**	91.54**	0.01	676.88**
Error	15	9.97	8.93	20.68	0.33	0.35	1.94	28.33	23.02	6.28	2.19	0.01	10.02

*Significant at 5 % (p -value 0.05); **Significant at 1 % (p -value 0.01).

**Fig. 2.** Bar graph depicting the genetic variability parameters observed among the segregating lines.

Genetic variability parameters

The genetic variability parameters are shown in Fig. 2. The NFG recorded the highest value of PCV (20.39 %) and GCV (20.03 %), indicating the presence of a higher magnitude of variation for the trait (17). Moderate values of PCV and GCV were shown by GY (16.39 %, 11.29 %), SP, NT, NPT and TW in lines of F₄ population. These traits with moderate values of PCV and GCV can also be used as selection criteria (18, 19). Low values of PCV and GCV were observed for number of DFF, PH, number of days to maturity, LB ratio and PF (20). It indicates the presence of lesser variation among the lines studied. The difference between the PCV and GCV values was minimum, indicating the traits under the study were less influenced by the environment (21).

High values of heritability coupled with high values of GAM were exhibited for NFG (96.59 %, 40.62 %), followed by SP (94.35 %, 29.74 %), TW (87.40 %, 27.26 %), NT (83.58 %, 23.55 %) and NPT (78.90 %, 20.87 %). These indicated the presence of additive gene action and the effectiveness of the selection method used (22). Moreover, high heritability with moderate GAM has been recorded for LB ratio (86.74 %, 16.49 %), followed by PF (81.51 %, 11.29 %) and PL (71.91 %, 16.61 %). Moderate heritability and GAM were recorded for GY (47.38 %, 16.05 %) and moderate and low GAM were recorded for PH (53.63 %, 8.67 %) (23). A moderate heritability of a trait indicates that the character can be passed on to its offspring, but it is still influenced by environmental factors. Traits with moderate heritability also have the opportunity to be used as a selection criterion in crop improvement programmes (24). High heritability indicates that variability is influenced by

genes, so it can be inherited to their offspring.

Trait association analysis

The association of grain yield was positively significant with NFG (0.84) followed by SP (0.78), NPT (0.76), NT (0.72), PL (0.59), PF (0.41), LB ratio (0.33) and TW (0.28). PH (-0.23), DFF (-0.097) and DM (-0.067) were negatively correlated with the grain yield. Improvement in grain yield is the top most important trait in rice breeding. It can be reached by direct selection of easily observable characters. However, this needs a good understanding of the association of different traits with grain yield and their association among themselves (25). The positive association indicates that the selection for these positively associated traits will bring direct improvement in grain yield (26).

A highly positive direct effect on yield was recorded by the NFG (0.7621), NPT (0.3809), PL (0.1771), DM (0.1737), NT (0.0498), LB ratio (0.0497) and TW (0.0207). The SP (-0.2438), DFF (-0.1441), PF (-0.1258) and PH (-0.0625) displayed a negative direct effect (27). Analysis of path coefficient measures the direct and indirect contributions of independent variables to the dependent variable. It is used to partition values of correlation coefficients into direct and indirect effects on dependent variable (28). Focusing on traits that have a positive effect on yield will be beneficial for boosting production. The results of the correlation are shown in Fig. 3 and the path analysis results are shown in Table 2.

Skewness and Kurtosis

Platykurtic and positively skewed distribution were recorded by DFF, NT, number of productive tillers, PL, SP, NFG, TW and GY.

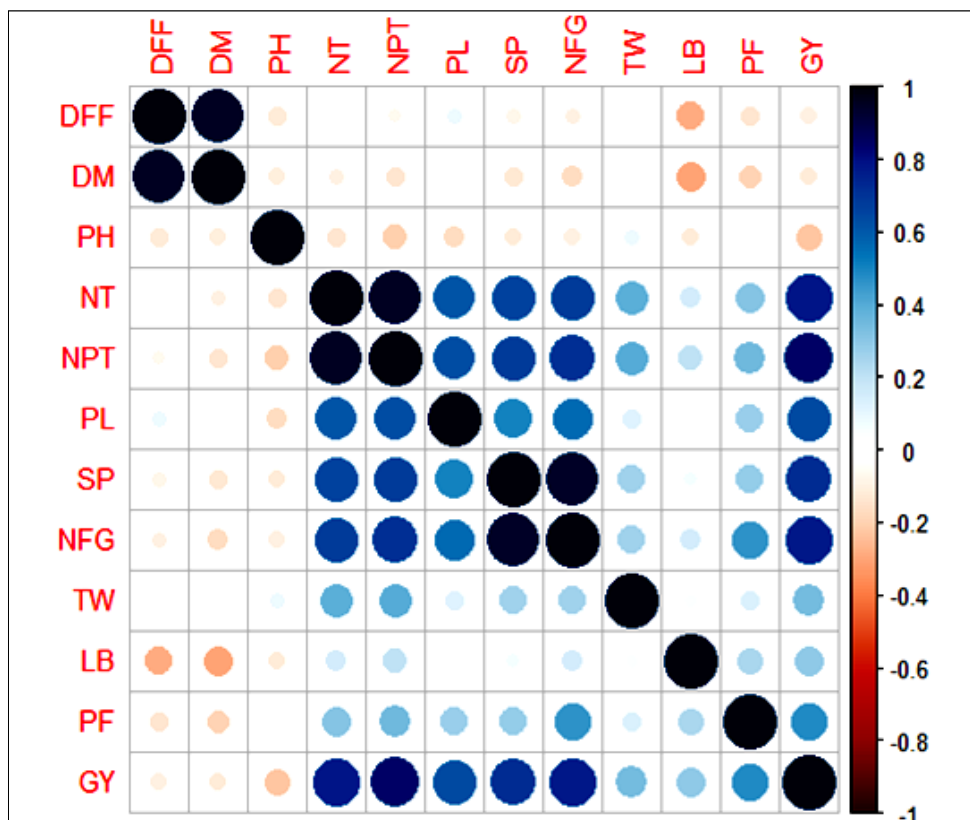


Fig. 3. Correlation among yield and yield contributing characters.

Table 2. Path analysis among yield and yield contributing characters

Effect of trait	Via trait										
	DFF	DM	PH	NT	NPT	PL	SP	NFG	PF	TW	L/B
DFF	-0.1441	-0.128	0.009	0.0059	0.0028	-0.0044	0.0318	0.0316	0.0074	-0.0011	0.0001
DM	0.1543	0.1737	-0.0203	-0.0124	-0.0125	-0.0006	-0.0348	-0.0337	-0.0098	0.013	0.007
PH	0.0039	0.0073	-0.0625	0.0133	0.0096	0.0153	0.0058	0.0041	-0.0007	0.0114	0.0155
NT	-0.002	-0.0036	-0.0106	0.0498	0.0438	0.019	0.0296	0.0288	0.0209	0.0077	0.0146
NPT	-0.0073	-0.0273	-0.0584	0.3351	0.3809	0.1454	0.2443	0.236	0.1775	0.0362	0.0959
PL	0.0054	-0.0006	-0.0433	0.0675	0.0676	0.1771	0.078	0.0837	0.0324	0.0319	0.022
SP	0.0538	0.0489	0.0227	-0.1451	-0.1564	-0.1074	-0.2438	-0.2245	-0.0921	-0.0649	-0.0505
NFG	-0.1674	-0.1478	-0.0499	0.4404	0.4723	-0.3603	0.7018	0.7621	0.3921	0.2408	0.1731
PF	0.0065	0.0071	-0.0015	-0.0529	-0.0586	-0.023	-0.0475	-0.0647	-0.1258	-0.0182	-0.0022
TW	0.0002	0.0015	-0.0038	0.0032	0.002	0.0037	0.0055	0.0065	0.003	0.0207	0.0014
LB	0.0001	0.002	-0.0124	0.0146	0.0125	0.0062	0.0103	0.0113	0.0009	0.0034	0.0497
r values	-0.0968	-0.0668	-0.2309	0.7194	0.764	0.5916	0.781	0.8413	0.4058	0.2809	0.3267

r: Correlation coefficient with grain yield per plant.

Maximizing the genetic gain concerning the traits with positively skewed distribution requires intense selection from the existing variability. Negatively skewed platykurtic distribution is shown by PF in lines of both the crosses and by DM. Positively skewed leptokurtic distribution was shown for TW and PH. These distribution components studies, such as skewness (third-degree statistic) and kurtosis (fourth-degree statistic) provide information regarding the nature of gene action (29) and the number of genes affecting the traits, respectively (30). The parameters d and h in the genetic expectations of skewness ($-3/2 d^2 h$) reflect additive gene effects and dominant gene effects, respectively. Both skewed and kurtosis have greater predictive potential than first and second-degree statistics in revealing interaction genetic effects (31). Most of the traits show a normal distribution. The

frequency distribution curve of PH and PL is shown in Fig. 4 and the rest of the traits are shown in Supplementary Fig. S1 & S2.

Grain quality parameters analysis

Based on the performance of the segregating population, the top 10 lines are identified and analyzed for quality parameters of rice. The high-yielding lines were outperforming the parents. Entry 44 (35.35 ppm, 11.79 ppm), 41 (33.85 ppm, 14.92 ppm) and 50 (31.36 ppm, 10.43 ppm) have outperformed all other lines in zinc and iron content respectively. The correlation analysis of grain quality parameters with grain yield shows that all the three traits, namely, zinc (0.37), iron (0.31) and amylose (0.35), have a weak positive correlation (Fig. 5). It shows the significant impact of grain quality parameters on grain quality (32). Whereas the correlation between

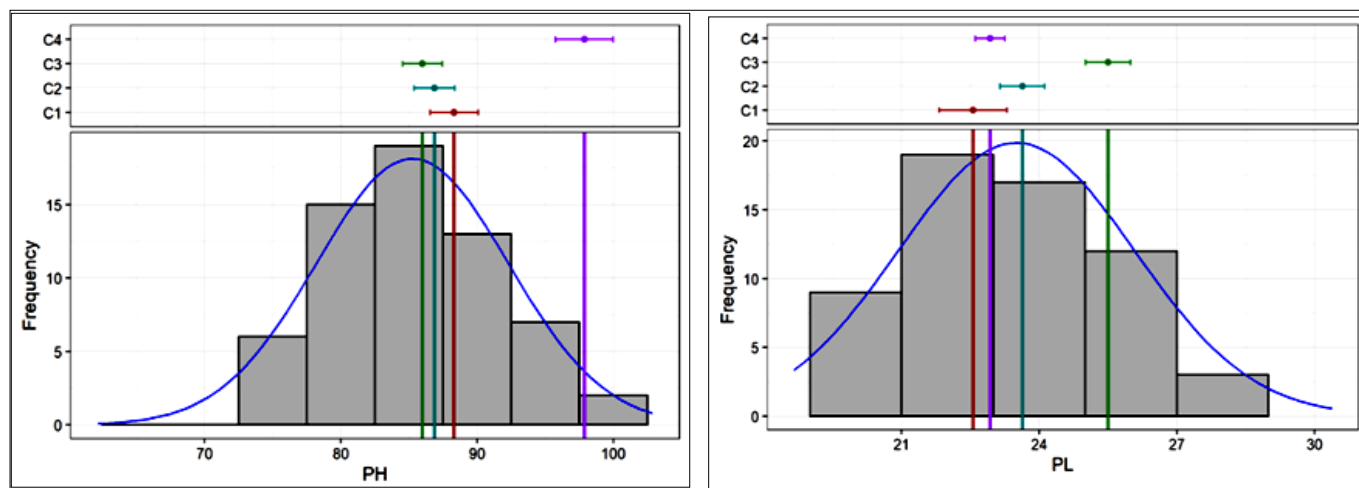


Fig. 4. Frequency distribution curve of PH and PL.

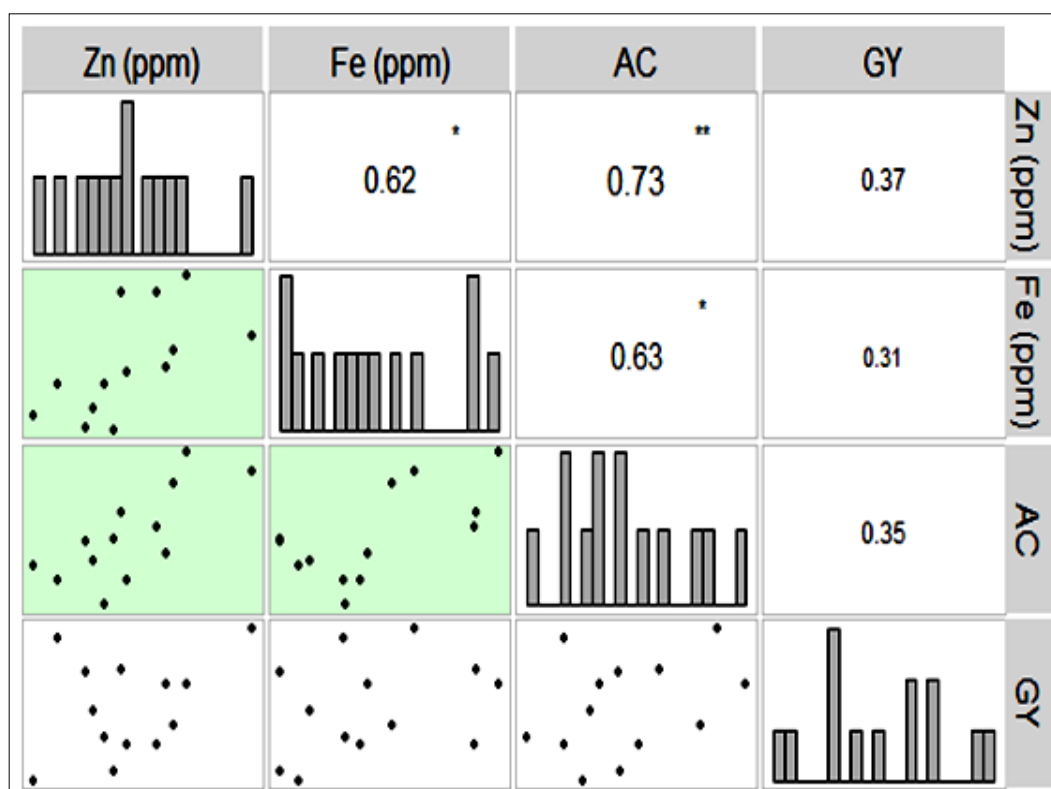


Fig. 5. Correlation of grain quality parameters with grain yield (Zn: zinc; Fe: iron; AC: amylose; GY: grain yield).

zinc and amylose is highly significant (0.73) and also amylose have a positive significant correlation (0.63) with iron (33).

Consumer preference is essential in the modern era. To satisfy the consumer and to have nutritional benefits, focusing on the quality aspects is a must. Zinc and iron are micronutrients that are essential for the body. Based on the amylose, the grain texture after cooking will be different. Hence, the high-yielding lines are analyzed for zinc, iron and amylose content. The lines identified for superior grain quality and yield also exhibited desirable grain types, such as slender and medium-slender forms, which the consumers most prefer (Fig. 6). The details of zinc, iron and amylose content of high-yielding lines are represented in Table 3.

It to the next generation are the key steps in crop improvement. Grain yield had a strong association with NFG, SP, NPT, NT, PL and TW and they have shown high values of heritability coupled with high values of genetic advance as % of mean, which indicate that they are governed by additive gene action. Rather than making a direct selection for making an indirect selection in segregating variable population of rice based on both GY and yield contributing characters like NPT, NFG and PF will be rewarding. Entry 44 (35.65 ppm, 11.79 ppm), 41 (33.35 ppm, 13.92 ppm) and 12 (30.43 ppm, 11.21 ppm) have outperformed all other lines in zinc and iron content respectively. The identified lines will be advanced through subsequent generations to develop homozygous lines for use in crop improvement programs.

Conclusion

Most of the traits have ample scope for variation for selection. High values of PCV and GCV were observed for NFG. Selecting by analyzing the segregating population for variation and forwarding

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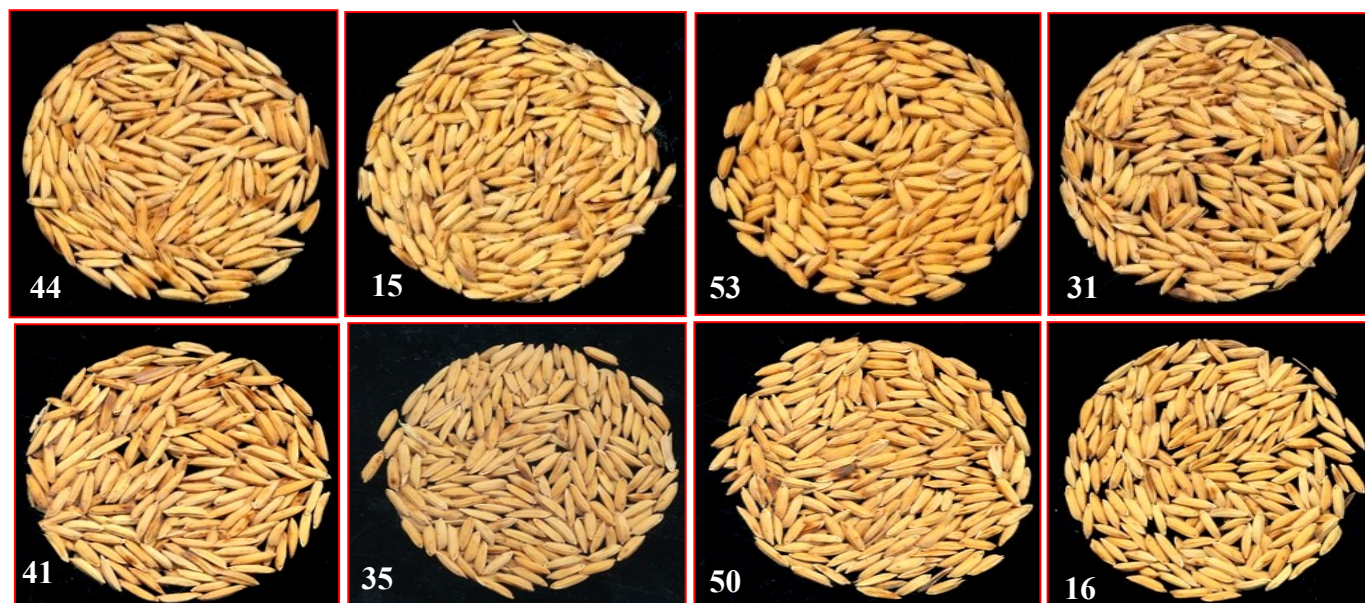


Fig. 6. Promising high-yielding lines with slender and medium slender grain type.

Table 3. Quality parameters data of high yielding lines

Entry	Zn (ppm)	Fe (ppm)	AC	GY
Tunga	22.81	10.01	18.28	36.81
KPR 1	27.06	13.33	23.5	34.6
KHP 13	24.6	8.43	21.3	34.48
44	35.65	11.79	26.6	37.67
15	29.99	10.62	20.3	33.5
53	25.21	9.18	19.75	31.6
31	25.85	10.07	16.3	29.6
41	31.35	13.92	28.2	33.58
35	21.17	8.86	19.3	26.5
50	27.36	10.43	18.3	29.2
16	29.3	13.31	22.4	29.15
12	30.43	11.21	25.68	30.5
18	26.5	8.4	21.36	27.2

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Authors' contributions

HMA carried out the research part of the paper. DL carried out the corrections. SKS participated in the sequence alignment. HBM participated in the table's arrangements. MG helped with the rearrangement of the subheadings and finishing. CNNB and JIT contributed to the modification of the manuscript. All authors have read and approved the final version.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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