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Short Title: Morphological characterization of rice germplasm for rice grain discoloration resistance

RESEARCH ARTICLE

Morphological characterization of rice germplasm for rice grain discoloration resistance

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Abstract

Rice grain discoloration is a complex disorder which occurs due to the infection of various microorganism(s) on glumes/kernels or both. A study was conducted on the discoloration of existing rice germplasm and potential sources for resistance to be utilized in future breeding programs. 200 rice lines (land races, approved varieties and advanced breeding lines) were collected from Plant Genetic Resources Institute (PGRI), National Agricultural Research Centre (NARC) Islamabad Pakistan; Rice Research Institute (RRI) at Kala Shah Kaku, Pakistan; International Rice Research Institute (IRRI), Philippines; China National Rice Research Institute (CNRRI), China and United States Department of Agriculture, United States of America. Rice germplasm were sown at Rice Research Institute (RRI), Kala Shah Kaku and tested in terms of their phenotype expression. The experiment was designed in a three replicated Alpha Lattice Design (ALD). Throughout the season the standard agronomic practices were followed as recommended by Punjab, Pakistan for transplanting (30 days after sowing), recommended dose of Nitrogen, Phosphorus, and Potassium (NPK) fertilizer (25:35:55 kg acre^{-1}) and irrigation management. The results indicated that there was a large phenotypic variation between the cultivars with Coefficient of Variation expressed as a percentage (CV%) ranging from 1.32 (days to maturity) to 54.7 (unfilled grains per panicle). Plant height ranged from 80.0 to 189.3 cm. Number of tillers per plant ranged from 13.3 to 48.7 with an average of 24.9 (Coefficient of Variation (CV)=24.53%) and there was a slight skewness (1.41) due to the small proportion of lines with a very high number of tillers. For seed morphology (50 g seeds of each cultivar), seed length ranging from 7.05 to 11.95 mm mean=9.22 mm and CV=9.26% seed width from 1.68 to 3.60 mm mean=2.28 mm and CV=13.75% and length-to-width ratio from 2.24 to 6.22 (mean=4.13; CV=19.08%), spanning grain shape classes from medium to extra slender. The 1000 grain weight ranged from 11.27 to 28.29 g with a mean of 19.82 g CV=12.99% and n=199. The heritability estimates for traits with high Analysis of Variance (ANOVA) ranged from 1.68% for days to maturity to 100% for 1000-Grain Weight. Seed length (h^2bs=89.33%), seed width 89.20% and length-to-width ratio of 88.88% had highest heritability values followed by flag leaf area of 81.32% and unfilled grains per panicle of 79.98%. Approximately 3% to 78% of germplasm collection was confirmed to be resistant, moderately resistant, moderately susceptible and susceptible respectively with the disease incidence ranges being Percentage Disease Index (PDI) ≤ 12%, PDI 12-26%, PDI 26-50% and PDI>50%. The mean percentage of PDI was 34.87%, indicating that most of the lines were susceptible to disease under the pathogen pressure. Discoloration of the grain was observed in all germplasm panels ranging from less than 5% up to more than 70% with a mean PDI of about 34.87%. Level of resistant lines 7% and moderately resistant lines 27% indicate presence of natural resistance in cultivated Pakistani germplasm. The germplasm lines with grain quality were identified as candidates to be used in

future breeding programs.

Keywords: Characterization, Grain quality, Disease incidence, Land races, Phenotype

Abbreviations: PGRI: Plant Genetic Resources Institute; NARC: National Agricultural Research Centre; RRI: Rice Research Institute; IRRI: International Rice Research Institute; CNRRI: China National Rice Research Institute; ALD: Alpha Lattice Design; NPK: Nitrogen, Phosphorus, and Potassium; CV: Coefficient of Variation; CV%: Coefficient of Variation expressed as a percentage; ANOVA: Analysis Of Variance; PDI: Percentage Disease Index; DSI: Disease Severity Index; h_{bs}^2: broad-sense heritability; IQR: Interquartile Range

Introduction

More than 50 diseases of rice can appear at different plant growth stages (Arshad, et al. 2009). Literature stated that following diseases have been isolated from rice grains in the nursery, and in the main field: Blast, bacterial leaf blight and tungro diseases are common diseases in the nursery stage. In the main growing regions, blast, sheath blight, brown spot and sheath rot, grain discoloration and false smut are reported to be very damaging to the crop (Nguefack, et al. 2007). Several factors are responsible for reduced rice grain yield per unit area of which diseases are some of the major factors.

Rice grain discoloration has been known as a complex pathological syndrome rather than a disease from a single organism and has been present for a long time. It is a more complex disorder which occurs due to the infection of various microorganism(s) on glumes/kernels or both. The primary cause of this disease is fungi, although bacteria have also been reported as a cause. Apart from microorganisms, other abiotic factors also produce such effects and cause abnormal colour changes in the rice grains (Biswas, 2003). In recent years, the importance of this disease has also been on the rise in rice producing areas around the world. It has been a concern, both in seed production and crop production (Mia, et al. 1994).

According to official statistics, in Pakistan, about 3.6 million hectares of land were cultivated for rice production and approximately 9.9 million tonnes of rice was produced in 2024-2025, accounting for about 0.6% of the national Gross Domestic Product (GDP) (Pakistan Bureau of Statistics, 2025). Any disease that affects the quality, marketability or export phytosanitary requirements of the grain then has consequences that extend beyond the field. Discoloration of grain causes change in grain morphology such as shape and size of individual grains and has a significant negative impact on yield, besides other problems associated with discoloration like interference in drying, shelling, milling and processing of rice (Ashfaq, et al. 2022). Expected economic losses in coming years are significant in Pakistan, if the disease is not well defined and managed (Ashfaq, et al. 2017). For all these reasons, it is important to accurately characterize rice germplasm in local environment, as this is a basic need for any rational breeding or management strategy.

Grain discoloration has been a continuously increasing issue in the rice industry of Pakistan which has been reported to affect the yield (up to 30%), quality, milling recovery, nutritional composition and export value of the grain (Ashfaq, et al. 2021). Germplasm diversity studies do exist but lacks comprehensive knowledge under climatic conditions of Pakistan. Therefore, the present study is at a right time to shift the focus of the Pakistani research work on grain discoloration from descriptive to confirmatory phase which will entail creation of an inventory of identification and classification of existing germplasm and resistant sources that could be applied in breeding programs and also be useful for the scientific community.

Materials and Methods

Experimental site

Present study was carried out at Research and Development Unit of Rice Research Institute Kala Shah Kaku (Sheikhupura) in district Sheikhupura, Punjab, Pakistan and Laboratory analyses were carried out at the Department of Plant Breeding and Genetics at Faculty of Agricultural Sciences at University of the Punjab in Lahore, Pakistan.

Plant material

The seeds of 200 rice lines (land races, approved commercial varieties and advanced breeding lines) were obtained from Plant Genetic Resources Institute (PGRI), NARC Islamabad Pakistan and Rice Research Institute at Kala Shah Kaku in Pakistan and International Rice Research Institute in Philippines also China National Rice Research Institute at China and United States Department of Agriculture.

50 g sample of rice seeds was used from each rice line for a study of seed parameters and morphological characterization and pathological tests for the isolation and identification of causal organisms causing discoloration of grain. For seed longevity and seed contamination seeds were stored in sealed polyethylene bags under 4°C.

Field experiment/phenotyping

Rice germplasm lines were planted at Rice Research Institute, Kala Shah Kaku and evaluated for their phenotype expression during two successive seasons. The experiment was designed in a three replicated ALD. The standard agronomic practices for rice cultivation as recommended for Punjab, Pakistan, were followed throughout the season including transplanting at 30 days after sowing, recommended dose of NPK fertilizer (25:35:55 kg $acre^{-1}$) and irrigation management as per the requirement.

The lines were screened for grain discoloration symptoms, detailed evaluation and confirmation of the extent of the discoloration. Number of infected seeds was estimated for each variety based on the disease severity/incidence of grain discoloration in a random panicle per variety at maturity.

Seed parameters

Standard procedure was followed to obtain the following morphological parameters for each germplasm line. Twenty seeds were randomly picked from each line for measurement (Tab. 1).

Table 1. Seed parameters recorded for rice germplasm lines.

S. No.	Seed parameter	Method/unit
1	Length of seed	Measured with digital Vernier caliper (mm)
2	Width of seed	Measured with digital Vernier caliper (mm)
3	Thickness of seed	Measured with digital Vernier caliper (mm)
4	Length-to-Width ratio (L/W)	Calculated from length and width values
5	1000-seed weight	Weighed on analytical balance (g)

Plant morphological traits

Morphological data were collected at suitable growth stage using standard evaluation criteria for rice recommended by IRRI, 2013. The following morphologic parameters were measured (Tab. 2):

Table 2. Plant morphological traits recorded at different growth stages.

S. No.	Morphological trait	Stage of measurement
1	Plant height (cm)	At maturity
2	Days to 50% flowering	Heading stage
3	Days to maturity	Harvest stage
4	Number of tillers per plant	At maturity
5	Panicle length (cm)	At maturity
6	Number of grains per panicle	After harvest
7	Weight of grains per panicle (g)	After harvest
8	Number of unfilled grains per panicle	After harvest
9	Percentage of seed set (%)	Calculated after harvest
10	Yield per hectare (kg/ha)	At harvest

Table 3. Disease severity scale for rice grain discoloration (Malavolta, et al. 2007).

Scale	Symptom description	% Grain surface affected
0	No symptoms-healthy grain	0% (absence of spots)
1	Very minor discoloration	0-25% (small spots)
2	Mild discoloration	25% occurrence of spots

3	Moderate discoloration	26-50% occurrence of spots
4	Severe discoloration	>50% occurrence of spots on grain surface

Assessment of grain discoloration disease

Severity and incidence of disease and grain discoloration were evaluated according to scale developed by Malavolta, et al. 2007. For visual assessment of the crop 1000 grains were randomly selected from each replication at crop maturity. Grains were classified based on the percent of the grain surface with discolored symptoms.

The PDI was calculated using the following formula:

PDI%=(Number of discolored grains/Total number of grains assessed) × 100

DSI was computed according to the formula:

DSI=Σ(Scale value × Number of grains in that scale)/(Total grains assessed × Maximum scale value) × 100

The grain discolored was visually evaluated in grains collected from each experimental plot. Post-harvest handling involved sun drying the panicles for 2-3 days and threshing and putting away the seeds in labelled cloth bags. The grains were classified as healthy or discolored by visual observation of the amount of glume surface discoloration. These grain samples were then classified by using the IRRI scale, (scale 0 being completely healthy and scale 9 being grains with more than 50% of glume surface affected) as shown in figure below. The 1000 seeds per replication were evaluated and classified in each scale for the calculation of PDI and DSI (Fig. 1).

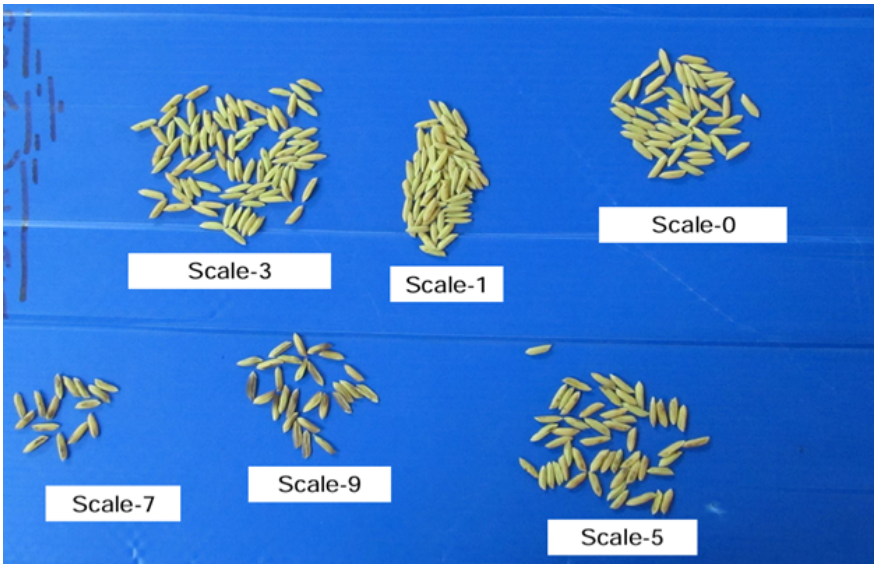


Figure 1. IRRI Scale (0 to 9) for measuring discoloration of rice grains.

Statistical analysis

All the data obtained from experimental field trials and laboratory tests were analyzed statistically. All quantitative traits were subjected to ANOVA to estimate the effects of treatments and genotype differences. Least Significant Difference (LSD) test was performed to carry out mean separation at $P \leq 0.05$. Pearson's correlation coefficients were calculated to provide information on the relationships between morphological and yield-related parameters. Principal Component Analysis (PCA) technique was used to determine major sources of variation among germplasm lines. SAS® Version 9.2 (Statistical Analysis System (SAS) Institute Inc., Cary, NC, USA) software was used for all statistical analyses (Tab. 4).

Table 4. Summary of statistical methods and software used in this study.

Analysis type	Symptom description	% Grain surface affected
aNOVA (Analysis of Vari-ance)	No symptoms-healthy grain	0% (absence of spots)
Mean comparison	Very minor discoloration	0-25% (small spots)

Correlation	Mild discoloration	25% occurrence of spots
Multivariate analysis	Severe discoloration	>50% occurrence of spots on grain surface

Results

Descriptive variation among rice germplasm lines

The descriptive statistics i.e. mean, minimum, maximum, range, Standard Deviation (SD), Standard Error (SE), Coefficient of Variation (CV%), skewness and kurtosis were calculated for all sixteen quantitative traits for 200 germplasm lines (Tab. 5). The results showed that there was a wide phenotypic difference among the cultivars, ranging from 1.32% (days to maturity) to 54.7% (unfilled grains per panicle) CV%. Plant height ranged from 80.0 to 189.3 cm with a mean of 129.5 ± 19.4 cm (CV=14.99%). The variability in the number of tillers per plant ranged from 13.3 to 48.7 with a mean of 24.9 (CV=24.53%; skewness=1.41) which means that a small proportion of lines had a much higher number of tillers than the typical range. Flag leaf area averaged 38.7 cm² (range: 18.06-63.49 cm2; CV=21.79%).

Table 5. Descriptive statistics for sixteen quantitative traits across 200 rice germplasm lines evaluated at Rice Research Institute (RRI), Kala Shah Kaku.

Trait	Unit	N	Mean	Min	Max	Range	SD	SE	CV (%)	Skew-ness	Kurto-sis
Plant height	cm	200	129.58	80.00	189.33	109.33	19.42	1.373	14.99	0.346	0.773
No. of tillers per plant	count	200	24.89	13.33	48.67	35.33	6.1	0.432	24.53	1.411	2.272
Flag leaf area	cm²	200	38.72	18.06	63.49	45.43	8.44	0.597	21.79	0.28	-0.19
Panicle length	cm	200	25.39	18.00	37.67	19.67	3.82	0.27	15.03	0.829	0.38
Panicle weight	g	200	2.79	0.95	6.69	5.74	0.99	0.07	35.45	0.66	1.44
Filled grains per panicle	count	200	103.12	35.33	227.33	192	32.57	2.303	31.59	0.72	1.882
Unfilled grains per panicle	count	200	48.92	6.33	179.33	173	26.74	1.891	54.67	2.175	6.958
Total grains per panicle	count	200	152.04	66.33	354	287.67	44.95	3.179	29.57	1.682	5.57
Seed setting	%	200	68.12	22.03	94.3	72.27	11.99	0.848	17.6	-0.83	0.65
Days to 50% bloom	days	200	81.00	73.00	85.67	12.67	2.42	0.171	2.99	-0.787	0.825
Days to maturity	days	200	110.26	107.00	113.67	6.67	1.46	0.103	1.32	-0.315	-0.321
Seed length	mm	200	9.22	7.05	11.95	4.9	0.85	0.06	9.26	0.097	0.187
Seed width	mm	200	2.28	1.68	3.60	1.92	0.31	0.022	13.75	0.535	0.796
Seed thickness	mm	200	1.77	1.43	2.14	0.71	0.12	0.008	6.77	-0.229	0.574
Length-to-width Ratio	ratio	200	4.13	2.24	6.22	3.98	0.79	0.056	19.08	0.314	-0.148
1000-Grain Weight	g	199	19.82	11.27	28.29	17.02	2.57	0.182	12.99	-0.025	0.596

Note: N: number of genotypes means; SD: Standard Deviation; SE: Standard Error; CV: Coefficient of Variation (%); Skewness and Kurtosis are Fisher's moment coefficients. Bold highlighted CV values (>30%) indicate traits with highest phenotypic diversity. 1000-Grain Weight: n=199 (single replicate per line). Seed Width: One replication excluded for plant ID 56 (typographic error).

This analysis indicated that filled grains per panicle as one of yield components was the most direct measurement of productive panicle capacity with range from 35.3 to 227.3, mean 103.1 ± 32.57 and CV 31.6%. Relative diversity of germplasm for specific trait was highest for unfilled grain per panicle which had the highest CV of 54.67%. Total grains per panicle ranged from 66.3 to 354.0 mean=152.04; CV=29.57%. Seed-setting percentage ranged between 22.03 and 94.30 mean 68.12 and CV 17.60 and skewness -0.830 and skewness was negative meaning that there were poor-setting lines at lower end.

The lowest CV values were obtained for phenological traits. Days to 50% bloom ranged from 73 to 85.67 days (mean=81.00 ± 2.42 days; CV=2.99%) and days to maturity ranged from 107 to 113.67 days (mean=110.26 ± 1.46 days; CV=1.32%). For seed morphology seed length ranging from 7.05 to 11.95 mm mean=9.22 mm and CV=9.26% seed width from 1.68 to 3.60 mm mean=2.28 mm and CV=13.75% and length-to-width ratio from 2.24 to 6.22 (mean=4.13; CV=19.08%), spanning grain shape classes from medium to extra slender. The 1000 grain weight ranged from 11.27 to 28.29 g with a mean of 19.82 g CV=12.99% and n=199.

Boxplot-based trait distribution

Boxplots were drawn for all 16 traits to visualize distribution of each trait among 200 germplasm lines as in Fig. 1 and the properties of the distributional including Interquartile Range (IQR), median, mean, whisker extent (1.5 × IQR) and individual outlier observations. The CV% value annotated in each panel provides a quick reference to relative variability.

Boxplots corroborated high CV values of yield-component traits reported in Tab. 5: The spreads were widest for yield-component traits such as unfilled grains per panicle and panicle weight and filled grains per panicle and most outliers were observed in higher end of each of these traits. Phenological traits (days to bloom, days to maturity) were characterized by a narrow and compact box which showed a high uniformity in germplasm for these traits. Seed morphology traits (seed length, seed width and seed thickness) moderately compact distributions with few outlying individuals like length-to-width ratio had a wider distribution indicating the range of bold to extra-slender grain types.

The distribution of seed-setting percentage showed a slight left skew (Skewness=-0.830; Fig. 2), visible in the boxplot as a median positioned above the midpoint of the box, with a longer lower whisker indicating a subset of lines with markedly reduced panicle fertility consistent with differential susceptibility to grain discoloration pathogens at flowering.

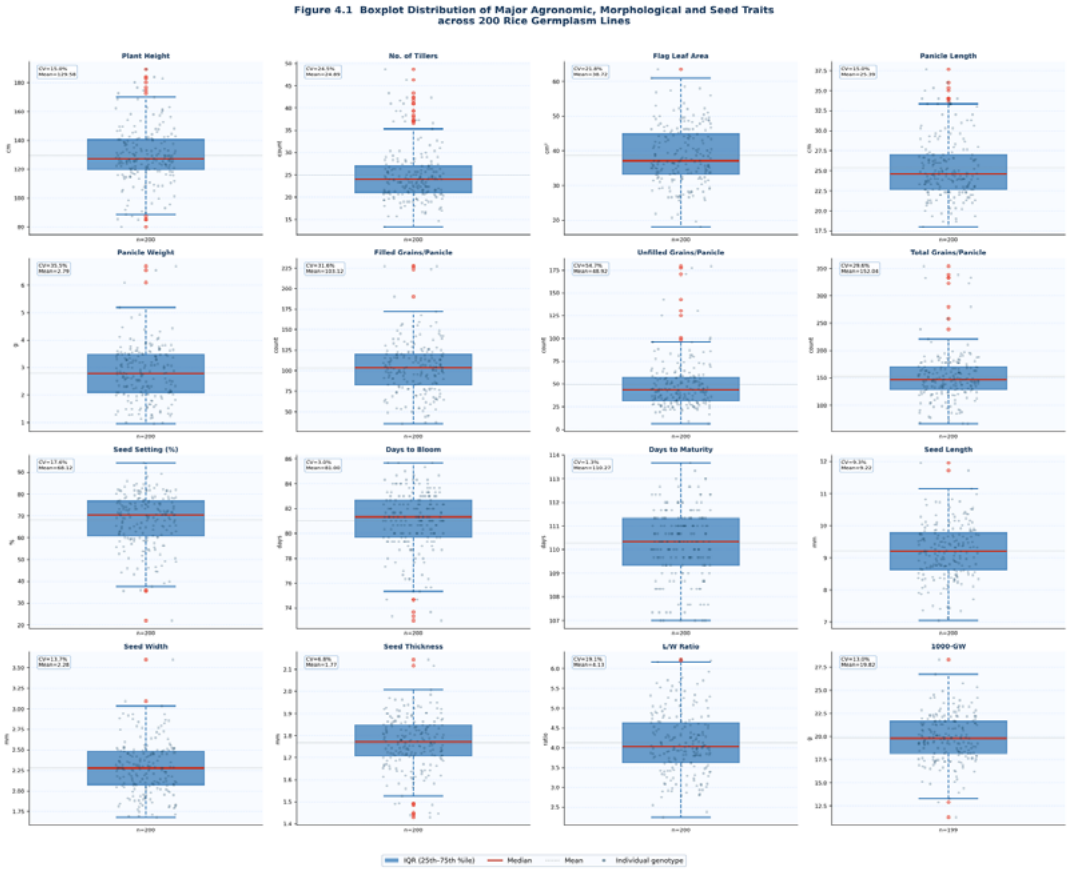


Figure 2. Boxplot distribution of sixteen quantitative traits across 200 rice germplasm lines. Each panel shows interquartile range (box), median (bold line), mean (dotted line), whiskers extending to 1.5 × IQR and individual genotype observations. CV (%) is annotated in each panel. Data from three-replicate field trials at Rice Research Institute (RRI), Kala Shah Kaku.

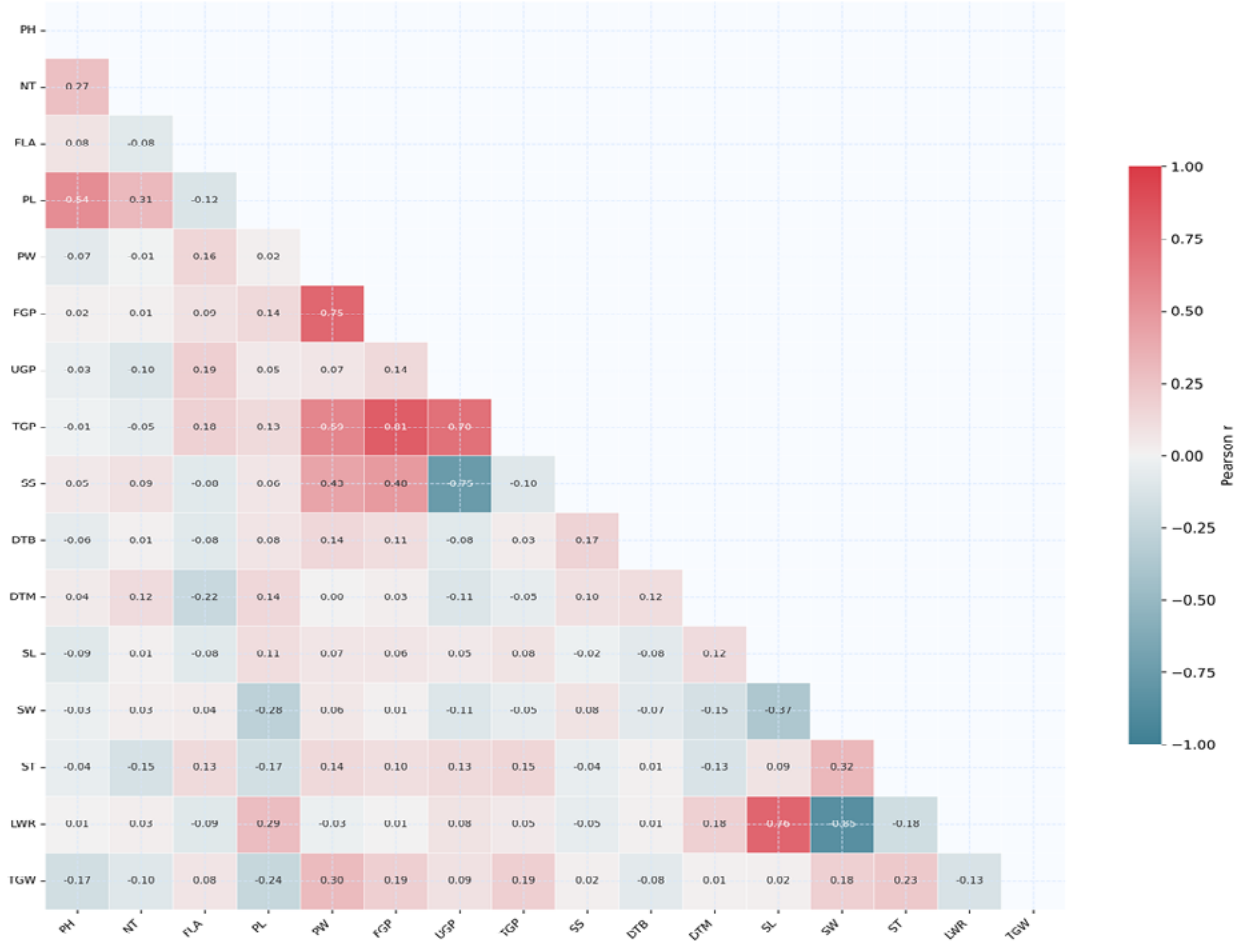


Figure 3. Boxplot distribution of sixteen quantitative traits across 200 rice germplasm lines. Each panel shows interquartile range (box), median (bold line), mean (dotted line), whiskers extending to 1.5 × IQR and individual genotype observations. CV (%) is annotated in each panel. Data from three-replicate field trials at Rice Research Institute (RRI), Kala Shah Kaku.

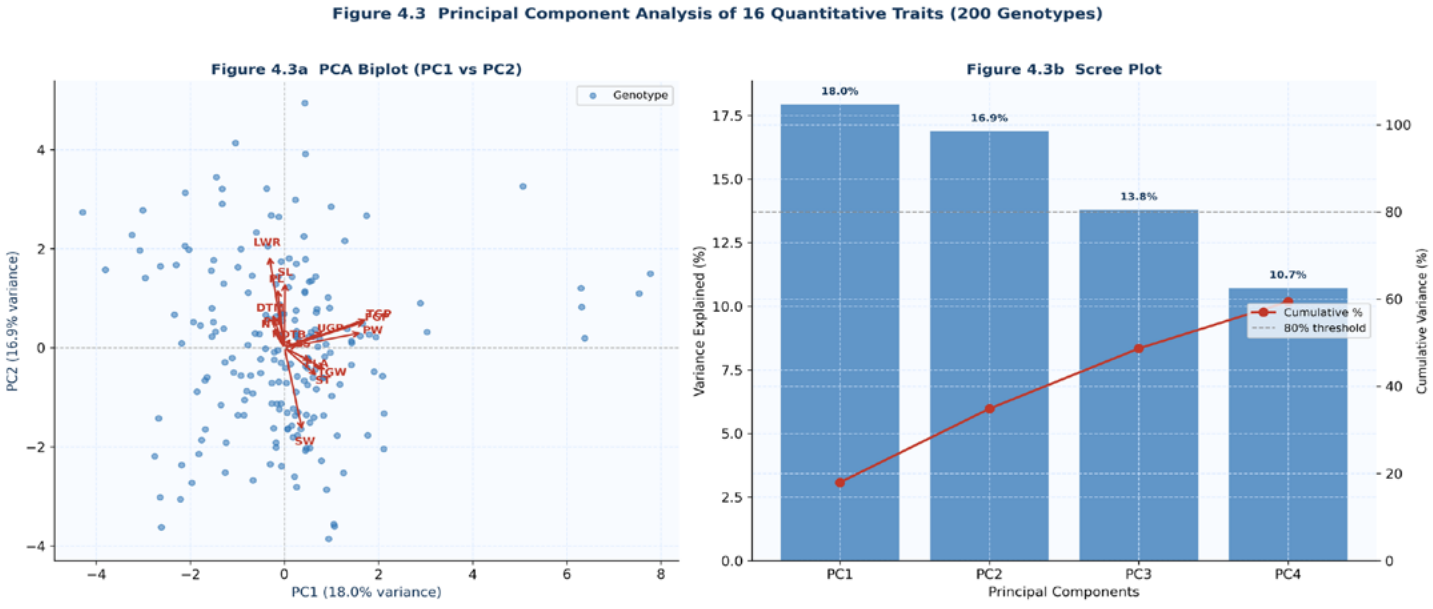


Figure 4. Left show PCA biplot of PC1 *vs.* PC2 for 200 rice germplasm lines; blue points=individual genotypes; red arrows=trait loading vectors and right show scree plot showing variance explained per component (bars) and cumulative variance (red line) and dashed line=80% cumulative variance threshold.

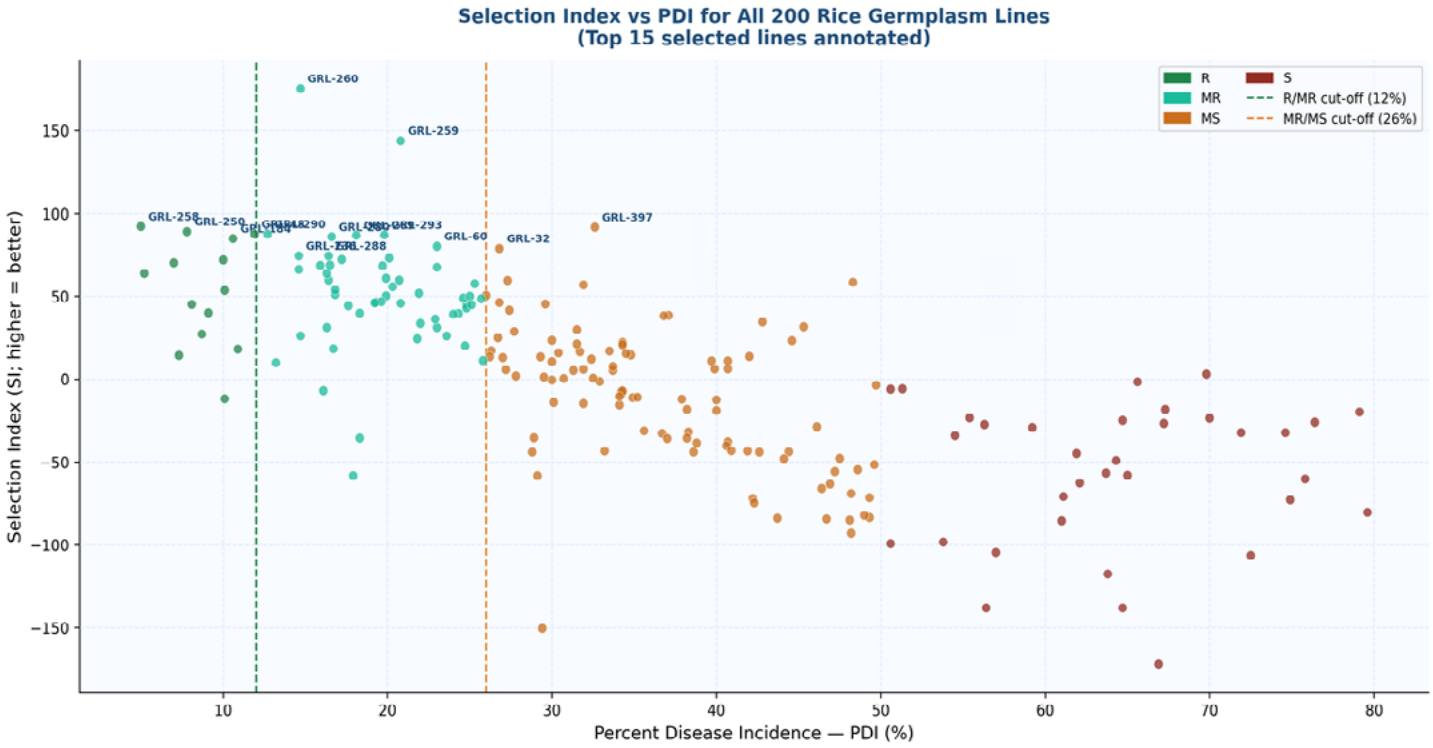


Figure 5. Selection Index (SI) vs. Percent Disease Incidence (PDI) for all 200 rice germplasm lines. Colour coding: Green=resistant, teal=moderately resistant, orange=moderately susceptible, red=susceptible. Top 15 lines are annotated. Dashed vertical lines indicate PDI thresholds (12% and 26%).

Analysis of variance for morphological, yield and seed traits

One-way ANOVA was conducted for each of the sixteen traits to determine whether statistically significant phenotypic differences existed among the 200 germplasm lines. The genotypic mean square (MSg) was tested against the error mean square (MSe) using the F-ratio at a significance level of $P \leq 0.05$. Results are presented in Tab. 6.

Table 6. One way ANOVA results for sixteen quantitative traits across 200 rice germplasm lines.

Trait	df (G)	MS (G)	df (Err)	MS (Err)	F-value
Plant height (cm)	199	1131.767	400	106.456	10.631
No. of tillers per plant	199	111.805	400	18.857	5.929
Flag leaf area (cm2)	199	213.534	400	15.188	14.059
Panicle length (cm)	199	43.694	400	5.203	8.398
Panicle weight (g)	199	2.936	400	0.331	8.878
Filled grains per panicle	199	3182.485	400	361.570	8.802
Unfilled grains per panicle	199	2145.570	400	165.217	12.986
Total grains per panicle	199	6062.766	400	513.927	11.797
Seed setting (%)	199	431.101	400	49.720	8.670
Days to 50% bloom	199	17.628	400	9.685	1.820

Days to maturity	199	6.366	400	6.055	1.051
Seed length (mm)	199	2.188	400	0.084	26.125
Seed width (mm)	199	0.294	399	0.011	26.727
Seed thickness (mm)	199	0.043	400	0.006	7.180
Length-to-width Ratio	199	1.861	400	0.075	24.985

Note: df (G): degrees of freedom for genotype effect; df (Err): degrees of freedom for error. Significance: *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05; NS: Non-Significant. F-values>10 and significance cells are highlighted. 1000-Grain weight had extremely high F owing to near-zero error mean square from single replication; this value should be interpreted with caution.

Fifteen of the sixteen traits showed highly significant genotypic variation (P ≤ 0.001). The only exception was days to maturity (F=1.051; P=0.336; NS), confirming that the collection was essentially uniform for crop duration under the prevailing growing conditions at RRI Kala Shah Kaku consistent with the very low CV (1.32%) reported in Tab. 5.

The highest F-values were record for seed length of 26.125 and seed width 26.727 and length to width ratio 24.98 indicating that seed morphology traits were most strongly differentiated among genotypes with genotypic mean square substantially exceeding error mean square. Flag leaf area 14.05 and unfilled grains per panicle F=12.98 and total grains per panicle 11.79 also showed high F-values. Plant height F=10.631 filled grains per panicle F=8.802 and panicle weight F=8.878 and seed setting F=8.670 were all highly significant. Days to 50% bloom showed a significant genotypic effect F=1.82; P ≤ 0.001 despite its low CV confirming that even modest phenological variation in collection was of genetic origin.

Genetic variability, heritability and genetic advance

All sixteen traits were estimated for variance component broad sense heritability (h_{bs}^2), Genotypic Coefficient of Variance (GCV) and Phenotypic Coefficient of Variance (PCV) and genetic advance under 5% selection GA as Tab. 7. These parameters can be used to estimate amount of observed variation that is due to genetic factors and the potential for response to selection.

Table 7. Variance components, broad sense heritability (h_{bs}^2), genotypic and phenotypic coefficients of variation (GCV, PCV) and Genetic Advance (GA) at 5% selection intensity for sixteen quantitative traits.

Trait	Mean	$\sigma^2 g$	$\sigma^2 e$	$\sigma^2 p$	$h^2 bs$ (%)	GCV (%)	PCV (%)	GA	GA (%)
Plant height (cm)	129.58	341.77	106.456	448.23	76.25	14.27	16.34	33.303	25.7
No. of tillers per plant	24.89	30.983	18.857	49.84	62.17	22.36	28.36	9.054	36.37
Flag leaf area (cm^2)	38.72	66.115	15.188	81.303	81.32	21	23.29	15.127	39.07
Panicle length (cm)	25.39	12.83	5.203	18.033	71.15	14.11	16.72	6.233	24.55
Panicle weight (g)	2.79	0.869	0.331	1.199	72.42	33.4	39.24	1.636	58.63
Filled grains per panicle	103.12	940.31	361.57	1301.9	72.23	29.74	34.99	53.763	52.14
Unfilled grains per panicle	48.92	660.12	165.217	825.33	79.98	52.52	58.73	47.403	96.9
Total grains per panicle	152.04	1849.6	513.927	2363.5	78.26	28.29	31.98	78.487	51.62
Seed setting (%)	68.12	127.13	49.72	176.85	71.89	16.55	19.52	19.721	28.95

Days to 50% bloom	81	2.648	9.685	12.333	21.47	2.01	4.34	1.555	1.92
Days to maturity	110.26	0.104	6.055	6.159	1.68	0.29	2.25	0.086	0.08
Seed length (mm)	9.22	0.701	0.084	0.785	89.33	9.09	9.61	1.633	17.72
Seed width (mm)	2.28	0.094	0.011	0.106	89.2	13.43	14.22	0.598	26.18
Seed thickness (mm)	1.77	0.012	0.006	0.018	67.32	6.28	7.65	0.188	10.63
Length-to-width ratio	4.13	0.596	0.075	0.67	88.88	18.7	19.83	1.501	36.37
1000-Grain weight (g)	19.82	6.624	0	6.624	100	12.99	12.99	5.309	26.79

Note: σ^2g is genotypic variance and σ^2e is error variance and σ^2p is phenotypic variance. $hbs^2=\sigma^2g/\sigma^2p \times 100$. GCV=($\sqrt{\sigma^2g}$ / Mean) × 100; PCV=($\sqrt{\sigma^2p}$/ Mean) × 100. GA=k × $\sqrt{\sigma^2p}$ × hbs^2 (k=2.063 at 5% selection intensity). $hbs^2 \geq 80\%$ and GA%>50% highlighted. 1000-Grain weight hbs^2=100% reflects single-replicate measurement; treat with caution.

Heritability estimates for traits with significant ANOVA ranged from 1.68% days to maturity to 100% 1000-grain weight. Seed length (h_{bs}^2= 89.33%), seed width 89.20% and length-to-width Ratio of 88.88% had highest heritability values followed by flag leaf area of 81.32% and Unfilled Grains per panicle of 79.98%. In such cases genotypic variance components are extremely large in comparison to the phenotypic variance component and therefore phenotypic selection would be quite reliable in such cases. Moderate to high heritability 60-80% of all yield-related traits namely plant height, total grains per panicle, panicle weight, filled grains per panicle, setting% and panicle length indicates that selection for these traits may be effective in this germplasm.

Days to 50% bloom (h_{bs}^2=21.47%) and days to maturity (h_{bs}^2=1.68%) had very low heritability, as expected by the insignificant ANOVA for maturity and the low CV for bloom date, suggesting that there is low genetic variation for phenological traits under the current growing conditions.

There was little difference between the corresponding GCV and PCV values for most traits, suggesting that genetic variance was greater than environmental variance with the greatest positive difference being for seed morphology traits. The range of genetic advance as a percentage of the mean (GA%) was between 0.08 % (days to maturity) and 96.90 % (unfilled grains per panicle). High GA% was also obtained for panicle weight (58.63%), filled grains per panicle (52.14%) and total grains per panicle (51.62%) which indicates that significant increase in yield component could be achieved in one cycle through selection.

Disease incidence and disease severity of rice grain discoloration

All 200 germplasm lines were evaluate for grain discoloration disease under natural field inoculation conditions across both growing seasons and disease assessment followed the Malavolta, et al. 2007 scale where 0=no symptoms and 4=more than 50% of glume surface affected per grain from which PDI and DSI were calculated as described in M&M section (Tab. 3).

PDI across 200 lines ranged from approximately 3% to 78% confirming a wide spectrum of susceptibility within germplasm collection and based on PDI threshold classification R: PDI≤12%; MR: PDI 12-26%; MS: PDI 26-50%; S: PDI>50% 14 lines were classified as Resistant 54 as moderately resistant and 98 as moderately susceptible and 34 as Susceptible. The mean PDI across the population was 34.87% which indicates that majority of lines showed some level of disease susceptibility under prevailing pathogen pressure at RRI Kala Shah Kaku.

Lines classified as resistant showing PDI values below 12% in both seasons confirming their consistence resistance across two years and lines in Susceptible category of PDI>50% showing severe grain discolor with visible fungal colonies on glume surface reduced seed weight and increase unfilled grain counts relative to resistant lines consistent with disease effects documented in the literature.

Correlation among disease, yield and seed traits

All possible two-way Pearson correlation coefficients were calculated for the 200 germplasm lines for the 16 quantitative traits (Tab. 8; Fig. 3). Correlation values P<0.05 (*), P<0.01 (**), P<0.001 (**) are considered significant, highly significant and very highly significant respectively.

Table 8. Eigenvalues and variance explained by the first eight principal components from PCA of sixteen quantitative traits (200 rice germplasm lines).

PC	Eigenvalue (approx.)	Variance (%)	Cumulative (%)	Major contributing traits
PC1	2.873	17.95%	17.95%	FGP, TGP, PW, TGW, UGP yield productivity axis
PC2	2.704	16.90%	34.85%	SL, LWR, SW, ST grain shape and morphology axis
PC3	2.21	13.81%	48.66%	DTB, DTM phenological axis
PC4	1.717	10.73%	59.40%	PH, PL, FLA plant stature axis
PC5	1.139	7.12%	66.52%	Mixed minor variance
PC6	1.086	6.79%	73.31%	Mixed minor variance
PC7	0.948	5.92%	79.23%	Mixed minor variance
PC8	0.808	5.05%	84.28%	Mixed minor variance

Note: PC: Principal Component; FGP: Filled Grains per Panicle; TGP: Total Grains per Panicle; PW: Panicle Weight; TGW: Thousand-Grain Weight; UGP: Unfilled Grains per Panicle; SL: Seed Length; LWR: Length-to-Width Ratio; SW: Seed Width; ST: Seed Thickness; DTB: Days to Booting; DTM: Days to Maturity; PH: Plant Height; PL: Panicle Length; FLA: Flag Leaf Area. PC1 and PC2 together explained 34.9% of total variation; the first four PCs cumulatively explained 59.4%. Eigenvalue approximated as (% variance × number of traits)/100. First four PCs were highlighted.

The relationship between the yield components followed the expected pattern with filled grains per panicle having strong positive correlation with total grains per panicle and panicle weight. Seed-setting percentage was negatively correlated with unfilled grains per panicle, indicating the direct relationship of grain sterility to panicle fertility in terms of arithmetics. As might be expected, the total grains/panicle was moderately correlated with filled and unfilled grains.

As expected, seed length was strongly and positively correlated with length to width ratio for seed morphology. The width of the seed had a moderate negative correlation with L/W ratio which is not surprising since wider grains would relate to lower L/W ratios or bolder grain shape. Thickness of seed was also correlated positively with the width of seed and 1000-grain weight, suggesting that seed that is physically plumper are associated with higher individual grain weight.

There was moderate positive correlation between plant height and panicle length which indicates that taller varieties produce longer panicles. The grain number per panicle was significantly correlated with flag leaf area, with filled and total grain numbers both being positively correlated with flag leaf area, thus showing that genotypes with higher flag leaf area at canopy level had better supporting leaf area for the yield of filled and total grain. Low correlations were obtained between phenological traits with most morphological and yield traits which are consistent with the near uniform distribution of these traits in this germplasm panel.

Principal component analysis

Principal Component Analysis (PCA) was performed on standardized trait data (z-scores) for all sixteen quantitative traits across the 200 germplasm lines to identify major axes of phenotypic variation and multivariate relationships among germplasm (Tab. 8; Fig. 4).

The first two principal components together explained 34.9% of total variation while first four components accounted for 59.4%. PC1 17.95% variance was primarily associated with yield-component traits including filled grains per panicle and total grains per panicle and panicle weight and 1000-Grain weight representing a grain productivity axis separating high-yielding from low yielding genotypes. PC2 with 16.90% variance was dominated by seed morphology traits like seed length, L/W ratio, seed width and seed thickness separating slender-grained from bold grained genotypes a distinction of direct commercial relevance for basmati-type rice. PC3 (13.81% variance) captured phenological variation (days to bloom, days to maturity), while PC4 (10.73% variance) reflected plant stature (plant height, panicle length, flag leaf area). The biplot of PC1 *vs*. PC2 shown a diffuse distribution of genotypes without clearly separated subgroups, consistent with the continuous and overlapping nature of phenotypic variation within this cultivated rice germplasm panel.

Ranking

All 200 germplasm lines were ranked by their SI score and SI ranged widely with highest ranking lines combining low PDI and high yield components and favorable SSR alleles and lowest-ranking lines penalized for high disease incidence and high unfilled grain

counts and low productivity. Fig. 3 shows the scatter of SI against PDI for all 200 lines and target selection zone is populated predominantly by R and MR lines.

Discussion

Phenotypic diversity and genetic variability

There was broad phenotypic variation among the 200 germplasm lines evaluated in this study with CV% ranging from 1.32% (days to maturity) to 54.67% (unfilled grains per panicle) for the sixteen quantitative traits evaluated (Tab. 5). This variation range is comparable with the results of Bibi, et al. 2021 and Rabbani, et al. 2010 obtained on rice germplasm from Pakistan where both landraces and improved varieties of exotic introductions were involved. Epidemiological significance was observed on the high coefficient of variation for the yield components especially on unfilled grains per panicle (54.67%), panicle weight (35.45%) and filled grains per panicle (31.59%) which is directly affected by fungal infection during flowering and grain filling stage. In the current study, the increase in sterility in the susceptible lines was reflected in a significantly decreased 1000-grain weight, head rice percentage and filled grain percentage for the five rice varieties, as shown by Phat, et al. 2005. The crop duration expressed in days to maturity was uniform and showed no significant variation (CV=1.32%) and also the non-significant ANOVA (F=1.051; P=0.336) which indicated phenotypic uniformity for the crop duration, suggested medium duration phenological adaptation of the germplasm to the cropping calendar of Punjab kharif crop (Carriger and Vallee, 2007).

The heritability (bs) of seed morphology traits was high for seed length 89.33%, seed width 89.20% and L/W ratio 88.88% indicating the reliability of phenotypic selection in this germplasm for grain shape (Tab. 5). They exhibited moderate to high heritability filled grain 72.23% and unfilled grain 79.98% and seed setting 71.89% and genetic advance of 52.14%, 96.90% and 28.95%. These GA% values indicate that substantial genetic gain can be achieved for these traits after a single selection cycle which have high heritability and high GA%. These estimates are similar to what Ashfaq and Khan, 2012 have reported in Pakistani rice germplasm and Bibi, et al. 2021 in rice germplasm of Bangladesh.

Ratio length-to-width (mean=4.13, range=2.24-6.22) represented the grain shape classes from medium-bold to extra-slender all of which included the entire commercial range of non-basmati to premium basmati grains. Characterization of confirmed Resistant lines with extra-slender grain shape particularly GRL-184; L/W=6.20; PDI = 10.6% and PDI=10.6% ; L/W=5.66; PDI=7.8% is directly relevant to rice export market in Pakistan where basmati quality grain commands a significant premium price and where resistance to grain discoloration is a prerequisite for producing premium grain that meets export phytosanitary standards.

Trait interrelationships and multivariate structure

The correlation analysis verified some significant agronomically related relations. The positive and high correlation between filled grains per panicle and panicle weight substantiates the selection of these traits together in yield improvement program. The negative association between unfilled grains per panicle and seed-setting percentage is consistent with a direct and positive association between pathogen induced grain sterility and reduced panicle fertility and hence the use of seed-setting percentage as a simple field indicator of grain discoloration susceptibility. The positive correlation between flag leaf area and filled grains per panicle agrees with the principle that more photosynthetic source capacity will maintain the grain filling under disease stress. The high positive correlation between seed length and L/W ratio with the negative correlation between seed width and L/W ratio verify that selection on L/W ratio (basmati grain shape) can be based mainly on seed length as the highest heritability (h_{bs}^2=89.33%) was found for seed length (Tab. 7).

The first two PCs accounted for 34.85% of the total variation with PC1 representing grain productivity (filled grains, total grains, panicle weight, 1000-grain weight) and PC2 representing grain morphology (seed length, L/W ratio, seed width) (Tab. 5; Fig. 2). The variance of the remaining 65% was distributed in the following components, implying the presence of a complex multivariate structure similar to Bibi, et al. 2021 and Rabbani, et al. 2010 who also found that multiple components were needed to explain the phenotypic variation in Pakistani rice germplasm. The diffuse biplot distribution supported the idea of continuous phenotypic variation with no distinct clusters of lines in a biplot region and the distribution of lines in different regions of the biplot showed that disease resistance is not genotypically limited to a single phenotypic background.

Disease incidence of grain discoloration

Discoloration of the grain was observed in all germplasm panels ranging from less than 5% up to more than 70% with a mean PDI of about 34.87% (Fig. 5). Level of Resistant lines 7% and Moderately Resistant lines 27% indicate presence of natural resistance in cultivated Pakistani germplasm but this is still not common in released varieties which is in accordance with the finding of Ashfaq, et al. 2017 who reported grain discoloration as a new emerging quality constraint in Pakistani rice production. Confirmed resistance in two seasons as in the present case is in accordance with suggestion of Chaudhry, et al. 2023 that testing for resistance be carried out over more than one growing season before advancing lines in breeding programs.

Conclusion

A wide range of phenotypic variability was observed in 200-line germplasm panel and heritability advance for yield components

indicated potential for phenotypic selection. The germplasm lines with grain quality were identified as candidates to be used in future breeding programs. The grain discoloration syndrome in Punjab, Pakistan was found to be caused by a multi-pathogen complex. Results of this study contribute to rice grain discoloration Patho-system characterization in Pakistan and help to generate germplasm for creating disease resistant commercial rice varieties.

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