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SHORT COMMUNICATION

Multivariate Analysis of Morphological and Biochemical Traits Revealed Some Promising Pigmented Rice Genotypes of North Western Himalayas for Future Breeding Program

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Abstract

Here, 140 pigmented rice genotypes from the North Western Himalayas were evaluated with the objective of addressing the concerns of nutritional insecurity faced by the population having rice as a staple food. Using an augmented block design, we assessed morphological traits alongside biochemical traits like total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity. The principal component analysis identified genotypes SR-2 and NBPGR-16 as promising for plant height and panicle length, while GS-608 excelled in grain yield and antioxidant activity. High TPC and TFC were found in Black Rice, SKUA-533-1, and ZAG-V4, with GS-596 and Chanab showing strong antioxidant activity. Furthermore, the results revealed substantial variability, indicating the potential for targeted breeding to enhance yield and nutritional quality. This study highlights promising pigmented genotypes with superior agronomic performance and health-promoting properties, providing a foundation for breeding programs aimed at improving food security and public health through biofortified rice.

Keywords: Pigmented rice, Genetic Variability, Flavonoids, Phenols, Antioxidants.

Introduction

Rice (*Oryza sativa* L.) is indispensable for global food security, serving as a staple food for over half the world's population, particularly in Asia, Africa, and Latin America. With an estimated global population increase to 9.7 billion by 2050 and amid evolving climate challenges, enhancing rice yield and quality has become a critical priority. Although rice breeders have made strides in yield improvement, progress is constrained by limited genetic diversity within cultivated rice (Xing *et al*., 2010). To bridge this knowledge gap, it is essential to explore untapped genetic variability in diverse rice germplasm (Devi *et al*., 2017).

Beyond yield, the nutritional quality of rice is gaining importance. White rice contains significantly fewer bioactive compounds compared to pigmented rice varieties (Unpublished data). Black rice exhibits the highest levels of flavonoids, followed by red rice, while white rice has the lowest content. Additionally, the antioxidant capacity of red and black rice is notably high, primarily due to the presence of catechin in red rice and quercetin in black rice (Chen *et al*., 2022). These compounds have been traditionally valued for therapeutic purposes. This study advances prior research by providing a comprehensive analysis of 140 diverse rice genotypes, both pigmented and non-pigmented, from Kashmir Valley and Northeast India. While previous studies, such as those by Kaur *et al*. (2018), addressed limited genotypic diversity of North Western Himalayas, especially Kashmir valley, our work encompasses a broader genetic spectrum, including landraces, pigmented, aromatic, and some released varieties from the North-Western Himalayas. This expanded genetic framework highlights the untapped potential of pigmented rice for biofortification and lays a foundation for identifying key quantitative trait loci (QTLs). These findings will guide the development of targeted breeding strategies, such as crossing high-yielding varieties with nutritionally superior pigmented genotypes, to bridge existing gaps in nutritional quality and yield stability across diverse agro-climatic zones.

This study was conducted over two consecutive kharif seasons, from 15th June to 8th October 2022 and from 7th June to 15th October 2023, at the experimental field of

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MRCFC Khudwani, SKUAST-Kashmir, India. A total of 144 pigmented rice genotypes, including four checks from Kashmir Valley and Northeast India, were evaluated. The genotypes were planted in an augmented block design with seven blocks and four check varieties, following recommended agronomic practices with 20 x 10 cm spacing. Five plants were randomly selected and tagged from each line of individual genotypes for observations at different growth stages. Traits measured included grain yield, 1000 seed weight, spikelets per panicle, number of tillers, plant height (cm), panicle length (cm), days to 50% flowering, phenols, flavonoids and antioxidant content. Data analysis was done using ANOVA in the R package 'augmented RCBD' to assess variability, heritability, and genetic parameters. Further, trait variability was evaluated through range, mean, and coefficient of variation, with correlations and principal component analysis employed to explore trait associations. For biochemical parameters, total phenolic content (TPC) and total flavonoid content (TFC) estimation were carried out using the method proposed by Shen *et al*. (2008) with slight modifications. DPPH method (2, 2-diphenyl-1-picrylhydrazyl) was used for antioxidant activity estimation (Oki *et al*., 2005). Further, the population structure analysis was done using the Bayesian clustering method in STRUCTURE version 2.3.4 (Pritchard *et al*. 2000) by analysis of SSR-based marker data. The length of the burn-in period and Markov Chain Monte Carlo (MCMC) were set at 100,000 iterations (Evanno *et al*. 2005). To obtain an accurate estimation of the number of populations, three runs were performed for each K-value, ranging from 1 to 10. Further, Delta K (Figure 1b) values were calculated and the appropriate K value was estimated by implementing Evanno *et al*. (2005) method using the STRUCTURE Harvester program (available at http://taylor0.biology.ucla.edu/structureHarvester/).

The study revealed significant phenotypic and genotypic variability across multiple rice genotypes, which highlights promising opportunities for enhancing key traits. Phenotypic analysis showed a wide range of variability, with traits like days to 50% flowering, plant height, tiller number per plant, grain yield, spikelets per panicle, panicle length, and 1000 seed weight displaying notable variation. ANOVA results (Table 1) demonstrated significant differences for both adjusted and unadjusted sums of squares, underscoring the presence of substantial genetic diversity, consistent with findings by Bairwa *et al*., 2023; El-Agoury *et al*., 2024. This aligns with prior studies suggesting that phenotypic variability is crucial for selection in crop breeding (Naseem *et al*., 2014).

In terms of genetic parameters (Table 2), the study found that phenotypic coefficient of variation (PCV) values exceeded genotypic coefficient of variation (GCV) across all traits, indicating a notable environmental influence. Heritability in the broad sense, combined with genetic advance, was particularly high for traits such as grain yield and spikelets per panicle, suggesting that these traits could be reliably improved through selection. Traits with high heritability and genetic advance indicate a strong genetic basis, making them suitable targets for breeding. However, for traits with lower heritability, such as the number of effective tillers, environmental factors likely play a more substantial role, complicating the reliability of direct selection.

In the biochemical analysis (Figure 1a), pigmented rice genotypes (black, red, purple, brown) showed high levels of bioactive compounds, with black rice exhibiting the highest phenolic (upto 1563.59 mg GAE/100 g) and flavonoid contents (523.69 mg RE/100 g), along with antioxidant activity of 94.68%. These results align with previous observations that pigmented rice tends to have higher bioactive content compared to non-pigmented varieties, suggesting their potential for enhancing nutritional value. Notably, even non-pigmented white rice genotypes demonstrated significant variation in phenolic and flavonoid contents, with certain genotypes surpassing

Table 1: ANOVA for augmented block design for seven traits in 140 pigmented rice genotypes

Source	*Df*	*50F*	*PH*	*PL*	*NT*	*SPP*	*GY*	*1000 SW*
Treatment (ignoring Blocks)	143	64.0 4**	328.89 **	7.46**	3.70 **	783.41 **	151.41 **	18.38 **
Treatment (eliminating Blocks)	143	44.46**	246.39**	6.52**	3.27^{ns}	606.27**	125.94**	17.42**
Block (eliminating Treatments)	6	1.17^{ns}	9.82^{ns}	0.54^{ns}	0.31^{ns}	313.49^{ns}	0.58^{ns}	0.39^{ns}
Block (ignoring Treatments)	6	467.86**	1976.04**	23.08**	10.51**	4535.33**	607.48**	23.16**
Checks	3	680.92**	2294.65**	57.11**	29.14**	2690.30**	433.34**	74.12**
Test entries vs. Checks	1	1.09^{ns}	00.0 **	45.87 **	1.87^{ns}	6.34^{ns}	418.14**	421.74 **
Test entries	139	51.18 **	288.83**	6.12**	3.17^{ns}	747.84**	143.40**	14.27**
Error	18	1.61	6.20	1.01	1.84	206.86	0.59	0.38

PH= Plant Height, PL= Panicle Length, NT= Number of tillers per plant, SPP= Spikelets per Panicle, DF= Days to 50% flowering, GF= Grain yield, SY= Seed yield, 1000 SW= 1000 seed weight

Table 2: Descriptive and genetic variability analysis for seven traits in 140 pigmented rice genotypes

Trait	*Mean*	*Range*		*Coefficient of variation*		*h2(%)*	*GA*	*GA%*
		Min	*Max*	*GCV (%)*	*PCV (%)*			
50F	95.77	79.94	113.44	7.35 (Low)	7.47 (Low)	96.86 (High)	14.30	14.93 (Medium)
PH	114.43	76.84	147.50	14.69 (Medium)	14.85 (Medium)	97.85 (High)	34.31	29.98 (High)
PL	21.17	14.82	27.15	10.68 (Medium)	11.68 (Medium)	83.49 (High)	4.26	20.12 (High)
NT	11.69	7.33	17.88	9.85 (Low)	15.23 (Medium)	41.83 (Medium)	1.54	13.14 (Medium)
SPP	117.83	51.38	188.67	19.74 (Medium)	23.21 (High)	72.34 (High)	40.81	34.63 (High)
GY	25.78	5.57	57.27	46.35 (High)	46.45 (High)	99.59 (High)	24.60	95.43 (High)
1000 SW	24.49	8.69	34.75	15.22 (Medium)	15.43 (Medium)	97.33 (High)	7.59	30.97 (High)

PH= Plant Height, PL= Panicle Length, NT= Number of tillers per plant, SPP= Spikelets per Panicle, DF= Days to 50% flowering, GF= Grain yield, SY= Seed yield, 1000 SW= 1000 seed weight, (PCV %) Phenotypic coefficient of variation percentage, (GCV %) Genotypic coefficient of variation percentage, (h2%) Heritability percentage, GA Genetic Advance, GA% Genetic Advance as percentage mean.

pigmented ones. This suggests that some white rice varieties may also serve as valuable nutritional resources (Figure 1a). Correlation analysis revealed a strong positive association between flavonoid content and antioxidant activity, indicating that flavonoids significantly contribute to antioxidant properties. Morphological traits, especially grain yield, positively correlated with panicle length, days to 50% flowering, number of effective tillers, 1000 seed weight, and spikelets per panicle, supporting the study indicated that these traits are critical for yield improvement (Anisuzzaman *et al.*, 2023). Conversely, a negative correlation was observed between plant height and tiller number, highlighting potential trade-offs between plant architecture and yield traits. Principal component analysis (PCA) identified three significant components explaining 61.35% of the total variance. The first two components, which captured nearly 50% of this variance, were strongly associated with plant height, panicle length, and antioxidant activity, suggesting these traits as key differentiators among genotypes. The genotype-by-trait (GT) biplot (Figure 2) further supported this, as genotypes with high PC scores for yield and biochemical traits clustered together. In conclusion, based on multivariate analysis and genetic evaluation, we identified several potential rice genotypes with exceptional performance as GS-596, Chanab, Zag-V4, and NBPGR-11-1, that showed high antioxidant activity. For flavonoid content, Black Rice was the top performer, followed by SKUA-544, Zag-V3, Zag-V4, and NBPGR-21, while ZAG-V5, ZAG-V4, black rice, GS-596, and Jehlum excelled in phenolic content. From PCA results we found SR-2, NBPGR-16, NBPGR-2, NBPGR-34, and NBPGR-5 contributed most towards plant height, panicle length, and 50% flowering. Further traits like grain yield and antioxidant activity having dominant contributions in PC2 were represented by GS-608, GS-480, GS-484, GS-474, and GS-621. For PC3, phenolic and flavonoid content were key traits, with black rice, SKUA-533-1, ZAG-V4, ZAG-V15, and SKUA-556 as leading genotypes. These genotypes hold strong potential for breeding programs aimed at improving rice varieties for both nutritional quality and agronomic performance.

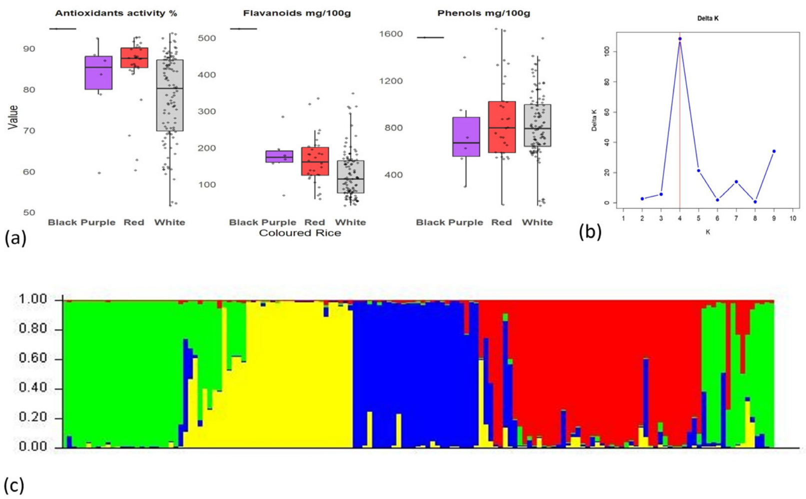

Figure 1: (a): Distribution of phenols, flavonoids and antioxidant activity in colored rice germplasm., (b): Delta K showing the number of populations, (c): Population structure of 147 rice genotypes.

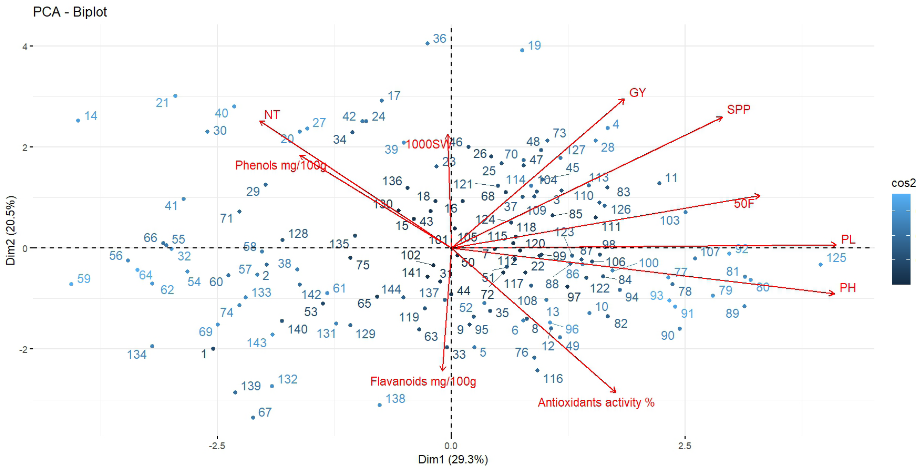

Figure 2: Genotype by trait (GT) biplot based on the individual genotypic data explaining the contribution of 10 traits to the total variation. The biplot was based on singular value decomposition of trait-standardized data ("Scaling = 1, Centering = 2") and trait-focused singular value partition ("SVP = 2")

The population structure analysis grouped the population into four sub-populations (Figure 1c), reflecting their diverse genetic backgrounds. Landraces predominantly belonged to the green cluster, indicating high genetic uniformity with minimal admixture. Indian collections were largely assigned to the yellow cluster, with some genotypes showing admixture, suggesting historical gene flow. Advanced breeding lines exhibited significant admixture, highlighting their derivation from diverse parental sources. Similarly, released varieties displayed contributions from all clusters, reflecting efforts to combine genetic resources for improved traits. Collections from international sources primarily clustered in the red group, indicating their distinct genetic composition. This emphasized the importance of utilizing diverse genetic resources, particularly the landraces and exotic collections, in breeding programs to enhance genetic diversity and adaptability. Preserving unique landraces and leveraging exotic collections can contribute to sustainable crop improvement efforts.

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Competing Interests

The authors declare they have no conflicts of interest.

References

Anisuzzaman M, Rafii M, Zaman HU and Islam R (2023) Genetic assessment of selected rice (Oryza sativa L) genotypes based on agro-morphological traits in tropical climate: Malaysia. Journal *of Biotechnology and Bioresearch* 4(5): 622-639.

Bairwa, R. K., Yadav, M. C., Kushwaha, A., & Joshi, M. A. (2023). Morphological and molecular analyses of grain traits in aromatic rice landrace accessions from Indo-Gangetic plain region of India. *Indian Journal of Plant Genetic Resources*, *36*(02), 290-300.

Chen, X., Yang, Y., Yang, X., Zhu, G., Lu, X., Jia, F., and Wu, X. (2022). Investigation of flavonoid components and their associated antioxidant capacity in different pigmented rice varieties. *Food Research International*, 161, 111726.

Devi KR, Chandra BS, Lingaiah N, Hari Y and Venkanna V (2017) Analysis of variability, correlation and path coefficient studies for yield and quality traits in rice (*Oryza sativa* L.). Agricultural Science Digest – *A Research Journal* 37(1): 1–9.

El-Agoury RY, El-Hashash EF, Abou El-Enin MM, Sakr SM, Essa WM, El Sherbiny HA, and El-Absy KM (2024) Combining Ability, Heterosis and Multivariate Analysis for Physiological and Agronomic Traits of Rice Genotypes Under Normal and Water Stress Conditions. *Agricultural Research* 13(1): 10-25.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular ecology*, 14(8), 2611-2620.

Kaur, P., Singh, N., Pal, P., & Kaur, A. (2018). Variation in composition, protein and pasting characteristics of different pigmented and non-pigmented rice (*Oryza sativa* L.) grown in Indian Himalayan region. *Journal of food science and technology*, 55, 3809-3820.

Naseem I, Khan AS and Akhter M (2014) Correlation and path coefficient studies of some yield related traits in rice (*Oryza sativa* L.). *International Journal of Scientific and Research Publications* 4(4): 1-5.

Oki T, Masuda M, Nagai S, Take'ichi M, Kobayashi M, Nishiba Y, Sugawara T, Suda I and Sato T (2005) Radical-scavenging activity of red and black rice. *Journal of Agricultural and Food Chemistry* 53(3): 359–365.

Shen Y, Jin L, Xiao P, Lu Y and Bao J (2009) Total phenolics, flavonoids, and antioxidant capacity in rice grain and their relations to grain color, size, and weight. *Journal of Cereal Science* 49(1): 106–111.

Xing Y and Zhang Q (2010) Genetic and molecular bases of rice yield. *Annual Review of Plant Biology* 61: 421–442.