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Genetic Variability and Correlation Studies in Red Rice (*Oryza sativa* **L.) for Yield Related Traits and Grain Zinc, Iron and Protein Content**

Navya B Hulmani ^a , Shridevi A Jakkeral a* , Shashikala S Kolakar ^a , Basavaraj I Halingali ^b and Dhananjaya B. C. ^c

^a Department of Genetics and Plant Breeding, College of Agriculture, KSNUAHS, Shivamogga, India. ^b Department of Statistics, College of Agriculture, KSNUAHS, Shivamogga, India. ^c Department of Soil Science, College of Agriculture, KSNUAHS, Shivamogga, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Nutrient deficiencies, particularly zinc (Zn), iron (Fe) and protein, pose severe health risks especially in children and lactating women. Red rice is significantly rich in iron, zinc and protein compared to white rice. The present investigation was undertaken in this context to elucidate information on variability and character association in red rice genotypes to identify effective selection criteria for grain yield and quality improvement of red rice genotypes. **Study Design:** Randomized complete block design with two replications.

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^{}Corresponding author: E-mail: jakshridevi25@gmail.com;*

Place and Duration of Study: Zonal Agricultural and Horticultural Research Station, Brahmavar, Udupi during Kharif 2023.

Methodology: A total of 108 red rice genotypes were used in the study and observations were recorded for ten characters. Protein was estimated by Kjeldhal method, zinc and iron content by Atomic absorption spectrophotometer (AAS) method. The data collected was subjected to statistical procedures using R software.

Results: Analysis of variance revealed significant variation among the genotypes for all the characters studied. Grain Zn (11.4-44.7ppm), Fe (13.11-37.12ppm) and protein (5.0-11.43%) showed wide range of variability. High genotypic and phenotypic coefficient of variation was observed for Zn, number of productive tillers per plant, Fe, grain yield per plant, number of grains per panicle and plant height. Correlation studies revealed that grain yield per plant exhibited significant positive association with yield component traits. Protein and Fe content had negative association with grain yield.

Conclusion: The traits that have high PCV, GCV, heritability and genetic advance as per cent mean are controlled by additive gene action which is very valuable for selection. The traits that are positively correlated with grain yield can be used for indirect selection which aids crop improvement. Karthika, Bilihallga and Shahara genotypes have high protein, iron and zinc respectively, which can be used in the further breeding program to develop nutritionally rich varieties.

Keywords: Red rice; zinc; iron; protein; variability; correlation.

1. INTRODUCTION

Rice (*Oryza Sativa* L.) is one of the most important cereal crops in the developing world. It is the staple food of over half the world's population and fulfils over 21% of total caloric requirements (Hossain M., 2004) and more than 90% of the world's rice is cultivated and consumed in Asia. It is a crucial dietary and food security source for many Asian countries (Singh KS, et al., 2020). People generally prefer white rice for its desirable eating qualities such as appearance, tenderness, taste and quicker cooking time. However, polishing removes the bran layer, aleurone, embryo, and part of the endosperm, significantly reducing its nutritional content. For example, iron content decreases from 8.8 ppm to 4.75 ppm, and zinc drops from 33 ppm to 18 ppm, along with other nutrients (Pillai MA, et al., 2011, Modgil R, 2016). Nutrient deficiencies, particularly zinc, iron and protein, pose severe health risks, especially to children and lactating women. Approximately two billion people worldwide suffer from iron and zinc deficiency (Grember VK, et al., 2014) leading to stunted growth and high child mortality, anemia and developmental issues respectively, protein deficiency causes kwashiorkor and marasmus. Therefore, red rice which is significantly richer in iron, zinc, manganese, protein, vitamins and minerals compared to white rice can be used to overcome these deficiencies. Red rice is defined as rice that has a red bran layer. The red hue comes from proanthocyanidin pigment (Oki TM, et al., 2002). Red rice contains 2-3 times more iron and zinc content compared to white rice

(Ramaiah K, and Rao, 1953), and its bran is rich in manganese, enhancing its antioxidant properties (Kaneda I, et al., 2007). Additionally, red rice has higher levels of protein, vitamins B1, B2, C, as well as N, P, K, S, Mg, Ca and edible cellulose compared to white rice (Jing MA, et al., 2000). It contains flavonoids, which are proanthocyanidins, which are beneficial not only as antioxidants but also have anti-inflammatory, anticancer, immunomodulatory, cardioprotective and antithrombotic properties (Oliveira DAC, et al., 2020). Unlike white polished rice, red rice releases sugars slowly thus helping to stabilize blood sugar in a sustained manner. This trait makes it a better option for people who are suffering from diabetes mellitus, it also have low glycemic index thereby preventing the risk of diabetes type 2 (Priya, et al., 2019). Assessment of variability and knowledge of correlation among yield, its attributing traits and grain zinc, iron and protein content is essential for successful yield and quality improvement through breeding. The present investigation was undertaken in this context to elucidate information on variability, heritability, genetic advance and character associations in red rice genotypes to identify effective selection criteria for grain yield and quality improvement of red rice genotypes.

2. MATERIALS AND METHODS

The experimental material comprised of 108 red rice genotypes collected from Zonal Agricultural and Horticultural Research Station (ZAHRS), Brahmavar, Udupi District, Karnataka and College of Agriculture, Shivamogga. During Kharif 2023, all the red rice genotypes were sown at ZAHRS, using the wet nursery method to raise seedlings. Twenty-one-day-old healthy seedlings from each genotype, along with checks, were transplanted into a well-prepared puddled field in a Randomized Complete Block Design (RCBD) with two replications. Each genotype was transplanted separately in a 5 square meter area with a spacing of 20 cm between rows and 15 cm between plants. The required cultural operations and plant protection measures as per the package of practice were taken up to ensure uniform and healthy crops. For each genotype observations were recorded from five randomly selected plants in both the replications for days to maturity, plant height, panicle length, number of productive tillers per plant, number of grains per panicle and test weight; and for quality characters, namely, protein, iron and zinc content one gram of the powered sample was used for analysis. Observations for test weight, protein, iron and zinc content were obtained from random grain samples drawn from each plot in each genotype and replication using standard procedures. Protein content was estimated by the Kjeldhal method as given by Piper, (1966), zinc and iron content was estimated by AAS method using the procedure as suggested by Lindsay and Norvell (Lindsay and Norvell, 1978) and the data collected was subjected to standard statistical procedures where the mean values were used. Variability and correlation analysis was done by using the RStudio Version 4.3.1 software. The package used for analysis was "variability".

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance for Yield and Yield Related Traits

Analysis of variance revealed highly significant differences among all 108 red rice genotypes for all the evaluated traits. The significant mean sum of squares for all traits indicates that the genotypes were genetically diverse and displayed considerable variability in their mean performance for the studied characters (Table 1). Similar results were recorded by Ratnam et al. (Ratnam et al., 2023).

3.2 Genetic Variability Studies

Phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h² broad sense) and genetic advance as per cent of mean (GAM) is estimated (Table 2). Grain Zn, Fe and protein content showed wide range of variability which ranged from 11.4-

44.7 ppm, 13.11-37.12 ppm and 5.0-11.43%, respectively. Comparable findings were reported by Gogoi et al., (2024) for Zn and Fe content, Agustin et al., (2021) for protein content. High GCV and PCV values were recorded in Zn content, number of productive tillers per plant, Fe content, grain yield per plant, number of grains per panicle and plant height. A higher magnitude of both PCV and GCV for these traits suggests that there is better opportunity for selecting these traits during crop improvement, as they exhibit substantial genetic variability. The results were in accordance with Jasmine et al., (2022) where they reported high GCV and PCV for number of productive tillers per plant, number of grains per panicle, Fe content and grain yield per plant, Paramanik et al., (2023) for number of grains per panicle, grain yield per plant, Zn and Fe content.

High heritability along with high GAM were recorded for plant height, Zn content, Fe content, test weight, protein content, number of grains per panicle, productive tillers per plant, grain yield per plant and panicle length. High value of heritability with high GAM suggests that these traits are governed by additive gene action and environmental factors had minimal impact on these traits, making them suitable for selection. Deepak et al., (2023) reported parallel results for number of grains per panicle, grain yield per plant, panicle length and plant height, Singh et al., (2020) for number of grains per panicle, grain yield per plant, protein content, Zn and Fe content.

3.3 Estimation of Correlation Coefficients

Genotypic and phenotypic correlation coefficients for yield attributing traits and grain zinc, iron and protein content in red rice genotypes is estimated (Table 3). The results revealed that Grain yield per plant displayed positive and significant correlation with number of productive tillers per plant, number of grains per panicle and test weight at both phenotypic and genotypic levels. Therefore, the selection of these traits will indirectly enhance the grain yield. Similarly, grain yield exhibited non-significant positive association with Zn content and days to maturity at both the levels and panicle length only at genotypic level. Grain yield per plant had negative and significant association with protein content indicating the practical difficulty of improving the grain yield and protein simultaneously. Grain yield exhibited nonsignificant negative association with Fe content and plant height at both phenotypic and genotypic levels and panicle length only at

Source of variation	d.f.	DΜ	PH	PL	PT	NG	TW	GY		Ζn	Fe
Replication		0.66	0.39	5.77	1.04	18.37	1.00	10.08	0.11	1.19	. 44
Genotype	107	80.60**	832.62**	24.45**	16.97**	1425.93**	22.68**	86.31**	$5.03**$	166.08**	54.64**
Error	107	2.70	1.81	3.12	1.12	78.30	0.30	7.32	0.18	0.86	0.46
CD (5 %)		3.25	2.67	3.50	2.10	17.54	1.09	5.36	0.84	1.84	1.35
CD (1 %)		4.31	3.53	4.63	2.78	23.20	1.44	7.10	1.12	2.43	1.78
CV		1.22	.38	7.76	10.39	8.34	2.17	10.70	4.25	4.16	3.46

Table 1. Analysis of variance for grain yield, its attributing traits, grain zinc, iron and protein content in red rice genotypes

** Significant at 1% level d.f. – degrees of freedom DM- Days to maturity, PH- Plant height (cm), PL- Panicle length (cm), PT- Productive tillers per plant, NG- Number of grains *per panicle, TW- Test weight (g), P- Protein content (g/100g), Zn- Zinc content (ppm), Fe- Iron content (ppm), GY- Grain yield per plant (g)*

Characters	Mean	Range		GCV (%)	PCV (%)	Heritability (%)	GAM (%)	
		Min	Max					
Days to maturity	133.73	113.5	152	4.66	4.82	93.50	9.29	
Plant height (cm)	97.15	52	149.9	20.97	21.02	99.56	43.12	
Panicle length (cm)	22.76	13.95	34.15	14.34	16.31	77.31	25.97	
Number of productive tillers per plant	10.20	5.5	19.5	27.57	29.46	87.55	53.15	
Number of grains per panicle	106.02	56	186.5	24.48	25.86	89.58	47.73	
Test weight (g)	25.10	18.5	31.57	13.17	13.35	97.35	26.78	
Grain yield per plant (g)	25.28	11.17	45.4	24.85	27.06	84.34	47.02	
Protein content (g/100g)	8.14	5.00	11.43	19.11	19.82	92.97	37.97	
Zinc content (ppm)	22.31	11.4	44.7	40.73	40.94	98.96	83.47	
fron content (ppm)	19.66	13.11	37.12	26.46	26.69	98.31	54.06	

Table 2. Genetic variability parameters for grain yield, its attributing traits, grain zinc, iron and protein content in red rice genotypes

**GCV- Genotypic coefficient of variation, PCV- Phenotypic coefficient of variation, GAM- Genetic advance as percent of mean*

	DM	PH	PL	PТ	NG.	TW		Zn	Fe	GY
DM		-0.0554	$-0.1452*$	0.0974	0.0016	-0.0445	0.0173	-0.0682	$-0.1951**$	0.0638
PH	-0.0574		$0.584**$	$-0.1689*$	0.0358	0.0691	0.0525	$0.3563**$	-0.1023	-0.0949
PL	-0.1563	$0.6299**$		-0.1324	0.0584	0.0604	-0.0308	$0.2738**$	-0.0539	-0.0321
PT	0.1239	-0.1807	-0.1183		$-0.1528*$	-0.1266	0.0908	0.1248	0.0257	$0.5593**$
ΝG	0.0044	0.0418	0.0763	-0.17		0.0514	$-0.1915***$	-0.0332	0.0013	$0.6021**$
TW	-0.0513	0.0694	0.0633	-0.1326	0.0509		$-0.148*$	0.0008	$-0.1462*$	$0.3216**$
P.	0.0164	0.0589	-0.0393	0.1272	$-0.2237*$	-0.1587		$0.1901**$	0.0647	$-0.1467*$
Zn	-0.0767	$0.3573**$	0.2979**	0.1316	-0.0325	0	$0.2026*$		-0.0665	0.0886
Fe	$-0.2013*$	-0.103	-0.0545	0.0292	-0.0042	-0.1485	0.0672	-0.067		-0.0832
GY	0.0854	-0.1023	0.0078	$0.5329**$	$0.6048**$	$0.3459**$	-0.1495	0.0972	-0.0938	

Table 3. Genotypic and phenotypic correlation coefficients for grain yield, its attributing traits and grain zinc, iron and protein content in red rice genotypes

**and ** Significant at 5 % and 1 % respectively, Above diagonal values indicate phenotypic correlation coefficients, Below diagonal values indicate genotypic correlation* coefficients, DM- Days to maturity, PH- Plant height (cm), PL- Panicle length (cm), PT- Productive tillers per plant, NG- Number of grains per panicle, TW- Test weight (g), P-*Protein content (g/100g), Zn- Zinc content (ppm), Fe- Iron content (ppm), GY- Grain yield per plant (g)*

phenotypic level. Similar results were recorded by Prasannakumari et al., (2020) for number of productive tillers, number of grains per panicle, test weight and Fe content. Yu et al., (2009) for protein content. Thuy et al., (2023) recorded significant negative association of grain yield with protein content, non-significant positive association with Zn content and non-significant negative association with Fe content and panicle length.

Grain yield and protein and iron content have a negative correlation due to the physiological and biochemical relationship between carbon and nitrogen (N) in plants. Grain protein content (GPC) is inversely related to grain yield, this is because grain protein concentration and grain yield are closely linked through shared N pathways, genotypes with higher GPC tend to require a greater amount of N in grain, which causes increased translocation of N from vegetative organs, particularly leaves, and as a consequence of promoted leaf senescence and reduced N concentration in leaves, the canopy photosynthetic rate may decline, resulting in reduced grain yield (Fukai and Mitchell, 2023). Managing genetic trade-offs demands a deep understanding of the genetic mechanisms and their interactions. Advanced molecular techniques like genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping can identify crucial genetic regions linked to both high nutrient content and yield. Furthermore, the integration of CRISPR/Cas9 gene-editing technology offers a precise method for manipulating specific genes, potentially mitigating negative trade-offs. By targeting and modifying particular alleles, breeders can enhance desired traits while reducing adverse impacts on yield (Huang, 2024).

4. CONCLUSION

The results revealed high PCV, GCV, heritability and genetic advance as per cent mean (Zn, Fe, Protein) which indicates that the traits are controlled by additive gene action which is very valuable for selection in further breeding program. Number of productive tillers per plant, number of grains per panicle and test weight can be considered as effective for selection to improve grain yield as these traits had positive and significant correlation with grain yield per plant. Positive association of protein with Zn content indicates the probability of simultaneous effectual selection for both the traits. Simultaneous improvement of both grain yield and quality traits is difficult as protein and iron

content were negatively correlated with grain yield. The genotypes identified for higher protein (Karthika), iron (Bilihallaga) and zinc content (Shahara) could be recommended in quality improvement to utilize them as donor parents to transfer these traits to genotypes that are well adapted to local ecological niches. Two genotypes BMR-2 and BMR-31 were identified for high yield potential. Even one per cent higher protein content in the studied genotypes over the existing genotypes is deemed a valuable improvement, which can help address protein deficiencies.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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