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Genetic variability, heritability, genetic advance and path coefficients for grain protein content, quality traits and grain yield in rice (*Oryza sativa* L.) germplasm lines

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Abstract

The present study was carried out to discern the extent of genetic variability in one hundred and fortyseven (147) germplasm lines of rice, for grain protein content, quality traits and yield. Analysis of variance (ANOVA) has shown highly significant differences for all the ten (10) characters, demonstrating the existence of genetic variability among the evaluated germplasm lines. The estimates of PCV were only marginally higher than GCV for all the characters showing smaller environmental influence on the trait expression, therefore genetic improvement through direct selection is possible for the evaluated traits. But, milling % and kernel breadth exhibited moderate PCV and GCV values indicating significant environmental influence on their expression. Overall, the heritability (>80.0%) and genetic advance as percent of mean (20.0%) was high for all the characters except milling % (47.8%; 5.1%). High heritability coupled with genetic advance indicates the influence of additive gene action and selection would be effective in such traits. Protein yield per plant, Length: Breadth ratio and head rice recovery exerted high direct positive effect on grain protein content.

Keywords: Rice, protein, quality and yield

Introduction

Rice is an important cereal that feeds approximately half of the human population, and also provides for over 21% of caloric needs and up to 76% of the caloric intake of people especially in Southeast Asia (Zhao *et al.*, 2020)^[19]. People regularly consume polished (or milled) rice, which is mostly endosperm as both embryo and bran layers are removed during processing. Therefore, the nutritional value of polished (or milled) rice is decided by rice endosperm, which is mostly comprised of starch (70–80%), with proteins (7-10%) and lipids (<1%; Yang *et al.*, 2019)^[17]. It is generally known that rice endosperm lacks many micronutrients or phytonutrients. Hidden hunger is pervasive in low- and middle-income countries and holds signifcant individual, social, and economic consequences (Ruel-Bergeron *et al.*, 2015)^[11]. Development of nutritionally enriched varieties (i.e., increased protein content, Fe, Zn etc.) with desirable grain quality is a viable and cost-effective approach to increase grain nutritional value. This can be achieved either through conventional breeding or genetic engineering, which is shown improve nutritional quality of crops and impact human health (Zhao *et al.*, 2020)^[19].

Rice provides a substantial share of the protein intake for millions of people (Muthayya *et al.*, 2014)^[9]. The total food protein production per hectare of rice is next only to that of wheat. The protein digestibility and biological value of rice is higher than other cereals like wheat, corn and barley (Amagliani *et al.*, 2017)^[1]. Rice proteins are usually considered to possess hypoallergenic, anti-oxidative, anti-hypertensive, anticancer and anti-obesity activities. Further, proteins considerably influence structural, functional and grain quality and nutritional (including grain quality) properties of rice (Amagliani *et al.*, 2017)^[1]. Therefore, developing nutritionally enriched varieties with desirable grain quality and high productivity is essential to meet the demands of growing human population. The current trends of population growth and agricultural production suggests that the demand for food across the globe would nearly triple by 2030 (Shivani *et al.*, 2021)^[13]. Addressing food and nutritional security is a major challenge owing to a constant increase in human population.

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An understanding of the existing genetic variability in a crop is very essential for starting crop improvement programme. Information on heritability and genetic advance of traits and their association helps the plant breeder to identify traits for effective selection (Misra 1991)^[8]. Heritability explains whether differences observed among individuals is due to the genetic makeup or due to environmental forces. Genetic advance or response to selection explains the degree of gain obtained from phenotypic selection for a character. Estimates of heritability coupled with genetic advance are useful and reliable than considering them individually (Johnson et al., 1955) [7]. The major objective of the present study was to estimate the genetic variability for grain protein content, quality traits and grain yield along with heritability, and genetic advance that would help in selection and carrying breeding programmes for grain protein content and other traits.

Material and Methods

One hundred and forty-seven (147) germplasm lines collected from across India were evaluated for yield, grain protein content (GPC) and quality traits during Kharif 2019 and 2020 in simple lattice design with two replications at ICAR-Indian Institute of Rice Research (ICAR-IIRR) Farm, ICRISAT campus, Hyderabad. Twenty-five days old seedlings were transplanted by adopting a spacing of 20 cm between the rows and 15 cm between the plants. All the standard and recommended package of practices for the crop nursery and main crop were taken up during the experimentation. Single plant yield (SPY) data was collected from on five randomly selected plants in each genotype in each replication and the same seed was used for the estimation of grain protein content (GPC) and other quality traits (hulling %, milling %, head rice recovery [HRR %], kernel length [KL; mm], kernel breadth [KB; mm], length: Breadth ratio [L:B], amylose content [AC;

%], and gel consistency [GC]).

The mean of five plants for each metric trait was considered for statistical analysis using SAS software. The analysis of variance (ANOVA) was done using SAS 9.3. The genotypic and phenotype variances were calculated as per the formulae proposed by Burton and Devane (1953)^[3]. Heritability in broad sense (h^2) was calculated as suggested by Johnson *et* al., (1955)^[7]. From the heritability estimates, the genetic advance (GA) was calculated by the formula given by Johnson et al., (1955) [7]. Robinson et al., (1949) [101], categorized heritability values as low: 0-30%, moderate: 30-60% and >60% as high. The GPC was determined by the standard micro-Kjeldahl method (Yoshida et al., 1976)^[18]. The grain protein content was calculated by multiplying percent nitrogen content by 5.95. The grain quality analysis was performed at the "Grain Quality Laboratory" at ICAR-Indian Institute of Rice Research, Hyderabad as per Aravind et al., (2021)^[2].

Results and Discussion

Selection is of key importance in development of any crop and knowledge about selection criteria for any crop is essential for the plant breeder for taking any crop improvement programme. Also, the existence of genetic variability is crucial in any germplasm to be utilized in the breeding programme. Analysis of variance (ANOVA) revealed highly significant differences among the 147 germplasm lines for all the traits studied (Table 1), showing the existence of sufficient amount of genetic variability among the evaluated genotypes. The coefficients of variation (both genotypic and phenotypic; GCV, PCV), heritability in broad sense (h^2) and genetic advance as per cent of mean were estimated for all the ten characters (Table 2). The graphical representation of variability parameters is shown in Fig 1 and Fig 2.

Source of Variation	d.f	Milling (%)	HRR (%)	KL	KB	L:B	AC	GC	SPY	PYP	GPC
Replication	2	78.40	94.73	1.06	0.19	0.20	20.01	35.60	28.49	0.24	3.39
Treatment	146	3515.0*	38730.2*	189.8*	23.2*	68.5*	5608.8*	138766.2*	15309.5*	128.4*	2089.3*
Error	292	1874.19	1853.40	20.42	3.50	3.82	372.51	276.39	206.28	3.05	64.59

* Significance at 5% level

HRR – head rice recovery %; KL – kernel length; KB – kernel breadth; L:B – length: breadth ratio; AC – amylose content; GC – gel consistency; SPY – single plant yield; PYP – protein yield per plant; GPC – grain protein content.

The results revealed that the PCV estimates were slightly higher compared to the GCV values for all the characters evaluated indicating a lesser amount of influence of environmental factors on the expression of these traits. Since the characters are not hugely influenced by the environment, the traits can be used for direct selection. The magnitude of PCV and GCV estimates were moderate for six traits *viz.*, milling %, HRR, KL, KB, L:B and AC (Table 2; Fig 1 & Fig 2). Similar findings were reported by Dhanwani *et al.*, (2013)^[5] and Suresh *et al.*, (2016)^[15] for milling %, HRR, KL, KB, and L:B; Thakur and Pandey (2020)^[16] for AC. Higher estimates of GCV and PCV were observed for four characters *viz.*, GC, PYP, GPC and SPY, similar observations were reported by Dhanwani *et al.*, (2013)^[5] for GC and SPY while Thakur and Pandey (2020)^[16] reported similar results for GC and lower GCV and PCV values for GPC.

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Table 2.	Variability	narameters in	rice germ	nlasm lines
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Characters	Mean	Range	Coefficient of variation (%)		Heritability in broad sense (h ²)	Constin Advance noncent of mach (at 50/)		
			GCV (%)	PCV (%)	%	Genetic Auvance percent of mean (at 5 %)		
Milling (%)	67.60	55.4-75.24	3.59	5.19	47.83	5.11		
HRR (%)	54.67	18.9-69.8	16.99	17.61	93.15	33.79		
KL	5.62	3.95-7.52	11.39	12.33	85.43	21.69		
KB	2.36	1.67-2.98	9.39	10.48	80.32	17.34		
L/B ratio	2.42	1.64-4.06	16.14	16.83	92.07	31.91		
AC	24.11	8.94-29.12	14.59	15.33	90.66	28.62		
GC	36.22	22.0-100.0	49.11	49.19	99.70	101.03		

SPY	15.11 4.2-38.2	39.00	39.39	98.01	79.53
PYP	1.39 0.38-3.58	38.73	39.43	96.52	78.39
GPC	9.45 5.44-15.44	4 22.93	23.47	95.50	46.16

*HRR – head rice recovery %; KL – kernel length; KB – kernel breadth; L:B – length: Breadth ratio; AC – amylose content; GC – gel consistency; SPY – single plant yield; PYP – protein yield per plant; GPC – grain protein content



*HRR – head rice recovery %; KL – kernel length; KB – kernel breadth; L:B – length: breadth ratio; AC – amylose content; GC – gel consistency; SPY – single plant yield; PYP – protein yield per plant; GPC – grain protein content

Fig 1: Graphical representation of PCV and GCV

The reliability of the phenotypic value depends on the estimates of heritability for a particular character. Heritability measures the influence of genetic variability towards the phenotypic variability of a trait (Shivani et al., 2021)^[13]. The degree of genetic gain under selection is "Genetic Advance" (GA). Though heritability estimates are useful in predicting the genetic gain under selection, the combination of heritability and genetic advance estimates is much more helpful. High heritability coupled with high genetic advance estimates were recorded for eight of the traits viz., HRR, KL, L:B, AC, GC, PYP, GPC and SPY (Table 2; Fig. 2). The results are in agreement with those observed by Jaiswal and Dey (2007)^[6], Subbaiah et al., (2011)^[4] and Dhanwani et al., (2013) ^[5]. Therefore, the results show that the environmental influence on the expression of these characters is low and hence selection can be practiced. High heritability coupled

with moderate genetic advance estimates were observed for KB and milling %, similar findings were reported by Subbaiah *et al.*, (2011) ^[14] in CMS based rice hybrids and Dhanwani *et al.*, (2013) ^[5] in F₁ populations along with four parents in rice. KB and milling % could possibly be improved by selective breeding.

The path coefficient analysis results are presented in Table 3 (also Fig.3 a & b). The path coefficient results revealed that PYP exercised the highest positive direct effect on GPC followed by L:B, HRR, KB, milling %, AC, and GC, indicating that the selection for these characters could result in overall improvement in GPC directly. Therefore, during the selection programmes preference should be accorded to these traits to isolate superior genotypes with higher genetic potentiality for GPC. SPY and KL had a negative direct effects on GPC.



*HRR – head rice recovery %; KL – kernel length; KB – kernel breadth; L:B – length: breadth ratio; AC – amylose content; GC – gel consistency; SPY – single plant yield; PYP – protein yield per plant; GPC – grain protein content

Fig 2: Graphical representation of heritability (broad sense) and genetic advance as per cent of mean (5%)

Table 2: Genotypic and phenotypic path coefficients of GPC and other traits

Trait		Milling	HRR	KL	KB	L/B	AC	GC	РҮР	SPY
Milling	G	0.0084	0.0035	-0.0009	-0.0019	0	0.0002	-0.0006	0.0019	0.0014
	Р	-0.0107	-0.0046	-0.0015	-0.0012	-0.0017	-0.0019	0.0005	-0.0018	-0.0013
HRR	G	0.066	0.1578	-0.0106	-0.0721	0.0268	-0.0403	-0.0041	0.027	0.0091
	Р	0.0273	0.0637	0.0024	-0.018	0.0146	-0.01	-0.0016	0.0103	0.0033
KL	G	0.0923	0.0553	-0.8243	0.1339	-0.642	0.1042	-0.323	-0.1311	-0.2215
	Р	-0.0078	-0.0021	-0.0546	-0.0018	-0.0435	-0.0002	-0.0197	-0.0077	-0.0132
KB	G	-0.166	-0.3396	-0.1208	0.7432	-0.5529	0.1806	-0.0426	-0.1282	-0.088
	Р	-0.0032	0.0079	-0.0009	-0.0280	0.0145	-0.0096	0.0014	0.0043	0.0031
L/B	G	0.0066	0.2006	0.92	-0.8789	1.1814	-0.2989	0.374	0.2464	0.3162
	Р	0.0028	0.0041	0.0141	-0.0092	0.0177	-0.0026	0.0054	0.0034	0.0044
AC	G	0.0001	-0.0018	-0.0009	0.0017	-0.0018	0.0070	-0.0032	0.0001	0.0003
	Р	-0.0048	0.0042	-0.0001	-0.009	0.0039	-0.0265	0.0114	-0.0005	-0.0009
GC	G	-0.0003	-0.0001	0.0017	-0.0003	0.0014	-0.002	0.0044	0.0001	0.0011
	Р	0.0002	0.0001	-0.0017	0.0002	-0.0014	0.002	-0.0047	-0.0001	-0.0011
PYP	G	0.3582	0.2643	0.2453	-0.2661	0.3217	0.0304	0.0477	1.5428	1.2552
	Р	0.2524	0.2421	0.212	-0.2301	0.2915	0.0261	0.0459	1.4960	1.2105
SPY	G	-0.2591	-0.0901	-0.422	0.186	-0.4203	-0.0618	-0.3879	-1.2776	-1.5703
	Р	-0.1762	-0.0781	-0.3603	0.1641	-0.3748	-0.0513	-0.3653	-1.2086	-1.4936
GPC	G	0.1062	0.2498	-0.2124	-0.1545	-0.0855	-0.0807	-0.3353	0.2816	-0.2966
	P	0.0802	0.2373	-0.1905	-0.1329	-0.0792	-0.074	-0.3267	0.2953	-0.2887

*HRR – head rice recovery %; KL – kernel length; KB – kernel breadth; L:B – length: breadth ratio; AC – amylose content; GC – gel consistency; SPY – single plant yield; PYP – protein yield per plant; GPC – grain protein content



Fig 3: Path diagrams (A) – Phenotypical path for GPC (B) – genotypical path for GPC

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