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RESEARCH ARTICLE

Study of Maternal Influence and Identification of Stable Donors for Grain Protein Content in Rice (*Oryza sativa* L.)

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Abstract

An experiment was conducted during *kharif* 2021 at Paddy Breeding Station (PBS), Coimbatore, Regional Research Station (RRS), Paiyur and Tamil Nadu Rice Research Institute (TRRI), Aduthurai; and during *kharif* 2022 at Paddy Breeding Station (PBS), Coimbatore, to identify the stable donors and to determine the existence of maternal influence for grain protein content. Genetic diversity studies using a panel of 169 rice genotypes, including landraces and cultivated varieties, revealed significant variation in grain protein content. The genotypes IG74, Gandhasala, Ponkambi samba, Purple puttu and Burma kavuni were found to be promising and stable genotypes with higher grain protein content (>10%) across three locations. The genotypes IG74 and Gandhasala were found to be the best parents for the breeding programme to enhance grain protein content together with grain yield. The maternal influence on grain protein content was observed, which indicated that high protein donors could be recommended to be used as female parents.

Keywords: Combining ability, D^2 analysis, Maternal influence, Protein content, Rice, Stability

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Introduction

Rice (*Oryza sativa* L.) is the sole crop among cereals containing a storage protein called "Oryzenin" which have more balanced amino-acid profile when compared to the prolamin-rich storage protein. Rice grain protein content (GPC) plays a crucial role in determining the cooking, nutritional and eating quality (Chen *et al*., 2023). The protein components of rice are generally considered hypoallergenic and are suggested that the rice proteins might exert several health benefits with their anti-hypertensive, anti-oxidative, anti-cancer and anti-obesity activities (Amagliani *et al*., 2019). Approximately one-third of the global population is currently suffering from protein malnutrition. Though rice usually has the lowest grain protein content among cereal grains, the net protein utilization from rice is higher than that of the other cereal grains (Juliano, 1992). ICAR-NRRI developed India's first high-protein rice varieties, CR Dhan 310 (10.3% protein) and CR Dhan 311 (Mukul; ~10.1%), through classical backcross breeding in 2015–2016, demonstrating that high protein content can be combined with high grain yield (4.5 and 4.3 t/ha, respectively) (Mahender *et al.,* 2016). Both these high-protein varieties CRDhan 310 and CRDhan 311, were found to contain QTL (*qGPC1.1*) on chromosome 1.

The unique germplasm collections in India have several potential genes quite obvious to substantiate the nutritional needs of the global population. Hybridization among such diverse germplasm accessions helps to generate desirable recombinants for the rice improvement programme. A successful breeding programme requires knowledge of gene interactions, selection strategies, identifying the donor parents and choosing optimal parent combinations. Diversity could be well depicted by D^2 and Principal Component Analysis, which play a crucial role in the selection of parents for hybridization programme. Among several mating designs, Griffing's full diallel analysis involving the reciprocal crosses play unique role in determining the existence of maternal effect. Keeping these points in view, a study was taken up to improve grain protein content in rice through the selection of parents based on stability, PCA and D^2 analysis and development of F_1s to study the gene action and maternal influence on grain protein content.

Materials and Methods

To assess the G×E interaction on protein content, a panel containing 169 rice genotypes, which included landraces, improved lines and cultivated varieties were raised in an alpha lattice design, consisting of 13 blocks. Each genotype was raised in two rows with two replications adopting a spacing of 50 cm × 30 cm between the rows and plants with a single seedling per hill in each replication in three different environments *viz.,* Paddy Breeding Station (PBS), Coimbatore, Regional Research Station (RRS), and Paiyur and Tamil Nadu Rice Research Institute (TRRI), Aduthurai, during *kharif* season of 2021 (Table 1). The experimental material comprises landraces, improved lines and cultivated varieties of different origins. The following parameters were studied: DFF - days to 50% flowering (days), PH - plant height (cm), NPT - number of productive tillers (no.), PL - panicle length (cm), FLL - flag leaf length (cm), FLW - flag leaf width (cm), NFG - number of filled grains per panicle (no.), TGW - 1000-grain weight (g), SPY - single plant yield (g) and PC - protein content (%).

Grain protein content was estimated using high-throughput Near Infra-Red Spectroscopy (NIR; ZEUTEC Spectra Analyzer™), which was standardized and calibrated using the Kjeldahl method. Based on stability analysis (AMMI and GGE biplots), D^2 analysis and Principal Component Analysis (PCA), a total of six parents were selected with high protein (>10%) in IG74 and Gandhasala; medium (8-10%) in CO52; and low protein (<8%) in CRDhan315, TKM13 and Vellai chithiraikar. The selected lines were subjected to a 6 × 6 full diallel method to study direct and reciprocal combinations during *rabi* 2021. Thus, thirty hybrids and the respective parents were raised in PBS, Coimbatore, using a randomized block design with two replications during *kharif* 2022. The observations on ten agronomic traits were recorded using SES, IRRI, Philippines (2013) for both diversity and diallel studies. In the F_1 generation, grain protein content varied from 6.87 % (CRDhan315 × Vellai chithiraikar) to 11.66% (IG74 × TKM13) with an average of 8.69 %.

Stability analysis was performed based on AMMI (Gauch *et al*., 2008) and GGE biplots (Yan *et al*., 2000) using PBTools version 1.4. The genetic diversity was estimated by the Mahalanobis D^2analysis (Mahalanobis, 1936) using WINDOWSTAT version 7.1 and Principal Component Analysis (Pearson,1901) using GRAPES version 1.1.0 software. The diallel analysis, Method I (F_{1s} + F_{1s} reciprocal and parents) and Model I (fixed effect) of Griffing (1956) and Hayman's (1954) was performed to determine the gene action governing the protein content and other traits. The ANOVA and combining ability were performed using the TNAUSTAT statistical packages.

Results and Discussion

All 169 diverse rice genotypes displayed distinguished variation for grain protein and yield-related traits. Though all three environments had low levels of available soil nitrogen content, the Coimbatore location had relatively higher available soil nitrogen content with

Table 1: Meteorological data from different locations used in the study

Location	Latitude rainfall	Longitude	Altitude	Average	Soil type	N (kg/ha)	P (kg/ ha)	K (kg/ ha)
Coimbatore	11° N	77° E	427 m MSL	670 mm	Clayey	225	20.5	585
Paiyur	12°21′ N	78°18' E	490 m MSL	918 mm	Red loamy sand to sandy loam	112	14.7	329
Aduthurai	11° N	79° E	19.5 m MSL	1139 mm	Alluvial clay	194	20.3	615

medium P and high K than the other two locations (Table 1), which was reflected by higher grain yield and grain protein content as compared to the rest of the locations. As soil fertility status of RRS, Paiyur was relatively low, preferably in terms of available soil N, the yield level and grain protein content were also recorded to be low as compared with Coimbatore and Aduthurai. Nitrogen content is positively correlated with grain yield and grain protein content (Liang *et al*., 2021). The pooled mean of grain protein over three environments ranged from 6.45% in Varigarudan samba to 10.77% in IG74, followed by 10.69% in Gandhasala. The genotypes Gandhasala and IG74 have higher protein content (>10%) and greater single-plant yield, as previously reported by Dhanuja (2021) and Jangala *et al.,* (2022). And hence, these two genotypes could be used as donors to enhance protein content. Concerning the yield-related characters, the number of filled grains per panicle ranged from 38 (IG43) to 159 (Sembarai) and for thousand-grain weight, Mapillai samba recorded the highest (29.08g), and Jeeraga *samba* recorded the lowest (10.08 g).

The results of AMMI and GGE biplots indicated that, out of 169 genotypes, IG74, Gandhasala, Ponkambi samba, Purple puttu, and Burma kavuni performed stably with respect to increased protein content (>10%) across all three locations (Fig. 1). The PCA analysis also showed a similar trend (Fig. 2). Hence, these genotypes can be considered for further breeding purposes for grain protein improvement. The combined D^2 analysis across locations adopting Toucher clustering grouped the genotypes into eleven clusters with a cut-off value of 107.53 (Fig. 3). Most of the landraces were grouped in cluster III, and cluster V might be due to the close genetic relationship among themselves (Ariharasutharsan *et al*., 2022). It was quite interesting to observe that the parents selected based on mean performance were distinctive in such a way that they fell into different clusters *i.e.,* cluster IV (IG74) and cluster XI (Gandhasala) contain genotypes with high protein, and cluster V with low and medium protein (CRDhan315, Vellai chithiraikar; CO52, TKM13) and These genotypes with their unique traits can be utilized for hybridization to exploit transgressive segregants for grain protein content, leveraging their distinct nature to combine different alleles in a single population. The inter-D2 cluster distances were highest between clusters I and XI (D = 56.22), and the maximum intra-cluster distance observed was in cluster X (D = 21.64). Notably, maximum values of cluster mean were observed, including single plant yield (47.94 g) in cluster II, grain protein content (9.96%) in cluster VI, thousand grain weight (25.03 g) in cluster XI and number of filled grains (112.28 nos.) in cluster II. Therefore, it is suggested to cross cluster II with cluster IV to attain superior genotypes with enhanced yield and protein content simultaneously.

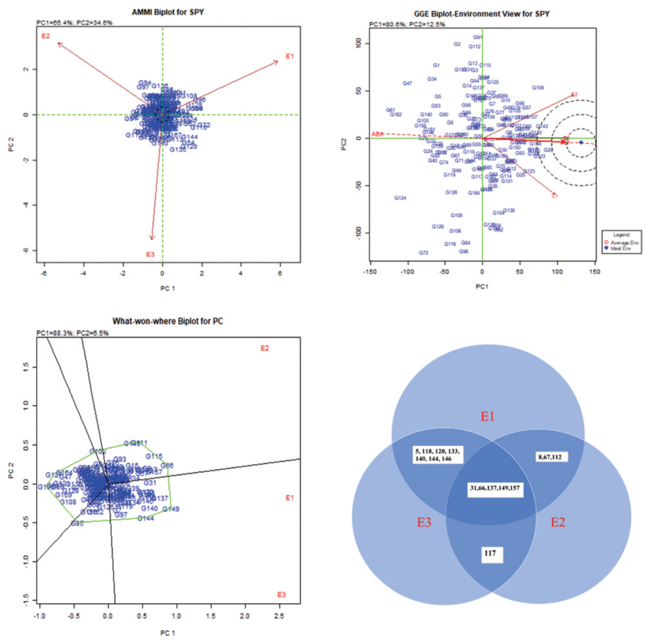

Fig. 1: AMMI and GGE biplots for Single plant yield and Which Won Where Biplot for PC and Representation of Stable genotypes using Venn diagram across three locations. Where, 31 - Purple puttu, 66 - IG74, 137 - Ponkambi samba, 149 - Gandhasala, 157 - Burma kavuni.

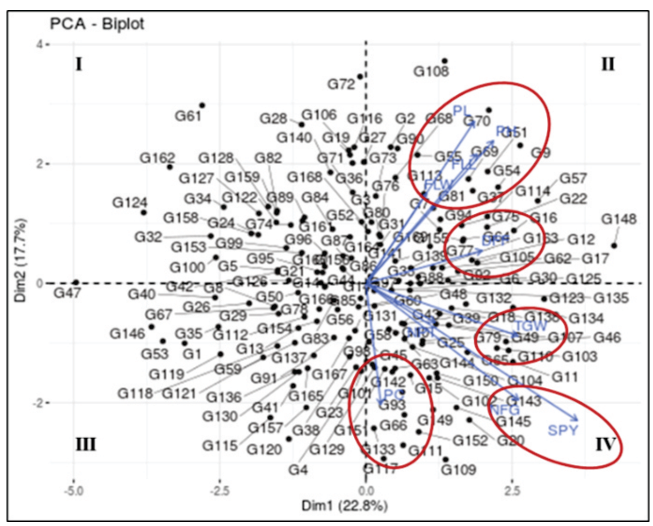

Fig. 2: PCA biplot of 169 rice accessions and 10 traits plotted by PC1 versus PC2 component.

Among the studied traits, the number of filled grains per panicle contributed the maximum to the genetic divergence with 71.72%, followed by thousand grain weight (9.59%), whereas the traits panicle length and flag leaf width showed very low contribution to the genetic divergence. The results suggested that for the selection of genetically diverse accessions, one should categorize the materials based on the traits such as number of filled grains per panicle and thousand-grain weight, as these traits exhibited maximum contribution towards divergence. The 4.28% contribution for PC indicates that protein content contributed less to total divergence but still possesses usable genetic variability. Clusters with higher mean protein content can be exploited through inter-cluster crosses to transfer favourable alleles. Such crosses enhance recombination and indirect selection, increasing the chances of obtaining transgressive segregants with improved protein content.

The same panel with169 genotypes was subjected to PCA analysis and was understood that four Principal Components PC1, PC2, PC3 and PC4 have contributed 68.37% of total variability with eigenvalues of more than one, indicating that these principal components play a major role in creation of variation (Fig. 2). The PC1 component accounting 24.03% of the total variation and has positive loading values for all the traits studied. The genotypes with high PC1 scores are desirable for these traits were identified from the PCA biplot. PCA may not show a clear grouping pattern like D^2 because PCA focuses on variance structure and dimensional reduction, whereas D^2 explicitly measures genetic divergence and enforces clustering. PCA highlights traits with high variance, while D^2 considers cumulative differences across all traits. Traits contributing strongly to divergence may not necessarily explain large variance in the first few principal components. The genotypes Chinthamani, Karuka, Sadai samba, Earapalli samba and Thattan samba on Quadrant 4 exhibited significantly superior values for number of filled grains per panicle, thousand grain weight, single plant yield and IG74. Gandhasala and Vadakathi *samba* exhibited superior grain protein content. Under favourable environments, in genotypes with efficient nutrient use, both yield and protein content may increase simultaneously, resulting in a positive association in PCA. The "yield-protein trade off" is because higher grain yield usually results from increased carbohydrate (starch) accumulation. If nitrogen uptake or remobilization does not increase proportionally, protein concentration becomes diluted, leading to lower grain protein content. Genotypes with favourable yield and grain protein content traits might be utilised in a hybridization program with genotypes from other groups to improve other traits that contribute to overall yield and protein content. Bhargavi *et al*., (2023) have also reported that the rice genotypes with increased protein content, single plant yield and 1000-grain weight may be ideal while making selection for creating genetic diversity. In the present study, the encircling made in biplot exhibited that number of filled grains, single plant yield and thousand grain weight possess close vector angle towards protein content, this might be due to increase in grain density and cell surface area leads to accumulation of more protein content in aleurone layer which is in accordance with the results of Wang *et al*., (2012). While panicle length had a negative association with the number of productive tillers, single plant yield, number of filled grains and protein trait.

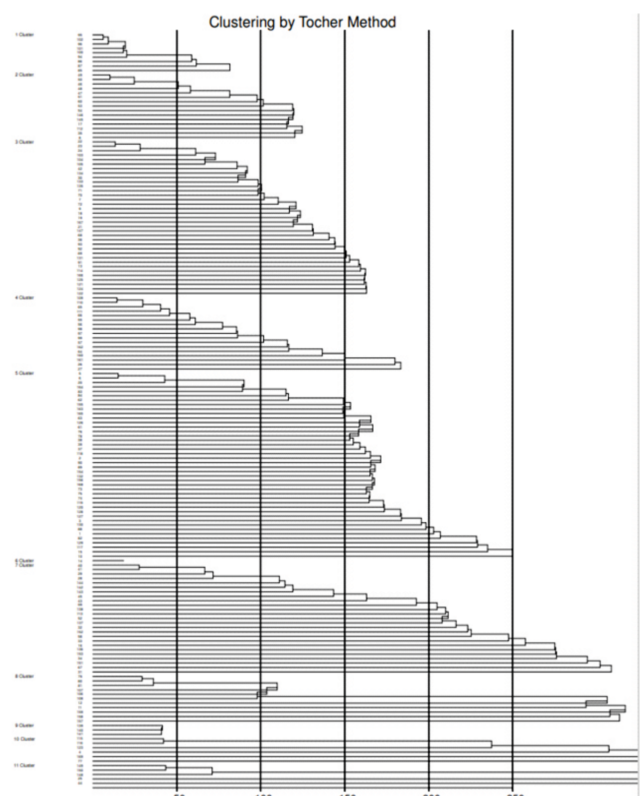

Fig. 3: Clustering of 169 rice genotypes for grain protein and yield related traits by Mahalonobis D^2 analysis over three environments.

The diallel analysis revealed that GCA and SCA variances were highly significant ($P \leqslant 0.01$) for all the traits, including grain protein content. This indicated the role of both additive and non-additive gene effects on the performance of hybrid combinations. In other studies, Bassuony *et al*., (2015) reported that grain protein content was governed by additive gene action and Lingaiah *et al*., (2021) reported that non-additive gene action. The parent IG74 had the highest significant *gca* for protein content, along with thousand grain weight and number of productive tillers with early duration, whereas the parent Gandhasala was adjudged to be the best combiner for protein content along with thousand grain weight. Hence, the good general combiners, IG74 and Gandhasala for grain

Table 2: General combining ability (*gca*) effects of parents for yield and grain protein content

Parents	DFF	PH	NPT	PL	FLL	FLW	NFG	TGW	SPY	TPDB	PC
IG74	-9.56**	-1.74	0.72*	-2.11**	-1.38**	-0.17**	-42.86**	0.33**	-0.07	-13.25**	0.95**
CO52	1.61**	-4.53**	-0.07	0.06	-0.18	0.05	19.60**	-0.08	2.40**	21.45**	-0.46**
TKM13	4.49**	-20.50**	0.10	-2.09**	-2.72**	0.10**	47.26**	-0.85**	-0.25	-12.39**	-0.42**
CRDhan315	1.69**	2.74**	0.51	1.97**	-0.19	0.06	3.51	0.03	-0.66	27.20**	-0.70**
Vellai chithiraikar	-1.18**	23.24**	-1.03**	3.40**	3.09**	-0.11**	-19.40**	1.02**	1.07**	-9.82**	-0.87**
Gandhasala	2.94**	0.78	-0.24	-1.23**	1.39**	0.07*	-8.11**	0.45**	-2.49**	-13.20**	1.50**

* Significant at 5% level, ** Significant at 1% level. DFF-Days to 50% flowering, PH-plant height, NPT-number of productive tillers, PL-panicle length, FLL-flag leaf length, FLW-flag leaf width, NFG-number of filled grains per panicle, TGW-1000 - grain weight, SPY-single plant yield, TPDB-total plant dry biomass, PC-protein content

protein content as well as for important yield-related traits, could be used in the breeding programme to exploit the additive genetic variance (Table 2).

The RCA (Reciprocal combining ability) variance was highly significant for grain protein content and other yield traits, indicating the contribution of the maternal effect on grain protein content, *i.e.* the influence of the maternal parent, to the phenotype of its progeny extends beyond the equal genetic contribution expected from each parent. The endosperm maternal effect for protein content was earlier narrated by (Shi *et al*., 2000; Anyanwu *et al*., 2015) since the grain protein mainly depends on triploid endosperm which derives two genomes from its maternal and one genome from its male parent. This also indicates the probable role of additive gene action for rice grain protein content. Therefore, care should be taken to fix the male and female parents due to the influence of maternal cytoplasm. While examining the mean performance and *rca* effect (Table 3) for grain protein content, the crosses having high *gca* × low *gca* (IG74 × TKM13), (IG74 × CRDhan315) and (Gandhasala × Vellai chithiraikar) were found to have significant mean performance as well as significantly influenced by maternal inheritance for protein content. This indicated that the donors for high protein content should only be used as female parents in the breeding programme to enhance grain protein content to exploit the maternal influence. In direct crosses, having low *gca* × high *gca,* (TKM13× IG74) and (CRDhan315 × Gandhasala), were found to have significant mean performance with a positive significant *sca* effect. These crosses can be

Table 3: Comparison of mean performance and maternal effects in direct and reciprocal cross for grain protein content

Reciprocal cross	Mean	Rca	Direct cross	Mean	SCA
IG74×CO52	10.13*	-0.35*	CO52× IG74	8.51	-0.69**
IG74×TKM13	11.66*	0.92**	TKM13× IG74	9.6*	0.76**
IG74× CRDhan315	9.77*	0.31*	CRDhan315× IG74	9.47*	-0.10
IG74× Vellai chithiraikar	7.63	1.10**	Vellai chithiraikar× IG74	7.7	-0.41*
IG74× Gandhasala	10.95*	0.27	Gandhasala× IG74	9.2	-0.17
CO52× TKM13	7.38	0.08	TKM13× CO52	7.8	-0.33
CO52× CRDhan315	7.48	0.09	CRDhan315× CO52	7.99	-0.76**
CO52× Vellai chithiraikar	7.61	0.70**	Vellai chithiraikar× CO52	7.1	0.07
Gandhasala× CO52	9.31	0.16*	CO52×Gandhasala	7.69	0.19
CRDhan315× TKM13	8.46	0.03	TKM13× CRDhan315	8.4	0.25
Vellaichithiraikar× TKM13	8.05	0.04	TKM13× Vellai chithiraikar	7.32	0.15
Gandhasala× TKM13	8.76	-0.10	TKM13 × Gandhasala	8.57	-0.72**
Vellaichithiraikar× CRDhan315	8.58	0.35*	CRDhan315 × Vellai chithiraikar	6.87	0.49**
Gandhasala×CRDhan315	8.5	0.12	CRDhan315 × Gandhasala	10.47*	0.48**
Gandhasala× Vellai chithiraikar	10.14*	0.61**	Vellai chithiraikar × Gandhasala	7.22	-0.10

*Significant at 5% level, ** Significant at 1% level adopted for recombination breeding in which the selection can be done in later generations to exploit both additive and nonadditive genetic effects. Interestingly, none of the high × high *gca* combination recorded a significant positive *sca* effect for protein content, depicting environmental interaction over the parental lines and hybrids. The lack of positive SCA in high × high GCA combinations for protein content is due to the predominance of additive gene action and environmental influence, rather than fixation alone. Such traits are better improved through selection in segregating generations rather than exploiting heterosis.

Considering the overall performance, IG74, Gandhasala, Ponkambi samba, Purple puttu and Burma kavuni were the stable high protein content genotypes across all locations. The genotypes IG74 and Gandhasala were found to be the best parents for improvement of grain protein content coupled with grain yield. Since the grain protein was influenced by additive as well non additive gene action, hybridization followed by postponement of selection in later generations could be rewarded. As the grain protein content was found to be influenced by maternal genes, the high protein-containing genotypes are recommended to be used as female parents in breeding programmes to cope with maternal influence.

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