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INHERITANCE OF PROTEIN PER GRAIN IN RICE

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ABSTRACT

Genetic control of the protein per grain (PPG) in rice (*Oryza sativa* L.) was investigated through nine-parent half-diallel analysis. Overall partial dominance of high PPG over the low was noted. However, at an individual loci, both the magnitude and the direction of dominance were inconsistent due to unequal distribution of dominant and recessive alleles, as well as the alleles with increasing and decreasing effects, in the parents. The dominant alleles were in excess of recessive, and a separate study had indicated that only the parents with the shikimate dehydrogenase-1^2 (Sdh-1^2) allozyme had an excess of alleles with increasing effects on PPG. Due to insignificant environmental variance recorded and the additive genetic component being larger than the dominance component, the heritabiltiy estimates both in broad sense (0.97) and narrow sense (0.71) were high, suggesting usefulness of early generation selection for the genetic improvement of PPG.

Key words: *Oryza sativa*, diallel analysis, seed protein content, protein per grain, heritability.

One-third of the world's population depends on rice (*Oryza sativa* L.) for over half of their caloric and protein requirements [1]. Wide variability for grain or brown (dehulled) rice protein (BRP) content (3–18%) [2] provides the opportunity for improving the nutritional value of high yielding cultivars through cross-breeding. Yet, inadequate information on genetics of the BRP content, coupled with lack of easily selectable and reliable markers, has hampered the efforts aimed at achieving high-protein rice. Due to complexity in the expression of the trait compounded with large environmental influences, little efforts have been made at understanding the genetics of BRP content [3]. Studies at the International Rice Research Institute (IRRI) have demonstrated that protein per grain (PPG) is much less influenced by environment, and compared with per cent BRP, is more effective as a selection criterion [4]. Subsequently, Sarreal [5] and Suprihatno [6] demonstrated that PPG also shows higher heritability values compared with per cent BRP. But information on the genetics of PPG is inadequate. The present study provides some details on this aspect.

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MATERIALS AND METHODS

Nine rice genotypes, viz., Kopansci Resista, Omirt-168, IR36, IR58, IR480-5-9-3-3, IR21313-36-3, IR 2153-338-3, Ping shan ta tswen ku (PSTTK), and IR19661-63-1, with diverse expression of PPG were crossed in all possible diallel combinations excluding reciprocals. The resulting 36 F_1 hybrids, along with the parents, were grown in randomized complete block design with four replications on irrigated Mahaas clay soils (Andaqueptic Haplaquolls) at the IRRI experimental farm, Los Banos, Philippines, during the 1986 wet season. A single row of 15 plants of each cross constituted the experimental plot. Four-week-old seedlings were transplanted one per hill, with 30 cm spacing between rows, and hill-to-hill distance of 25 cm. Sixty kg nitrogen was applied in two split doses (40 kg as basal dose at transplanting and 20 kg topdressed 65 days after seeding). A 5-cm water level was maintained throughout the cropping season, except during the last 15 days of the ripening phase. The panicles were harvested at maturity on individual plant basis from five randomly chosen plants per plot, excluding the border plants.

One hundred fully filled, healthy grains from each plant were randomly sorted out and their weight recorded. Ten grains were taken randomly from each of these, dehulled manually, and ground to fine powder. Each sample was subjected to the standard micro-Kjeldahl analysis to obtain N percentage. The BRP content was derived by multiplying N % with the factor 5.95, based on 16.8% N content of oryzenin, the major fraction of BRP [7]; and transposed to milligrams of PPG as follows [6]:

$$\text{PPG (mg)} = \frac{(\text{BRP \% x 100-grain weight, g})}{10}$$

Plot means were used for statistical and graphic analyses. The data were subjected to covariance (Wr), variance (Vr) graph analysis, and the genetic parameters were estimated following the procedures of [8, 9]. Analysis of standardized deviations graph of the parental means (Yr) and the parental order of dominance (Wr + Vr) was done by the method of Johnson and Aksel [10]. To test the adequacy of the additive–dominance model used and the overall assumptions of the diallel analysis, and to detect nonallelic interactions, the t^2 test and the test for heterogeneity of (Wr + Vr) and (Wr - Vr) were carried out following Allard [11].

RESULTS AND DISCUSSION

Cereal grains are mixoploids with three genetically distinct tissues involved [12], especially in hybrids. Since the grain proteins are predominantly concentrated in the triploid endosperm containing two maternal and a paternal genome, reciprocal differences for PPG in F_1 are inevitable. In F_2, such difference would have disappeared [13]. Mather and Jinks [14] also suggested the use of F_2 for reliable genetic analysis in hermaphrodite crops where

obtaining large quantities of F_2 is easier than getting F_1. As the embryonic and endosperm tissues are one meiotic cycle ahead of the plant that produced them, the crossed seed on the female parent itself would represent F_1 generation for PPG, and the seeds harvested from F_1 plants, the F_2 generation. Therefore, the PPG values obtained from the analysis of grain harvests of the F_1 plants represent F_2 generation, and hence, all the analysis and consequent interpretations were based on F_2 expectations.

The analysis of variance of plot means showed highly significant differences among the parents and their crosses. The mean PPG recorded in the parents and F_2 are summarized in Table 1. As expected, F_2 showed a larger spread (2.30 mg) compared to that of the parents (1.75 mg). Omirt-168 registered the highest PPG among the parents, and IR19661-63-1 the lowest. A close correspondence ($r = 0.93$) between the parental means and the array means suggests high prepotency of the parents in transmitting the PPG trait. The overall mean of F_2 (3.38 mg) was higher than that of the parents (3.29 mg), indicating partial dominance high PPG over low.

The basic assumptions underlying the diallel analysis [9] were fulfilled, as shown by nonsignificant t^2 (0.86). Further, the heterogeniety of (Wr + Vr) over the blocks implied occurrence of nonadditive gene action, and the nonsignificance of (Wr - Vr) differences over the arrays indicated that the observed nonadditive gene action was solely due to the dominance effects of the genes distributed independently among the parents (Table 2). This eliminated the need for χ^2 test for epistasis [14]. As a consequence, the regression of Wr on

Table 1. Protein per grain (mg) in the parents (in bold) and the F_2 in 9 x 9 diallel set of rice genotypes (mean of 4 replicates)

Female parent	Male Parents									Array mean
	Kopansci Resista	Omirt-168	IR36	IR58	IR480-5-9-3	IR21313-36-2	IR2153-338-3	Ping shan ta tswen ku	IR 19661-63-1	
Kopansci Resista	**4.15**	4.10	3.91	3.69	4.18	4.04	3.74	3.30	3.55	3.85
Omirt-168		**4.26**	4.11	4.12	4.71	4.20	3.94	3.77	4.15	4.15
IR36			**3.08**	3.16	3.79	2.76	2.79	2.46	3.16	3.25
IR58				**3.05**	3.61	2.82	2.93	2.62	3.11	3.12
IR480-5-9-3					**4.25**	3.27	3.38	3.01	4.09	3.81
IR21313-36-2						**2.74**	2.60	2.42	2.75	3.07
IR2153-338-3							**2.72**	2.45	2.86	3.05
Ping shan ta tswen ku								**2.83**	2.56	2.82
IR19661-63-1									**2.51**	3.19

Vr did not deviate significantly from unity, but did so from zero, implying absence of nonallelic interactions. Thus, validity of the additive–dominance model was established for the data analyzed, and we proceed for graphic analysis of Wr, Vr, and estimate the second-degree genetic parameters.

Table 2. Analysis of variance of covariance (Wr) and variance (Vr) estimates in 9 x 9 diallel cross for protein per grain in rice (4 replications)

Source	d.f.	MS	F
(Wr + Vr) array differences	8	0.207	6.53**
(Wr + Vr) block differences	27	0.032	
(Wr - Vr) array differences	8	0.002	0.03
(Wr - Vr) block differences	27	0.006	

**Significant at 1% level.

The regression line of Wr on Vr (b = 0.86) intercepted Wr axis above the origin (Fig. 1A). The point of interception was between the point of no-dominance (the point at which the tangent to the limiting parabola meets the Wr axis) and that of complete dominance (the point mid-way between the point of no-dominance and the orgin), indicating that dominance was partial. Omirt-168 was the most dominant parent, closely followed by Kopansci Resista. IR19661-63-1 was the most recessive parent, followed by IR21313-36-2. IR58, IR480-5-9-3, and PSTTK had more or less equal proportions of dominant and recessive alleles.

The theory of diallel analysis states that Yr is closely associated with the number of positive homozygotes, and (Wr + Vr) with the number of recessive homozygotes [9]. In the present context, the correlation between Yr and (Wr + Vr) was significant as well as negative (r = –0.75), indicating association of dominance with high PPG, corroborating the earlier inference from the F_2 means. The standardized deviations graph of Yr, (Wr + Vr) (Fig. 1B) confirms the results of Wr, Vr graph regarding the distribution of dominant and recessive alleles among the parents. In addition, it shows that the higher PPG in Omirt-168 and Kopansci Resista is governed by dominant alleles, and the lower PPG in IR19661-63-1 and IR 21313-36-2 is governed by recessive genes. However, PSTTK and IR480-5-9-3 having comparable dominance showed great difference in their mean PPG.

A separate study of the nitrogen-assimilating isozymes in rice indicated that the shikimate dehydrogenase-1^2 (Sdh-1^2) allele is associated with greater accumulation of oryzenin fraction, resulting in higher PPG [15]. This can explain the fact that IR480-5-9-3, along with Omirt-168 and Kopansci Resista, showed high PPG despite its comparable level of dominance with PSTTK, because it had the Sdh-1^2 allelozyme. Only the parents with Sdh-1^2 allozyme possess excess of alleles with increasing effect on PPG. Further, if the mean PPG of the three parents with Sdh-1^2 allozyme and their crosses are excluded, the overall mean of F_2 (2.76 mg) falls below the overall mean of the parents (2.82 mg), suggesting partial dominance of low PPG over high. This can probably explain why there are reports of the dominance of high grain protein content over low [16, 17], and vice versa [4, 6, 13].

The genetic components of variation estimated are presented in Table 3. D, the additive component; H_1 and H_2, the dominance components; and E, the mean of covariance of additive and dominance effects over the arrays, were significant, substantiating the adequacy of additive–dominance model employed. However, estimates of h^2, the dominance effect as the algebric sum over all loci in heterozygous phase in all the crosses; and E, the environmental component of variation, were unreliable because of their high standard errors.

Table 3. Estimates of genetic and environmental components of variation in F_2 of 9 x 9 diallel cross for protein per grain in rice

Component	Estimate ± S. E.
D	0.51 ± 0.02**
H_1	0.28 ± 0.05**
H_2	0.17 ± 0.04**
h^2	0.03 ± 0.03
F	–0.18 ± 0.05**
E	0.01 ± 0.01
$[H_1/4D]^{0.5}$	0.37
$[H_2/4H_1]$	0.15
$[h^2/H_2]$	0.17
$[(F/2)/D(H_1 - H_2)]^{1/2}$	$0.38^{1/2}$
[KD/KR]	0.62
Heritability (broad sense)	0.97
Heritability (narrow sense)	0.71

**Significant at 1% level.

Inequality of the H_1 and H_2 estimates ($H_1 > H_2$) indicates that the alleles with positive and negative effects are unequally distributed in the parents. The higher value of D than H suggests higher degree of additive action compared to that of dominance. The proportion $(H_1/4D)^{1/2}$, estimate of average degree of dominance over the loci was between 0 and 1, suggesting average partial dominance over all the loci across all the parents. This agrees with the partial dominance indicated by the intercept of the Wr, Vr regression line. The consistency of the level of dominance over all the loci in the parents is measured by the ratio $[(F/2)/D(H_1 - H_2)]^{1/2}$ [18]. In this context, the estimated ratio of 0.38 being less than unity implies that the level of dominance varied over the loci. The ratio of the total number of dominant to recessive alleles KD/KR was 0.62, suggesting that there are more recessive alleles controlling PPG in the parents than dominant ones. This is substantiated by the negative value of the parameter F.

The average frequency of alleles with positive and negative effects and ambivalence in the direction of dominance render the assumptions underlying the validity of the ratio h^2/H_2, (number of effective factors controlling the trait and exhibiting dominance in parents) unfulfilled, leading to invalid estimate of the parameter.

The high heritability values in broad sense (0.97) and narrow sense (0.71) were the consequence of nonsignificant environmental variance recorded, and higher proportion of the additive effects noted. This corroborates earlier reports regarding low influence of environment on PPG and also higher heritability values of PPG [4–6]. Such high heritability indicates that early generation selection for PPG can be effective at achieving high PPG genotypes. Further, partial dominance of high PPG should be taken cautiously, as at individual loci the magnitude and direction of dominance are ambivalent [19]. Under such circumstances, Sdh-1^2 allozyme can be a very useful marker for selecting appropriate

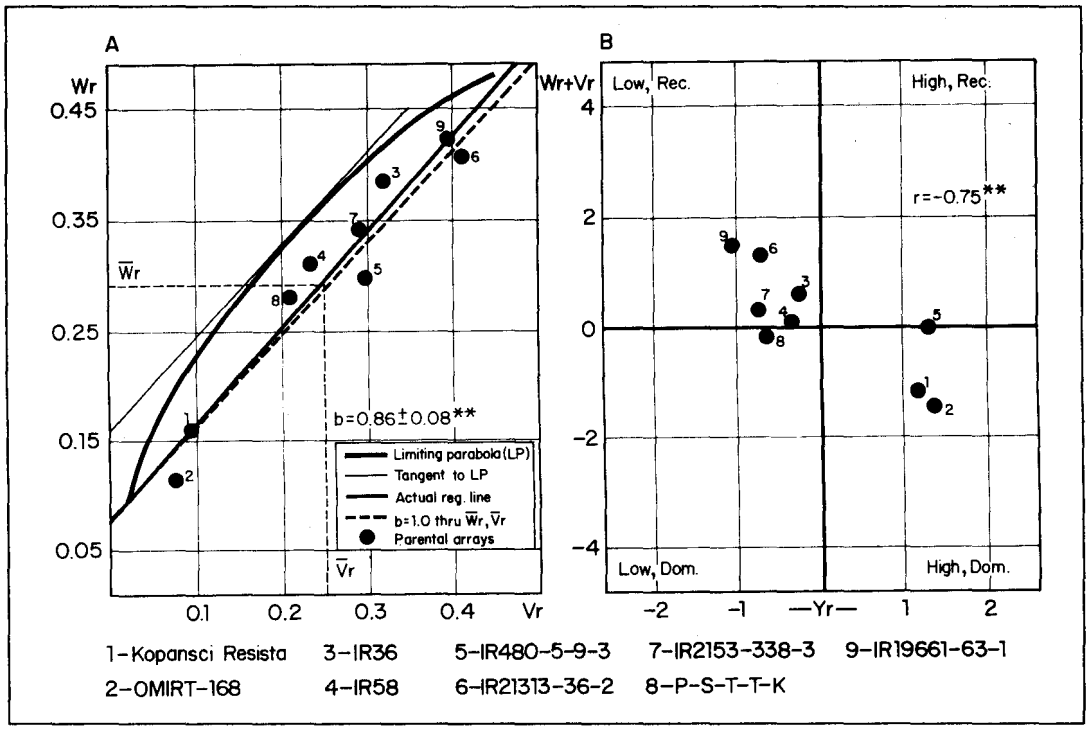

Fig. 1. [A] Covariance (Wr), variance (Vr) graph; and [B] standardized deviations plot of parental means (Yr), and the parental order of dominance (Wr + Vr) for protein per grain trait in 9 x 9 parental F_2 diallel cross of rice genotypes.

parents and for subsequent early-generation selection of high PPG homozygotes, as has been shown by Shenoy et al. [20].

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