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Genetic Analysis for Protein Content in Rice (*Oryza sativa* L.) Varieties

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Abstract: Seven rice (*Oryza sativa* L.) varieties, including Deilmani, Hassani, Shahpasand, Saleh, Sepidrod, Neda and IRFAON-215 were used in a partial diallel mating system. The genotypes contain parental and their hybrids varieties were arranged in a randomised complete block design with three replications. Protein content was determined by Kjeldahl extraction methods for the determination of nitrogen. Analysis of variance showed highly significant differences among genotypes. The unit slope of the regression lines suggested that all the diallel assumptions have been met to additive-dominance model. The significance of (a) and (b) components showed the presence of additive and dominance effects. The positive values of the F component indicated excess of dominant alleles were present in the genetic material. A moderate estimate of heritability in narrow sense represents fixable, additive heritable variation, which indicated that response to selection should be rapid for this trait. Although the diallel analysis revealed the importance of both additive and non-additive gene effects in controlling the observed variation, the effects of the former appeared to be less pronounced. Using biparental or recurrent selection in segregating material, followed by conventional selection, is likely to lead to substantial trait improvement.

Key words: Rice · Diallel method · Protein content · Genetic components

INTRODUCTION

Rice is one of the most important crops in the world and a staple food for more than half of humanity that is the principal source of protein in the most rice eating countries. Protein content can define most of the physicochemical properties of cooked rice [1]. Breeders have given more attention to the improvement of rice quality traits such as morphological and physicochemical characteristics of the grain [2]. In rice whole grain, in addition to amylose and amylopectin, there are the other components such as proteins, lipids, pentosans and minerals (phosphorous, calcium, ferrum, zinc, manganese, copper and silica). The primary chemical components of the grain are starch, protein and lipids. These components causes to used rice whole grain, flour or starch. The most abundant are protein and lipids [3]. Juliano [4] stated rice is unique among cereals because it has a storage protein made of *oryzenin*, which has a more balanced amino-acid profile compared to the prolamin-rich storage proteins. Indica rices have a protein content that varies from 4.9 to 19.3% and japonica rices may contain 5.9 to 16.5% [5]. *Japonica* rice had 20% prolamin and *indica* rice had 30% as reported by Hibino *et al*. [6].

Protein content of rice has been studied by some of researchers and they indicated that this trait is quantitative trait [7, 8]. Protein content was controlled by genetic effects of triploid endosperm, cytoplasm and diploid maternal plant [9]. Shi *et al*. [10] reported that protein content was controlled by genetic main effects as well as GE interaction effects; they found the embryo interaction effects as effects of triploid endosperm, cytoplasm and diploid maternal plant.

The protein content and composition of amino acids in indica rice had shown high heritability and significant variation among genotypes [11]. Won *et al*. [12] indicated the effects of dominance were highly significant for protein content, indicating the importance of the dominance gene action on protein content.

The objectives of this study were to estimate genetic components of variance and heritability for protein traits of *indica* rice.

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Table 2: Analysis of variance for Protein content (%)

Source of Variation	d. f.	MS
Replication	2	0.176^{ns}
Genotype (G)	27	2.208**
Error	54	0.132
CV%		5.05%

ns: not significant, **: Significant at 1% of probability level

MATERIALS AND METHODS

Plant Materials and Experimental Design: Seven rice (*Oryza sativa* L.) varieties, including Deilmani, Hassani, Shahpasand, Saleh, Sepidrod, Neda and IRFAON-215, were used in a partial diallel mating system. Some characteristics of the selected parents are shown in Table (1). In the choice of these parental lines, we considered quality and some of agronomic traits. Plants were grown at the Rice Research Institute of Iran in Rasht. Seedlings were transplanted to the field 25 days after sowing the seeds in the nursery. The genotypes contain parental and their hybrids varieties were arranged in a randomised complete block design with three replications, using 25×25 cm spacing in four-row plots of three m in length. This arrangement was chosen due to uniformity of the field. A suitable rate of chemical fertiliser, containing nitrogen (200 kg/ha) and phosphorus (100 kg/ha), was applied. Nitrogen was applied in three stages as follows: one-third as a basal rate at the time of transplanting the seedlings, one-third at 30 days after transplantation and the rest at the time of panicle initiation. Phosphorus was used at the transplanting stage. Two hand weedings were done 30 and 50 days after transplanting the seedlings. A permanent flood water level was maintained at 10 cm.

Measurement of Protein Content: The measurement of protein content was carried out in the laboratory of the Department of Agronomy and Plant Breeding in Islamic Azad University of Rasht (Guilan Province), Iran. After harvesting the rough rice, a clean 150 g sample from each plot was hulled with a Satake laboratory huller (Satake Engineering Co. Ltd, Japan) and milled for 30 s with an M. C. Gill miller (Baldor Electric Company, USA). Then, 50 g samples were ground into rice flour with a Cyclone Sample Mill (UDY Corporation, USA) and thoroughly mixed. Protein content percentages (PC) were determined by Kjeldahl extraction methods for the determination of nitrogen [13]. A factor of 5.95 was used to convert nitrogen% to protein.

Statistical Analysis: The data was analyzed by Fisher's analysis of variance. A significant difference in protein content was assumed to imply that genetic differences were present. Simple additive–dominance model approach of Hayman [14, 15] was followed for genetic analysis and for the estimation of components of genetic variation.

A diallel analysis using the protein content data was carried out according to Hayman [14] to obtain the mean squares components. The following statistical model was used:

$$Yrm = m + Jr + Jm + Kr - Km + Krm + Erm$$

Where, Jrm=L+Lr+Lm+Lrm; m= general mean; Jr, Jm= average deviation from general mean due to the r^{th} and m^{th} parents, respectively; Jrm= residual difference of the mean of the reciprocal crossings; Kr, Km = difference between the r^{th} or m^{th} parent effect used as male of female in the crossing; Krm = residual difference in the reciprocal difference in the mr^{th} order; L = average dominance deviation; Lr = dominance deviation due to the r^{th} parent; Lrm = deviation of the residual difference from the mean of the reciprocal crossings rm; Erm = average experimental error.

The slope of the regression line 'b' and the Y-intercept 'a' were obtained from the relationship where:

Wr = a + b Vr; a = Wr - b Vr; and b = Cov. Vr. Wr / Var. Vr. Where, Vr = Variance of all the offspring in each parental array, when an array consisted of the parental mean and mean values of all the crosses involving that parent; Wr = the covariance between parents and their offspring of the offspring in each parental array with non-recurring parent. The significance of difference of 'b' from zero and from unity was tested using 't' value of (b-0)/SE (b) and 1-b/SE (b), respectively, with n-2 degrees of freedom. The significance of first calculated 't' implies that 'b' is significantly different from zero. Hence, a second 't' test is applied to detect whether 'b' deviates from unity or not. Non-significant 't' at n-2 degrees of freedom indicates that the calculated value of the regression coefficient does not deviate significantly from unity. This satisfies the assumption regarding the adequacy of the additive-dominance model. Conclusively, if regression coefficients were significantly different from 0, but not from 1, indicated the gene action fitted into additive-dominance model. While the differences b from 1 revealed that the gene action was not suitable well to additive-dominance model.

Calculation for the analysis of (Wr-Vr) and the following genetic components, ratios and estimators were included in the dial 98 program [16]. (Wr + Vr) = an estimator of the order of dominance of the parents as indicated by the relative values of each parent. Low values of (Wr + Vr) indicate high levels of dominance while high values indicate low dominance. D = component of variation due to additive effects of genes, Fr = Co-variance among additive and dominant effects in the hybrids with i^{th} parent, F = Fr mean, relative frequency of the recessive and dominant genes in the population, an indicator of excess of dominant or recessive genes in the parent. A positive sign indicates an excess of dominant alleles of dominant effects on the parents while a negative sign indicates the same of recessive alleles. A value of F = 0 indicates that either no genes exhibited dominance or that the dominant and recessive alleles of each gene are distributed equally among the parents.

H_1= variation due to the dominance effects of genes, H_2= variation caused by dominance effects, corrected for gene distribution, H_1- H_2= Theoretically H_1-H_2>0. If H_1=H_2, the genes with positive and negative effects are present at equal frequencies. h^2 = the summation of dominance deviation over all loci or total genetic dominance component relative to heterozygous loci. When the frequency of dominant and recessive alleles is equal, then H_1=H_2=h^2. Significance of h^2 confirms that dominance is unidirectional. E=environmental component as estimated by the error mean square from the analysis of variance. $(H1/D)^{0.5}$= a weighted measure of the average degree of dominance at each locus with a value of zero indicating no dominance, a value of 1 indicating complete dominance and a value grater than 1 indicating over-dominance. Partial dominance results in a value between 0 and 1. The dominance component H1 is used in this ratio because it has the same coefficient as D, Hayman [14].

$H_2/4H_1$ = an estimator of the average frequency of negative versus positive alleles at loci exhibiting dominance. It has a maximum value of 0.25 when p = q = 0.5. Value less than 0.25 indicate that the additive components do not contain all dominance effects. Therefore, the above ratio of average degree of dominance would not be accurate Mather and Jinks [17].

$\frac{KD}{DR}=\frac{(4DH_1)^{1/2}+F}{(4DH_1)^{1/2}-F}$ = A ratio of the total number of dominates genes to recessive genes in all the parents.

h^2/H_2 = number of gene groups which control the traits and show some degree of dominance.

$h_n^2=\frac{D-F+H_1+H_2}{D-F+H_1-(1/2)H_2+2E}=\frac{(1/2)D}{(1/2)D+(1/4)H_1+E}$, narrow sense heritability

$h_b^2=\frac{D-F+H_1-(1/2)H_2}{D-F+H_1-(1/2)H_2+2E}=\frac{(1/2)D+(1/4)H_1}{(1/2)D+(1/4)H_1+E}$, broad sense heritability.

The above estimators were calculated only when the genetic components in the respective ratios were significantly different from zero. The t test was used for testing the significance of these parameters and an estimate was considered significant at the 5% probability level, when its division by the respective standard deviation was greater than 1.96 [18].

RESULTS

Analysis of variance showed highly significant differences among genotypes with respect to protein content. The replication effect was not significant for this trait (Table 2).

Table 3 indicated variation among the seven parents and their progenies in terms of protein content. Protein content varied from 5.54% (Sepidrod) to 8.3% (Shahpasand). The average ranges of protein content in progenies were similar to those in the parental groups with the exception of a few combinations. Protein content ranged from 5.27% (N×IR) to 9.11% in Sh×H. Only three combinations had more or less protein content than the parent with the highest value (N×IR, Sh×H and D×H).

The regression coefficient (b) of the covariance of parents and successors (Wr) on the variance of the series or parent varieties (Vr) (b=0.818±0.36) indicated that the regression coefficient depart significantly from zero and not from unity, suggesting no non-allelic interaction and an independence of genes distribution among the parents (table 4). This not difference to one and a significant difference to zero, is required conditions in the model according to Hayman [14]. Consequently, the unit slope of the regression lines suggested that all the diallel assumptions have been met Mather and Jinks [17].

Therefore, the analysis of the diallel crossings according to Hayman [14, 15] carried out for protein content; this approach provides very valuable information about the genetic properties of the investigated trait.

The data in Table (5) contains the significance levels of the diallel analysis of variance components. The significance of (a) and (b) components showed

Table 1: Information of some of important traits on parental varieties

Varieties	Origin	Grain type	Stature	Duration (d)	Grain yield (g/plant)
Shahpasand	Iran (land race)	Long	Tall	116	31.15
Hassani	Iran (land race)	Coarse	Tall	105	23.95
Deilmani	Iran (land race)	Fine	Tall	120	26.56
Sepidrod	Iran (Improved cultivar)	Long	Dwarf	115	42.89
Neda	Iran (Improved cultivar)	Medium	Dwarf	125	35.83
Saleh	Iran (Improved cultivar)	Long	Semi-dwarf	112	31.00
IRFAON-215	IRRI (line)	Coarse	Semi-dwarf	135	35.09

Table 3: Means of protein content (%)in seven parents and their partial diallel progenies in rice

Genotype	IRFAON-215	Saleh	Deilamani	Neda	Sepidrod	Hassani	Shahpasand
Shahpasand	6.03	8.06	7.90	5.79	8.03	9.11	8.3
Hassani	6.06	8.08	8.47	7.38	7.8	7.61	
Sepidrod	5.83	7.29	7.38	8.27	5.54		
Neda	5.27	6.43	7.38	7.99			
Deilamani	6.00	7.61	8.15				
Saleh	6.43	7.40					
IRFAON-215	6.00						
LSD1%=0.77							
LSD5%=0.58	μ=7.20						

Table 4: Test of regression coefficients of Wr/Vr for Protein content (%) in rice

Character	values
$b \pm s_b$	0.818±0.36
H_0: β=0, t value	2.288**
H_0: β=1, t value	0.509^{ns}

ns: Not significant, **: Significant at the 1% probability level.

Table 5: Estimation of genetic parameters for Protein content (%) in rice

Source of Variation	d. f.	MS
Replication	2	0.18^{ns}
Additive effect (a)	6	5.68**
Dominance effect (b)	21	1.21**
Directional dominance effect (b_1)	1	0.14
Gene distribution among the Parents (b_2)	6	2.41**
Effects of specific genes (b_3)	14	0.77**
Error	54	0.13

**: Significant at the 1% probability level.

the presence of additive and dominance effects. The b_1 item's that showed directional dominance, was non significant. The (b_2) portion of the (b) item was significant, which showed symmetrical distribution of genes. The (b_3) item was significant and indicated effects of specific genes.

With regard to the genetic components estimated by the diallel analysis (Table 6), the additive component (D) was significant. This confirmed the additive effects of the genes. Dominance components (H1 and H2) were significant, which showed dominance effects of genes. The positive values of the F component indicated excess of dominant alleles were present in the genetic material. The non-significance for the component h^2 confirmed that dominance was not unidirectional. The ratio $(H1/D)^{0.5}$ measured the overall degree of dominance, which was in the range of partial dominance. The ratio $H_2/4H_1$, estimated the frequency of negative versus positive alleles at loci exhibiting dominance, it was less than 0.25, which indicated that the additive components did not contain all the dominance effects. Estimates for the ratio of dominance to recessive genes in the parents [KD/(KD+KR)] were less than 1.0, which indicated the presence of an excess of recessive genes in the parents.

Table 6: Estimates of genetic parameters for Protein Content (%) in rice

Genetic parameters	Protein content (%)
Additive variance (D)	1.1135±0.2375
Dominance variance (H_1)	2.8490±0.3816
Dominance variance (H_2)	1.7933±0.2223
Relative frequency of dominant and recessive alleles (F)	0.6592±0.3368
Square of difference parents versus all all (h^2)	0.0083±0.0818
Environmental variance, whole (E)	0.0669±0.0133
average degree of dominance ($(H1/D)^{0.5}$)	0.95±0.178
Proportion of dominance genes (kd/(kd+kr))	0.5925±0.0351
Average direction of dominance (h)	-0.1967±0.1816
Broad-sense heritability (h^2b)	0.947±0.011
Narrow-sense heritability h^2n	0.594±0.011
Proportion of dominance and recessive genes ($H_2/4H_1$)	0.157±0.008

Table 7: Estimation of dominance ratios of parents for Protein content (%) in rice

Parents	Dominance ratios
SH	0.457
H	0.513
SP	0.317
N	0.596
D	0.615
SA	0.731
IR	0.919

SH: Shahpasand; H: Hassani; SP: Sepidrod; N: Neda; D: deilamani; SA: Saleh; IR: IRFAON-215.

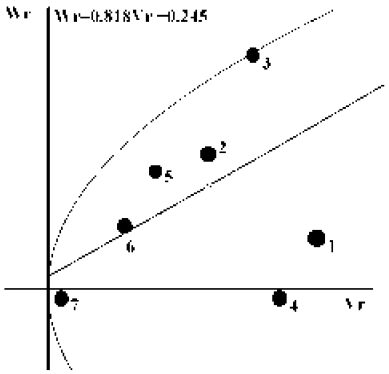

Fig. 1: Regression of Wr/Vr for protein content in rice. 1: Shahpasand; 2: Hassani; 3: Sepidrod; 4: Neda; 5: deilamani; 6: Saleh; 7: IRFAON-215.

The more than 1.0, indicated the presence of an excess of dominant genes in the parents. A moderate estimate of heritability in narrow sense (0.60) represents fixable, additive heritable variation, which indicated that response to selection should be rapid for this character. The moderate estimate of heritability was also revealed the role of dominance gene action in controlling of protein content. This offers a lot of scope for improvement of the character through individual plant selection. According to Hayman [19], epistasis can decrease or increase degree of dominance, which also effect on heritability estimates.

The distribution of the parent varieties for protein content was presented in Fig. (1), because the regression line of the wr-axis cuts in the positive area, one can assume a partial dominance of the genes controlling this characteristic. This result comes from the significant dominance ratio (Table 6). Moreover, IRFAON215 and Saleh revealed the most dominant genes for protein content; in contrast Sepidrod displayed the most recessive genes as shown in Table (7).

DISCUSSION

In order to increase rice protein content, breeders must understand the mechanism of inheritance of this trait and the genetic worth of the parents or the hybrid vigour of crosses. The efficiency of rice breeding programs based on protein content can be increased with the knowledge of the genetic mechanism. Protein content was controlled by additive and non-additive effects and exhibited moderate narrow-sense heritability. This result is similar to report of El-Kadyand El-Hissewy [20] due to contrlloling of protein content by additive and non-additive actions. Won *et al*. [12] indicated the effects of dominance were highly significant for protein content, indicating the importance of the dominance gene action on protein content. It seems that improvement of protein content of rice would be possible through selection for this trait by utilizing the genetic material under study.

The inheritance pattern of variation for protein content in the seven rice genotypes was revealed by diallel data. Although genetic variation appeared to be influenced predominantly by genes with additive and dominance effects, the presence of a significant additive component is encouraging. Based on narrow sense heritability and expected genetic gain because of selection, a potentially useful advance in high protein content.

An autogamous crop like rice, exploitation of non-additive genetic variance as such would be impractical. However, using biparental or recurrent selection in segregating material, followed by conventional selection, is likely to lead to substantial trait improvement. Further, advancing of segregating material through bulk, pedigree, single seed descent or single pod descent methods, as suggested by Gupta and Dahiya [21], would be rewarding.

REFERENCES

1. Juliano, B.O., 1993. Rice in Human Nutrition. FAO Food and Nutrition Series No. 26. International Rice Research Institute, Manila, Philippines.
2. Jiang, S.L., J.G., Wu, N.B., Thang, Y., Feng, X.E. Yang and C.H. Shi, 2008. Genotypic variation of mineral elements contents in rice (*Oryza sativa* L.). Eur. Food. Res. Technol., 228: 115-122.
3. Ring, S.G., 1995. Stiff tests for designer starches. Chem. Brit., 4: 303-307.
4. Juliano, B.O., 1985. Criteria and tests for rice grain qualities. In: Rice Chemistry and Technology, 2nd, ed., American Association of Cereal Chemists, St Paul, N, pp: 443-524.
5. Lin, R., Y. Luo, D. Liu and C. Huang, 1993. Determination and analysis on principal qualitative characters of rice gemplasm. In: Ying, C. (Ed.). Rice Germplasm Resources in China, Agricultural Science and Technology publisher of China, Beijing, pp: 83-93.
6. Hibino, T., T. Kidzu, T. Masumura, K. Ohtsubo, K. Tanaka, K. Kawabata and S. Fujii, 1989. Amino acid composition of rice prolamin polypeptides. Agric. Biol. Chem., 53: 313-318.
7. Gupta, M.P., P.K. Gupta, I.B. Singh and P. Singh, 1988. Genetic analysis for quality characters in rice. Genetika, 20(2): 141-146.
8. Shenoy, V.V., D.V. Seshu and J.S.K. Sachan, 1991. Inheritance of protein per grain in rice. Indian J. Genet., 51(2): 214-220.
9. Shi, C.H., J.M. Xue, Y.G. Yu, X.E. Yang and J. Zhu, 1996. Analysis of genetic effects for nutrient quality traits in indica rice. Theor. Appl. Genet., 92(8): 1099-1102.
10. Shi, C.H., J. Zhu, X.E. Yang, Y.G. Yu and J. Wu, 1999. Genetic analysis for protein content in indica rice. Euphytica, 107(2): 135-140.
11. Chai, Q.H., M.T. Shi and R.C. Yang, 1995. Genetic analysis of the protein content and the composition of amino acid in early indica rice. J. Fujian. Agr. Univ. 24: 149-153.
12. Won, J.G., T. Yoshida and Y. Uchimura, 2002. Genetic Effects on Amylose and Protein Contents in the Crossed Rice Seeds. Plant. Prod. Sci., 5(1): 17-21.
13. AOAC, 1990. AOAC Official Method 990.03. 1995. Protein (crude) in animal feed, combustion method, 16th ed. Association of Official Analytical Chemists, Arlington, VA.
14. Hayman, B.I., 1954a. The theory and analysis of diallel crosses. Genet., 39: 789-809.
15. Hayman, B.I., 1954b. The analysis of variance of diallel crosses. Biomet., 10: 235-245.
16. Ukai, Y., 2006. DIAL98. A package of progams for the analyses of a full and half diallel table with the methods by Hayman (1954), Griffing (1954) and others. http://lbm.ab.a.u-tokyo.ac.jp/ ~ukai/ dial98.html.
17. Mather, K. and J.L. Jinks, 1971. Biometrical Genetics. Chapman and Hall Ltd., London, UK.
18. Singh, T.K. and B.D. Chaudary, 1979. Biometrical methods in quantitative genetic analysis. Kayani Puplishers, Ludhiana, pp: 304.
19. Hayman, B.I., 1957. Interaction, heterosis and diallel crosses. II. Genet., 42: 336-355.
20. El-Kady, A. and A. El-Hissewy, 1999. Heterosis and combining ability for protein content in rice. Egypt. J. Appl., 14: 64-71.
21. Gupta, K.R. and B.S. Dahiya, 1986. Inheritance of pod yield traits in pea. Crop Improv, 13(1): 45-48.