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Exploratory Studies on Genetic Variability and Genetic Control for Protein and Micronutrient Content in F₄ and F₅ Generation of Rice (*Oryza sativa* L.)

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Abstract: Estimates of variability and presence of genetic interaction of protein and micronutrient (Iron, Copper, manganese, Zinc) characters were analyzed in segregating generations derived from a cross between BPT×HPR 14 of rice. Total Grain Protein Content (GPC) showed high heritability coupled with a moderate genetic advance in both generations. From F₄ to F₅, heritability estimates showed consistency indicating fixation of loci controlling protein content. According to estimated coefficients of skewness and Kurtosis indicated the presence of duplicate gene interactions in the expression of total grain protein content, copper and iron content whereas complementary gene interaction appears to be absent in the expression of zinc and manganese content. The results indicate the mild selection can be employed for traits like protein content, copper and iron content in segregating generation. The method of using skewness and kurtosis for detecting additive epistasis is very simple and can be used for crop improvement.

Key words: Heritability, kurtosis, skewness, gene interaction, micronutrients

INTRODUCTION

Protein and micronutrients play an important role in human nutrition in terms of a balanced diet. Protein malnutrition has a direct impact on human growth and development. Micronutrients play role in metabolic and physiological processes of the human body. For instance, iron (Fe) is an important component of hemoglobin and myoglobin; zinc stimulates the activities of many enzymes in the human body and is closely related to intelligence development in children; Manganese is the active radical and cofactor of many enzymes in the human body (Zhang *et al.*, 2004). Rice is the main staple food crop for the half of the world's population. Rice provides 20% of the world's dietary energy supply while wheat supplies 19% and maize 5% (Kennedy and Burlingame, 2003). Even though rice provides 14% of the global protein, unfortunately over three billion people in the world are malnourished. The genetic variation in protein content provides a basis for breeding protein content. However, manipulating this trait in traditional breeding is difficult because such substantial variation is quantitatively inherited (Shenoy *et al.*, 1991).

Estimates of the amount of variability for different characters and their heritable components available in the population are essential for dynamic and efficient plant breeding. It becomes necessary to split the overall variability into heritable and non heritable components

with the help of genetic parameters for increasing the selection efficiency.

The present study aims at the estimation of measures of variability, heritability and genetic advance for total protein content and micronutrient content in segregating generations of rice grain. Also it aims to know the genetics of the traits under study using third and fourth degree statistics in segregating generations of rice.

MATERIALS AND METHODS

In this study we used two segregating population derived from the cross between BPT 5204 and HPR 14. BPT 5204 used as Female parent is a medium slender with consumer acceptance whereas HPR 14 is a land race with high protein content used for hybridization with single seed decent method followed. The experimental material comprised of 100 F₄ progenies of the cross BPT 5204/HPR 14 and 95 F₅ progenies of the above cross planted along with parents. The F₄ experiment plots were grown in Zonal Agricultural Research Station, Hiriyur during 2008 (dry season) and F₅ generation were grown in K-block, GKVK, UAS, Bangalore during wet season 2008.

Trait measurement: All harvested seeds from single plant were dried and dehusked. The dehusked rice samples from each line were used for estimation of protein content and

micronutrient using Near Infrared Reflectance Spectroscopy (NIRS, Foss, Germany) facility available at National Seed Project (NSP), Dharwar.

Statistical analysis: Mean values of each line were used for statistical analysis. Phenotypic and genotypic coefficient of variability (PCV and GCV), Broad sense heritability (h^2) and genetic advance as percent mean (GAPM) were calculated as per Burton and De Vane (1953).

Skewness (K_3), the third degree statistics and kurtosis (K_4), the fourth degree statistics were estimated (Snedecor and Cochran, 1967) to understand the nature of distribution of different traits:

$$K_3 = \frac{n}{(n-1)(n-2)} \sum_{j=1}^n (Y_j - \bar{Y})^3$$

$$K_4 = \frac{n}{(n-1)(n-2)(n-3)} \left\{ (n+1) \sum_{j=1}^n (Y_j - \bar{Y})^4 - \frac{3(n-1)}{n} \left[\sum_{j=1}^n (Y_j - \bar{Y})^2 \right]^2 \right\}$$

where, n-sample size, Y_j - observational value of jth sample, \bar{Y} -sample mean

For a normal distribution, skewness is equal to zero in the absence of gene interaction; it is greater and smaller than zero in the presence of average complementary and duplicate interactions, respectively. Also, gene interaction can be detected by studying fourth degree statistics, kurtosis which is always negative or near to zero in the absence of gene interaction and is positive only in the presence of gene interaction (Choo and Reinbergs, 1982).

The standard error for coefficients of skewness and kurtosis were estimated as per Fisher (1950) as follows:

$$SEK_3 = \sqrt{\frac{6n(n-1)}{(n-2)(n+1)(n+3)}}$$

$$SEK_4 = \sqrt{\frac{24n(n-1)^2}{(n-3)(n-2)(n+5)(n+3)}}$$

Frequency distribution for each trait was considered significantly deviates from zero at the 0.05 probability level if that value was greater than twice of its standard error.

RESULT AND DISCUSSION

Genetic variability for protein and micronutrient studies in F_4 and F_5 generation: The estimates of range, PCV and GCV, heritability, GA as percent mean (Table 1) indicated

stabilized variability and decrease in the mean of F_5 progenies as compared to the F_4 progenies for all characters except iron content in F_5 generation. The estimates of PCV and GCV, broad sense heritability and genetic advance of F_5 progenies increased for total grain protein content, Manganese and Zinc content but decreased for iron content and copper content.

A gradual reduction or stabilized variability and increase in the mean of the advance progenies in self pollinated crops were a normal feature. The cosmetic variation in protein content and most of micronutrient content from the F_4 to F_5 generation indicates the fixation of loci controlling protein content at F_5 generation itself. The estimates of coefficients of variability of F_5 progenies increased for total grain protein content, Zinc and Manganese content. This indicated that the estimates of coefficient of variability were highly influenced by the environmental factors. The variability estimates ultimately influenced the heritability and genetic advance. The decrease in the coefficient of variability for copper and iron content was observed in F_5 generation. Since the estimates of heritability and genetic advance are dependent on the estimates of CV, their values also decreased in F_5 progenies.

The estimates of heritability and genetic advance of F_5 progenies for total grain protein content, Zinc and Manganese content showed subtle increase as compared to the F_4 progenies. This indicated early fixation of the genotypes leading homozygosity for the concerned traits. Though, high heritability coupled with high genetic advance is an indication of preponderance of additive genetic variance (Panse, 1957), it is difficult to confirm in the absence of appropriate genetic parameters. However, it suggests that for the above characters selection could be effective. Tsuzuki and Furusho (1986) reported 58.8% heritability for protein content in rice. Garcia-Oliveira *et al.* (2009) reported medium to high heritability for microelements in set recombinant inbred lines of rice.

On the other hand, the estimates of heritability and genetic advance for copper and iron content decreased in F_5 progenies, thus indicating the involvement of a large number of loci in the inheritance of these traits and the major role of non additive genes (Roy, 2000). For these above characters selection could be ineffective. A high proportion of PCV as compared to the GCV may be mainly responsible for reduced heritability estimates of F_4 progenies.

Genetics of traits: The study of distribution using skewness and kurtosis provides information about nature of gene action (Fisher *et al.*, 1932) and number of genes

Table 1: Estimates of genetic variability parameters for grain protein content and micronutrient content in F₄ and F₅ segregating population of BPT 5204 X HPR 14

Traits	Generation	Mean±SE	Range		PCV (%)	GCV(%)	h ² (%)	GA (% mean)
			Lowest	Highest				
Total grain protein (%)	F ₄	10.46±0.13	8.15	12.92	10.70	7.36	47.33	10.43
	F ₅	10.16±0.13	7.39	12.81	11.07	7.65	47.83	10.90
Copper (ppm)	F ₄	5.88±0.09	3.58	7.58	14.03	11.41	66.07	19.10
	F ₅	5.89±0.08	3.58	7.58	12.98	10.09	60.43	16.16
Zinc(ppm)	F ₄	20.30±0.20	8.64	30.51	25.66	25.15	96.06	50.78
	F ₅	17.36±0.18	8.60	30.51	26.15	26.09	99.49	53.60
Manganese (ppm)	F ₄	62.66±0.16	29.60	90.35	12.19	11.26	82.60	21.09
	F ₅	62.40±0.16	29.94	91.94	12.28	11.30	99.71	40.64
Iron (ppm)	F ₄	49.59±1.10	28.62	70.38	21.32	20.81	95.33	41.86
	F ₅	50.51±1.04	27.62	69.38	20.13	19.61	94.95	39.37

GCV: Genotypic coefficient of variation (%), PCV: Phenotypic coefficient of variation (%), h²: Heritability in broad sense (%), GA: Genetic advance

Table 2: The coefficients of Skewness and Kurtosis of macro and micronutrient in F₄ generation of rice

Trait	Generation	Sample size	Skewness	Kurtosis
Total grain protein content	F ₄	91	-0.21*	-0.57
	F ₅	85	-0.18*	1.98*
Copper	F ₄	95	-0.24*	0.13
	F ₅	95	-0.12	0.08
Zinc	F ₄	95	0.11	-1.02
	F ₅	95	-0.10	-0.82*
Manganese	F ₄	95	0.17	-0.19
	F ₅	95	0.01	-0.32
Iron	F ₄	95	-0.26*	-0.67
	F ₅	95	-0.41*	-0.50

*Significant at p = 0.05

controlling the traits (Robson, 1956), respectively. Positive skewness is associated with complementary gene interactions while negative skewness is associated with duplicate (additive×additive) gene interactions. The genes controlling the trait with skewed distribution tend to be predominantly dominant irrespective of whether they have increasing or decreasing effect on the trait. The traits with leptokurtic and platykurtic distribution are controlled by fewer and large number of genes, respectively. Kurtosis is negative or close to zero in the absence of gene interaction and is positive in the presence of gene interactions (Pooni *et al.*, 1977; Choo and Reinbergs, 1982; Kotch *et al.*, 1992). Studies on the amount of gene interaction are undoubtedly needed so as to increase the efficiency of our selection and breeding programs. Selection intensity could be higher under complementary than under duplicate relationship. Probably, progress in improving population performance may be greater under complementary interaction than under duplicate gene interaction (Choo and Reinbergs, 1982). The skewness and kurtosis coefficients are given in Table 2.

Negatively skewed and leptokurtic distribution was an evidence for involvement of fewer number of dominant genes with increasing effects and also coefficient of skewness significantly deviates from zero indicating presence of duplicate gene interaction in the inheritance of total protein content and iron content in both

generation. For total grain protein content in F₅ generation positive and significant coefficient of kurtosis observed, it indicates the presence of gene interaction for the trait. Genetic gain in respect of all the above mentioned traits showing negatively skewed distribution will be rapid under mild selection from the existing variability (Roy, 2000).

Positively skewed and leptokurtic distribution suggested the involvement of relatively fewer number of segregating genes with majority of them having decreasing effects in the inheritance of zinc and manganese content in F₄ generation. The coefficient of skewness for zinc and manganese content was not significantly different from zero, indicating that absence of additive epistasis interaction for the above traits. This conclusion is further substantiated by a non-significant copper content and negative coefficients of kurtosis in the total grain protein content, zinc, manganese, iron content in F₄ generation. Maximizing the genetic gain in respect of these traits with positively skewed distribution requires intense selection from the existing variability (Roy, 2000).

In F₅ generation, negative non significant coefficient of skewness and significant coefficient of kurtosis was observed indicating the presence of gene interaction for zinc content. Coefficient of skewness for the manganese content in F₅ generation was nearly zero and negative value of kurtosis indicating the absence of gene interaction. Whereas, copper content showed negatively skewed and leptokurtic distribution in both generation. It indicates the involvement of fewer number of dominant genes with majority of them having increasing effects and also coefficient of skewness significantly deviates from zero indicating presence of duplicate gene interaction in the inheritance in F₄ generation but in F₅ generation coefficient of skewness and kurtosis were non significant indicates the absence of gene interaction.

In the present study, presence of duplicate gene interaction in the expression of total grain protein content,

copper and iron content where as complementary gene interaction appears to be absent in the expression of zinc and manganese content. It indicates the mild selection can be employed for traits like protein content, copper and iron content in segregating generation. The method of using skewness and kurtosis for detecting additive epistasis is very simple and can be used for crop improvement.

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