RESEARCH PAPER

Diallel analysis of soluble protein in root nodules and seeds in blackgram [*Vigna mungo* (L.) Hepper]

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Abstract

The study was done to see the inheritance of soluble protein in two quantitative characters, such as seeds and nodules per plant in Blackgram [*Vigna mungo* (L.) Hepper]. Dominance and additivity were detected for soluble protein in both of the characters. Soluble protein in seeds was found to be regulated by partial dominance whereas, the same in nodule character showed over dominance. The asymmetry in the proportion of genes with positive and negative effects in the parents was indicated for soluble protein in both of the characters. The soluble protein in seeds indicated the presence of an excess of dominant genes in the parents. On the other hand, the same in nodules indicated the presence of an equal proportion of dominant and recessive genes. The negative dominant genes were controlling the character whereas, only one group of dominant genes was found to the same condition in both of the characters. This study also suggested that dominance effect over all loci in heterozygous phase was important for the soluble protein in seeds yield per plant but the dominance effect over all loci in heterozygous phase was important for the total amount of soluble protein in nodules. The general combining ability and specific combining ability were found to be significant for the soluble protein. It was noted that the line 20 (E2025) was good general combiner for the same. The crosses 5 (EML013) × 17 (E86325) and 5 (EMLO13) × 20 (E2025) for soluble protein in nodules and 5 (EMLO 13) × 21 (E86309), 17 (E86325) × 21 (E86309) in seeds were found to be the best combiners as judged from the specific combining ability effects.

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Introduction

Grain legumes are among the most important crops in many countries, since they provide one third of the dietary protein for human consumption. A pulse is an annual leguminous crop produce from one to twelve seeds of variable size, shape, and color within a pod. Pulses are used for food and animal feed. Among the pulses, Blackgram [*Vigna mungo* (L.) Hepper] is very nutritious and its pods are considered to be significant source of thiamin, riboflavin and niacin. Other vitamins of the B-complex group as well as ascorbic acid, vitamin-K and tokoferols are also present. Blackgram is a self-pollinating diploid (2n = 2x = 22) grain legume that is grown widely on the Indian subcontinent and to a lesser extent in Thailand, Australia, other Asian site and South Pacific countries. The tap root produces a branched root system with smooth, rounded nodules. The bean is boiled and eaten whole or after splitting into 'Dal' (In Bangladesh); prepared like this, it has an unusual mucilaginous texture. Ground into flour or paste, it is also extensively used in South Indian culinary preparation like dosa, idli, vada and papadum. Blackgram is also recommended for diabetics, as are other pulses. Besides in the presence of nodule on the roots of Blackgram as symbiotic association, containing nitrogen fixation bacteria (*Rhizobia* sp.) which help in management of soil fertility as well as save nitrate leaching during precipitation (Jones, 1939). For their nutritive values and economic importance varietal improvement of Blackgram through breeding methods is necessary. Therefore, in the present study examine to evaluate the nature of gene action, i.e., additivity and dominance inheritance of soluble protein in seed and root nodules of Blackgram. Present experiments were done according a diallel breeding method was conducted in four parents line of Blackgram.

Material and methods

At first, four lines of Blackgram [*Vigna mungo* (L.) Hepper] viz. line-5 (EMLO13), line-17 (E86325), line-20 (E2025) and line-21 (E86309) were collected from BARI regional station, Ishurdi, Pabna. Bangladesh. The experiment was done in the research field of Botanical Garden at University of Rajshahi, Rajshahi, Bangladesh during 2005 following completely randomized block design. The seeds of different line were shown randomly following individual plant randomization in replicated plots. Each plot had twenty five rows and having seven hills per plot. Data were collected on individual plant basis. Root nodules were collected after 60 days from the sowing of seeds. On the other hand, the seeds were collected in mature condition and dried up properly.

The collected experimental data were analyzed following two techniques of analyses as follows:

A. Biochemical analysis: Protein content of the different lines of root and seed was determined according to Lowry method, 1951 and
B. Biometrical analysis was done following diallel techniques of analysis of Hayman's , 1954 and method 2, model II of Griffing's, 1956 approaches.

Results and discussion

According to Hayman's, 1954b, analysis of variance of four parent half diallel with parents for testing the significance of genetic difference are shown in Table1. The items of Table 1 tested against their error mean square. The value of treatment was found to be significant for both characters. Significance of genotypic difference proved that, the parents, were used in this study, was significantly different from each other and the t^2-tests indicated probable fulfillment for all the postulated assumptions for chemical characteristics in both of the characters under study.

The location of array points for regression line provides information about the heterozygosity or homozygosity of recurrent parents (Dickinson and Jinks, 1956). Having the parental materials included in the present study, the array points stay on the regression line without any deviations, suggested that the parent materials were homozygous. Prudeck (1981) got heterozygosity by analyzing some traits in sweet pepper.

date of fruit repening in barley. Singh and Rai (1986) reported that in 8 × 8 half diallel cross in chilli partial dominance was important for fruit length, fruit number per plant and fruit yield per plant. Husain (1997) observed average over dominance for number of leaf at maximum flower and date of fruit ripening in Chilli. Ahmed et. al. (2000) also reported in seven-parent diallel cross in Chilli the presence of over dominance in most the characters under study.

Table 1. Analysis of variance of four parent half diallel for soluble protein in nodules and seeds.

Source	df (Nodule/Seed)	SS Nodule	SS Seed	MS Nodule	MS Seed	F Nodule	F Seed
Treatment	9	328.74	64.85	36.52	4.20	6.156*	10.46*
Replication	4	15.2575	4.351	3.814	1.08	0.642*	1.578*
Error	36	213.594	24.80	5.933	0.68		
Total	49	557.6	94.00				

The regression line drawn in Wr/Vr graphs (Fig. 1 and Fig. 3) indicated over dominance for protein in the characters nodule per plant and partial dominance in seed yield per plant. The results are in agreement with the findings of Krishnaswami et. al. (1964), while Dickson (1967) noted complete dominance. Partial dominance was noted by Islam et. al. (2000) for all the traits in seven parent diallel cross in chilli. The ratios of $(H_1/D)^{1/2}$ and V_1L_1/W_0L_{01} also indicated the partial dominance for chemical characteristics in seed yield per plant and over dominance for the same in nodule per plant. Johnson and Eunus (1964) got over dominance for number of leaf at maximum flower and

Table 2. Components of variation and their ratios obtained from four parents half diallel analysis for soluble protein in nodules and seeds.

	Components of Variation			Proportional values		
		Estimated Values		Proportion	Estimated Values	
Notation	Nodule	Seed			Nodule	Seed
D	5.003 ± 0.62	3.698 ± 0.22		$(H_1/D)^{1/2}$	1.786	0.797
F	-2.652 ± 1.61	3.026 ± 0.58		$H_2/4H_1$	0.232	0.178
H₁	15.972 ± 1.82	2.349 ± 0.65		$[(4DH_1)^{1/2} + F]/[(4DH_1)^{1/2} - F]$	0.74	3.109
H₂	14.84 ± 1.68	1.675 ± 0.60		h^2/H_2	0.403	-0.065
h²	5.995 ± 1.141	-0.109 ± 0.41		V_1L_1/W_0L_{01}	1.918	0.749
E	1.144 ± 0.28	0.145 ± 0.10				

The location of array points along the regression line in Wr/Vr graph depends on the relative proportion of dominant and recessive genes present in the recurrent parent of each array (Jinks, 1954 and Hayman's, 1654b). Having the excess of dominant genes, the

parent showed a low array variance and covariance and its position will near the point of origin. On the other hand, parent with excess of recessive genes will have large array variance and covariance and its position will the furthest from the point of origin on regression line. In this investigation, array 1 possessed dominant genes in excess for the character of soluble protein in seeds per plant and excess of recessive genes for the character of soluble protein in nodules per plant. For the same in both the characters array 2 possessed an excess dominant gene, while array 3 contained the recessive genes in excess for the same in seeds per plant. However, the same array contained dominant and recessive genes in equal proportion for the nodules per plant. Again dominant genes in excess were exhibited by the array 4 for the same in both the characters under study.

Table 3. Variance of parents (V_0L_0), array variance (V_r), array covariance (W_r), mean variance (V_1L_1), mean covariance (W_0L_{01}), corresponding covariance (W_{rei}), *standardized* deviation for parental measurement ($Y_r/$) and degree of dominance for soluble protein in nodules.

Array	V_r	W_r	W_{rei}	$Y_r/$	$(W_r + V_r)/$
1	12.207	8.038	7.983	-0.504	1.502
2	2.935	0.095	0.458	0.603	-1.04
3	7.720	4.352	4.342	1.249	0.295
4	3.626	1.318	1.0195	-1.349	-0.757
$V_0L_0 = 6.1482$		$V_1L_1 = 6.6224$		$W_0L_{01} = 3.4512$	

In order to determine whether dominance was due to positive and negative genes, a standardized deviation graph for parental measurements $Y_r/$ and the degree of dominance $(W_r + V_r)/$ was drawn and shown in Figure 2 and Figure 4 for soluble protein in nodules per plant and soluble protein in seeds per plant, respectively. Plus value of $(W_r + V_r)/$ denotes recessiveness, while minus value denotes dominance. On the other hand, plus value of $Y_r/$ denotes higher expression and minus value indicates lower expression. Figure 2 showed that the array 1

possessed recessive genes with negative effect, whereas the array 3 possessed recessive genes with positive effect. On the other-hand, the array 2 possessed dominant genes with positive effect, while the array 4 possessed dominant genes with negative effect. The value of correlation co-efficient was found to 0.98 suggesting the negative dominance genes were in excess. In case of Figure 4, dominance in arrays 2 and 4 were due to an express of negative genes and recessiveness in the array 3 due to an express of positive genes. The array1 exhibited dominance but it was due to slightly in excess of negative genes. The value of correlation co-efficient was found to be 0.97 suggesting the negative dominant genes are in excess.

Table 4. Variance of parents (V_0L_0), array variance (V_r), array covariance (W_r), mean variance (V_1L_1), mean covariance (W_0L_{01}), corresponding covariance (W_{rei}), standardized deviation for parental measurement ($Y_r/$) and degree of dominance for soluble protein in seeds.

Array	V_r	W_r	W_{rei}	$Y_r/$	$(W_r + V_r)/$
1	0.028	0.065	0.153	-0.120	-1.170
2	0.586	1.146	0.819	-0.405	-0.150
3	2.018	2.519	2.526	1.62	1.59
4	0.752	0.784	1.016	-1.093	-0.272
$V_0L_0 = 3.8438$		$V_1L_1 = 0.84654$		$W_0L_{01} = 1.12885$	

Again, according to Johnson and Aksel (1959), proportions of positive and negative dominant genes in the parents were measured by the co-efficient of correlation (r). A high minus value of r between the parent performance $Y_r/$ and the degree of dominance $(W_r + V_r)/$ indicated the presence of an excess of positive dominant genes and a high plus value indicates an excess of negative dominant genes present in the parents. Lower value of either sign indicates that both positive and negative genes tend towards equal proportion for dominance. In this study, the correlation coefficient showed an excess negative dominant genes controlling soluble protein in both nodules and seeds per plant.

Table 5. Analysis of variance for combining ability in method II (Model II) for soluble protein in nodules and seeds.

Item	DF		SS		MS		F	
	Nodules	Seeds	Nodules	Seeds	Nodules	Seeds	Nodules	Seeds
GCA	3	3	35.663	9.303	11.887	3.101	10.018*	22.52*
SCA	5	5	30.135	3.676	6.027	0.735	5.079*	5.337*
Error	36	36	42.718	4.960	1.186	0.137		

The components of variation and their proportions of two characters, upon which the graphical analysis were reflected and partly summarized by these proportion, are shown in Table 2. Component D, which measures additive variation, was highly significant for both the characters. The significant D indicated the importance of additive gene effect in conditioning these characters. The H_1 and H_2 components also showed highly significant for both the character. In this material, H_2 is less than H_1 indicated that dominance effect is important in controlling these character and the parents contained positive and negative dominant genes in equal proportion. Husain (1997) got additive variation in greater magnitude for plant height at maximum flower, number of fruit, fruit weight at harvest, number of secondary branch of maximum flower from a seven parent diallel cross in Chilli. Singh and Rai (1986) reported that non-additive gene effets i.e. dominace was important for fruit yield per plant and six related traits from 8×8 diallel cross in Chilli. Genetic analysis of Chilli revealed that fruit weight was controlled predominantly by additive gene effects while, dry matter content was controlled by non-additive gene effects in 10×10 diallel (Rao, 1984).

The significant value of the regression coefficient (b) for protein in each character indicated that non-allelic gene interaction was absent in controlling these characters. But Husain (1997) found involving non-allelic gene interaction in controlling some traits in seven-parent diallel in chilli.

Table 6. General combining ability effects (diagonal values), specific combining ability effects (above diagonal values) for soluble protein in nodules and seeds.

	EMLO13		E86325		E2025		E86309	
	Nodules	Seeds	Nodules	Seeds	Nodules	Seeds	Nodules	Seeds
EMLO13	**-0.096**	**-0.177**	1.3908	-0.1428	1.3528	-0.9442	-3.0619	0.7915
E86325			**0.9200**	**-0.1342**	-2.8300	0.1646	0.4306	0.8245
E2025					**1.1280**	**1.0081**	-1.4714	-0.6832
E863							**-1.050**	**-0.69**

The proportion, $H_2/4H_1$ measures average value of positive and negative genes i.e., uv over all loci in the parents. In case of unequal allelic frequencies i.e., u ≠ v at all loci estimated from the ratio $H_2/4H_1$ was less than its maximum value 0.25, which happens when u = v = 0.5 at all loci. The estimated value for $H_2/4H_1$ in the present investigation for the chemical characteristics in both the characters studied being less than 0.25 indicated asymmetry in the proportion of genes with positive and negative effects in the parents. Similar results were obtained by Husain (1997) on seven-parent diallel cross in Chilli. But Khaleque (1975) observed symmetrical distribution of genes in six-parent diallel cross in rice.
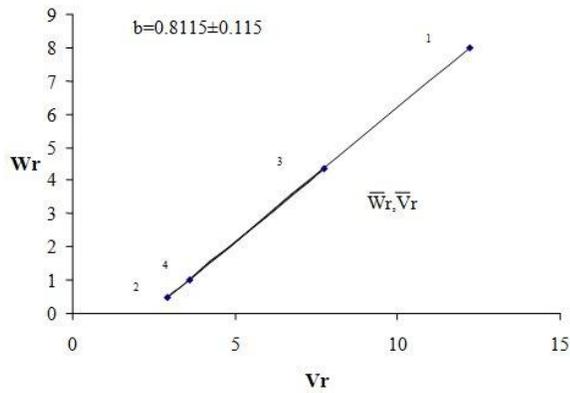
Fig. 1. Wr/Vr graph for soluble protein in nodules per plant in four parent half diallel.
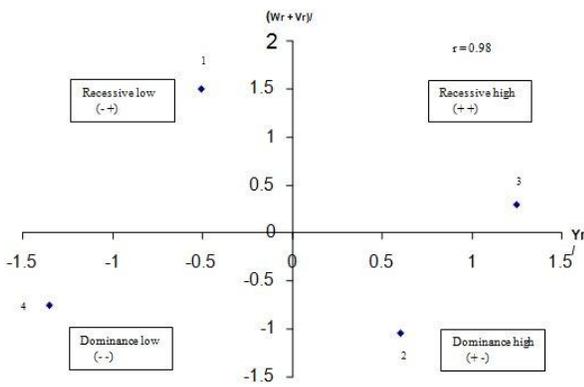
Fig. 2. Standardized deviation graph for soluble protein in nodules per plant in four parent half diallel.

The ratio $[(4DH_1)^{1/2} + F]/[(4DH_1)^{1/2} - F]$ determines the proportion of dominant and recessive gene in the parents. In this investigation the ratio was greater than one for soluble protein in seed yield per plant and less than one for soluble protein in nodule per plant. The seed character for the same indicated the presence of an excess of dominant genes in the parents and the nodule character indicated for the same the presence of an equal proportion of dominant and recessive genes. The parameter F is a measure of dominant and recessive genes present in parents. Significance exhibits dominance effects or if the dominance and recessive genes were distributed equally among the parents, F becomes zero or non-significant. The sign of F was thus an indicator of

relative frequencies of dominant and recessive alleles. In this study, non-significant F value for soluble protein in nodule character indicated that recessive and dominant alleles contributed equally and significant F value for the same in seed character indicated that dominant alleles were present in the parents. Husain (1997) observed an equal proportion of dominant and recessive genes for plant height at maximum flower, number of fruit, number of secondary branches at maximum flower and date of fruit ripening while for fruit weight at harvest and number of leaf at maximum flower observed that, dominant alleles were present in the parents. Ahmed *et. al.* (2000) estimated an equal proportion of dominant and recessive genes for date of flower opening, date of fruit set, date of fruit maturity and date of fruit ripening in Chilli.
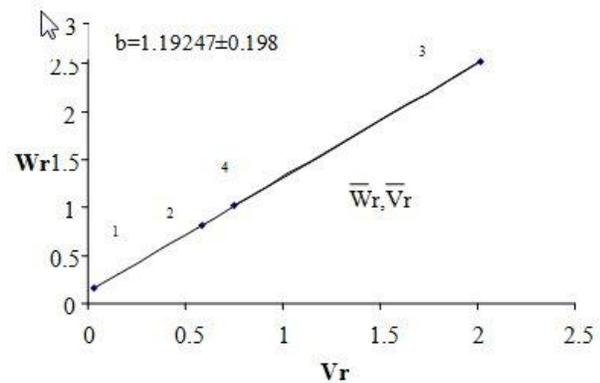
Fig. 3. Wr/Vr graph for soluble protein in seeds per plant in four parent half diallel.

The dominance effect estimated by the component, h^2. In this investigation, the non-significant value of h^2 suggested that dominance effect over all loci in heterozygous phase was important for the chemical character in seeds whereas, for the same in nodules indicated that dominance effect over all loci in heterozygous phase was not important by the significant value of h^2. An effective factor has been described by Mather as the smallest unit of hereditary materials that is capable of being recognized and detected through the methods of Biometrical Genetics (Mather, 1949). It may be a group of closely linked

genes or at the lower limit, a single gene. The ratio h^2/H_2 estimates the number of effective factors or groups of genes, which control the character and exhibit dominance.
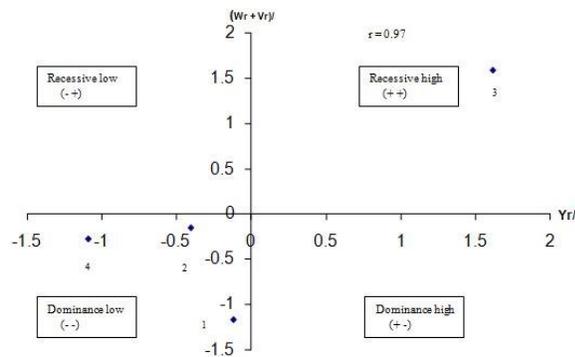
Fig. 4. Standardized deviation graph for soluble protein in seeds per plant in four parent half diallel.

When dominant genes of opposite effects appear in all combinations in parental lines instead of being isodirectionally distributed, the ratio h^2/H_2 was under estimated (Jinks, 1954; Cook and Mather, 1962). In this material, only one group of dominant genes was detected for chemical character in seeds and nodules both. Joarder and Eunus (1969, 1970a), Paul *et al.* (1976a) estimated 1 to 18 effective factors for the control of different quantitative traits in *Brassica campestris* L. and *B. Juncea.* Rahman and Eunus (1971) also reported 1 to 21 effective factors for the control of quantitative traits of jute. Only one group of dominant gene was detected in plant height at maximum flower, fruit weight at harvest, number of secondary branches at maximum flower and date of fruit ripening by Husain (1997) in Chilli. Similar result was found by Khaleque and Eunus (1977) to control some agronomic character in rice. For soluble protein the ratio, V_1L_1/W_0L_{01} in the present investigation showed over dominance for nodule and incomplete dominance for seed yield.

In the present study, the combining ability analysis in Blackgram showed that the variance due to general combining ability (gca) was significant for soluble protein in both the characters (Table 5). Islam *et. al.* (2000) observed same result in chilli. Variance due to Specific combining ability (sca) was also significant for the chemical character in both seeds and nodules. In the present materials gca was greater than sca suggested that inheritance of the character was predominantly governed by additive and additive × additive epistatic components of genetic variance (Griffing, 1956). Singh (1974) observed the similar nature of gene action for different yield attributes of *Corchorus capsularis,* Singh and Singh (1970) and Islam *et. al.* (2000) also found the similar results in brinjal and Chilli, respectively. Therefore, in the present investigation for fixation of additive genetic effects for soluble protein in both the characters, recurrent selection may be profitable as suggested by Paul *et. al.* (1976a).

It was noted from Table 6 that the line 20 (E2025) was general combiner for both the characters under study. The best combiner as observed from sca effects for the crosses, were 5 (EMLO13) × 17 (E86325) and 5 (EMLO13) × 20 (E2025) for total amount of soluble protein in nodules per plant and 5 (EMLO13) × 21 (E86309) and 17 (E86325) × 21 (E86309) for total amount of soluble protein in seeds per plant. These crosses may therefore, be utilized for the study of hybrid vigor and fixation of dominant genes in further breeding research.

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