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Shubham Sharma
Department of Genetics and Plant Breeding Indira Gandhi Krishi Vishwavidyalaya Raipur, Chhattisgarh, India

Dr. SK Nag
Department of Genetics and Plant Breeding Indira Gandhi Krishi Vishwavidyalaya Raipur, Chhattisgarh, India

Dr. Deepak Sharma
Department of Genetics and Plant Breeding Indira Gandhi Krishi Vishwavidyalaya Raipur, Chhattisgarh, India

SK Verma
Department of Genetics and Plant Breeding Indira Gandhi Krishi Vishwavidyalaya Raipur, Chhattisgarh, India

Corresponding Author:
Shubham Sharma
Department of Genetics and Plant Breeding Indira Gandhi Krishi Vishwavidyalaya Raipur, Chhattisgarh, India

Inheritance and linkage study in soybean (*Glycine max* L. Merill)

Shubham Sharma, Dr. SK Nag, Dr. Deepak Sharma and SK Verma

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Abstract
The primary goal of the present study was to investigate the inheritance patterns of qualitative traits such as flower color, leaf shape, pod pubescence color and seed hilum color in soybean. The study was carried out over two *kharif* seasons, 2021 and 2022, at the Research Cum Instructional farm in IGKV Raipur, India. Five parental lines((JS 97-52, JS 95-60, RVS 2012-11, RVSM 2011-35, and NRC 136) were crossed to produce F_1 progeny (JS 97-52 × JS 95-60, JS 97-52 × RVS 2012-11, JS 97-52 × RVSM 2011-35 and NRC 136 × RVSM 2011-35), which were then observed for their dominant traits. In the F_2 population, every individual plant was analyzed for the segregation of contrasting traits. The chi-square test was used to evaluate the observed and expected values of trait segregation. One primary gene was found to control the color of pod pubescence, leaf shape and flower color. The seed hilum color trait was found to be digenic in two crosses and monogenic in two other crosses. The test of independence showed that the characteristics flower color and pod pubescence color in the cross between JS 97-52 and RVS 2012-11 were linked and not independent.

Keywords: Inheritance, linkage, qualitative traits, segregation

Introduction
Soybean (*Glycine max* L. Merrill) is a leguminous and autogamous crop. It is a significant global source of oil, proteins, and phytochemicals that promote health for use in animal feed and human nutrition. Owing to its deep root structure and ability to fix nitrogen, soybean farming also increases soil productivity (Ali, 2010) [1].

Soybeans are regarded as one of the top oilseed crops in India. The genes that a plant received from its parents were measured using plant ratios and the chi-square test to determine how these genes interact or respond to a specific environment. For population development and improvement, soybean breeders employ selected markers such as leaf shape, flower color, and pod pubescence. These traits act as markers or indicators of homozygosity or heterozygosity during selection and are relevant to the phenotype of the plant population. Qualitative features were refined through selection and observation. These characteristics exhibit a Mendelian pattern of inheritance, are mostly determined by one or a small number of genes, and are hardly influenced by environmental factors (Kole *et al*. 2010) [4].

Knowledge of the gene (s) governing the qualitative attributes of soybeans and how they are inherited will help advance our understanding of the genetics of this crop and make it easier to apply this knowledge in subsequent studies. An essential feature that must be assessed for the effective gathering and conservation of soybean genetic resources is their qualitative characteristics (Oide and Ninomiya 2000) [8].

Materials and Methods
The current investigation was conducted in the kharif of 2021 and 2022 at, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) The climate was suitable for typical crop growth. Five parents-JS 97-52, JS 95-60, RVS 2012-11, RVSM 2011-35, and NRC 136 were employed in the current investigation. Numerous characteristics, including flower color, leaf shape, pod pubescence color and seed hilum color, were varied between these parents. The following crosses were created for the study: JS 97-52 × JS 95-60,JS 97-52 × RVS 2012-11, JS 97-52 × RVSM 2011-35 and NRC 136 × RVSM 2011-35.

In the current study, parents and F_1 observations were recorded during kharif season of 2021. Critical observation and documentation were done on the opposing features of the parents and the traits that manifested in the F_1.The F_2 population was raised in large quantities during the kharif 2022 season. Each individual plant in the population was observed for distinct contrasting features, and each trait was counted independently.

The chi square (χ^2) test was carried out to test goodness of fit. Null hypothesis (h_0) was formulated to test segregation of observed frequencies against expected ones. To determine whether there was any correlation between two characters, the test of independence (Panse and Sukhatme, 1954) was used to two characters in the F_2 population that were segregated simultaneously for the detection of linkage.

Result, Discussion and Conclusions

Inheritance studies

To study the gene interaction in this research, inheritance study was done for various characters in the above-mentioned crosses. In parents and F_1 generation, inheritance of various qualitative traits i.e. flower color, leaf shape, pod pubescence color and seed hilum color were recorded. In next generation, segregation pattern of F_2 for different traits were recorded. After that, statistical analysis was done with the help of goodness of fit. In this, chi square test was applied to find out how the observed value of a given phenomena was significantly different from the expected value. It is a valuable tool for the breeder to decide the observe data is according to an expected ratio or it agrees well with expected or theoretical frequency distribution. If observed χ^2 value is higher than expected χ^2 value, it is said to be significant otherwise it is said to be non significant and null hypothesis is accepted. So the overall summary of it is presented in the table 1, table 2 and table 3.

In the present study inheritance of flower color, leaf shape, pod pubescence color and seed hilum color of four crosses were worked out.

Table 1: List of parents and their distinguishing traits

S. No.	Parents	Distinguishing traits
1.	JS 97-52	White flower, tawny pod pubescence, broad leaf and black hilum
2.	JS 95-60	Purple flower, glabrous (Absence of pod pubescence), narrow leaf and grey hilum
3.	RVS 2012-11	Purple flower, glabrous (Absence of pod pubescence), narrow leaf and grey hilum
4.	RVSM 2011-35	Purple flower, glabrous (Absence of pod pubescence), narrow leaf and yellow hilum
5.	NRC 136	White flower, tawny pod pubescence, brown hilum and broad leaf

Table 2: Inheritance of traits in different crosses in soybean

S.N.	Name of cross	Trait	Expression in F_1	Observed value in F_2		Expected ratio	Expected value for F_2		Chi Square (χ^2)	P Value (0.05)
1	JS 97- 52 × JS 95-60	Flower color	Purple	345 Purple	135 White	3:1	360 Purple	120White	2.5	0.11
		Leaf shape	Broad	350 Broad	130 Narrow	3:1	360 Broad	120 Narrow	1.11	0.29
		Pod pubescence	Pubescent	348 Pubescent	132 Glabrous	3:1	360 Pubescent	120 Glabrous	1.6	0.21
		Pod pubescence color	Tawny	250 Tawny	98 Grey	3:1	261 Tawny	87 Grey	1.85	0.17
		Hilum color	Black	250 Black	100 Brown 130 Grey	9:3:4	270 Black	90 Brown 130 Grey	3.43	0.18
2	JS 97-52 × RVS 2012-11	Flower color	Purple	368 Purple	127 White	3:1	371.25 Purple	123.75 White	0.11	0.74
		Leaf shape	Broad	353 Broad	142 Narrow	3:1	371.25 Broad	123.75 Narrow	3.6	0.06
		Pod pubescence	Pubescent	367 Pubescent	128 Glabrous	3:1	371.25 Pubescent	123.75 Glabrous	0.19	0.66
		Pod pubescence color	Tawny	274 Tawny	93 Grey	3:1	275.25 Tawny	91.75 Grey	0.02	0.88
		Hilum color	Black	265 Black	100 Brown 130 Grey	9:3:4	278.44 Black	92.81 Brown 123.75 Grey	1.52	0.47
3	JS 97-52 × RVSM 2011-35	Flower color	Purple	365 Purple	140 White	3:1	378.75 Purple	126.25 White	2.0	0.16
		Leaf shape	Broad	360 Broad	145 Narrow	3:1	378.75 Broad	126.25 Narrow	3.71	0.05
		Pod pubescence	Pubescent	368 Pubescent	127 Glabrous	3:1	378.75 Pubescent	126.25 Glabrous	1.22	0.27
		Pod pubescence color	Tawny	265 Tawny	103 Grey	3:1	276 Tawny	92 Grey	1.75	0.18
		Hilum color	Black	362 Black	143 Yellow	3:1	378.75 Black	126.25 Yellow	2.96	0.08
4	NRC 136 × RVSM 2011-35	Flower color	Purple	385 Purple	112 White	3:1	372.75 Purple	124.25 White	1.61	0.20
		Leaf shape	Broad	390 Broad	107 Narrow	3:1	372.75 Broad	124.25 Narrow	3.19	0.07
		Pod pubescence	Pubescent	387 Pubescent	110 Glabrous	3:1	372.75 Pubescent	124.25 Glabrous	2.17	0.14
		Pod pubescence color	Tawny	302 Tawny	85 Grey	3:1	290.25 Tawny	96.75 Grey	1.90	0.17
		Hilum color	Black	380 Black	117 Yellow	3:1	372.75 Black	124.25 Yellow	0.56	0.45

The purple and white flower color pattern segregated in 3 Purple: 1 white with purple flowers predominate over white flowers indicating monogenic gene action. These results confirm the finding of Miku (1970) [6] and Raut *et al*. (1994) [13].

Broad leaf shapes were found to predominate over narrow leaf shape and 3 broad: one narrow ratio was obtained indicating monogenic gene action. The results of Bernard and Weiss (1973) [3], and Porter (2000) [12] are corroborated by this.

Pubescence was found to be dominating over glabrousness, while glabrousness and pubescence were found to separate in a 3 pubescent: 1 glabrous ratio. This is consistent with previous research by Ranjbar (1976) [14].

In the current study, the color of the pod pubescence was shown to segregate into 3 tawny: 1 grey ratio indicating monogenic gene action, with the tawny pod pubescence color being the prevalent feature. This is consistent with the previous research conducted by Pathak and Shukla (2021), Ranjbar (1976) [14], Singh (2009) [15], and Araujo *et al*. (2019) [2].

In two crosses seed hilum color segregated in 9 black: 3 brown: 4 grey ratio with supplementary gene action. Black hilum was recorded dominant over grey and brown hilum. This confirms the finding of Pathak and Shukla (2021) [10].

In two more crosses black and brown hilum color were found to be dominant over yellow hilum color respectively. Segregation fitted well in 3:1 ratio indicating monogenic gene action.

Table 3: Crosses showing their inheritance pattern and gene interactions present between the traits

S. N.	Crosses	Traits	Dominant Trait	Inheritance pattern	Gene interaction
1	JS 97-52 × JS 95-60	Flower color	Purple	Monogenic	Epistatic
		Leaf Shape	Broad	Monogenic	
		Flower color	Purple	Monogenic	Epistatic
		Pod pubescence	Pubescent	Monogenic	
		Flower color	Purple	Monogenic	Epistatic
		Pod pubescence color	Tawny	Monogenic	
		Flower color	Purple	Monogenic	Supplementary
		Hilum color	Black	Digenic	
		Leaf Shape	Broad	Monogenic	Epistatic
		Pod pubescence	Pubescent	Monogenic	
		Leaf Shape	Broad	Monogenic	Epistatic
		Pod pubescence color	Tawny	Monogenic	
		Leaf Shape	Broad	Monogenic	Supplementary
		Hilum color	Black	Digenic	
		Pod pubescence color	Tawny	Monogenic	Supplementary
		Hilum color	Black	Digenic	
2	JS 97-52 × RVS 2012-11	Flower color	Purple	Monogenic	Epistatic
		Leaf Shape	Broad	Monogenic	
		Flower color	Purple	Monogenic	Epistatic
		Pod pubescence	Pubescent	Monogenic	
		Flower color	Purple	Monogenic	Epistatic
		Pod pubescence color	Tawny	Monogenic	
		Flower color	Purple	Monogenic	Supplementary
		Hilum color	Black	Digenic	
		Leaf Shape	Broad	Monogenic	Epistatic
		Pod pubescence	Pubescent	Monogenic	
		Leaf Shape	Broad	Monogenic	Epistatic
		Pod pubescence color	Tawny	Monogenic	
		Leaf Shape	Broad	Monogenic	Supplementary
		Hilum color	Black	Digenic	
		Pod pubescence color	Tawny	Monogenic	Supplementary
		Hilum color	Black	Digenic	
3	JS 97-52 × RVSM 2011-35	Flower color	Purple	Monogenic	Epistatic
		Leaf Shape	Broad	Monogenic	
		Flower color	Purple	Monogenic	Epistatic
		Pod pubescence	Pubescent	Monogenic	
		Flower color	Purple	Monogenic	Epistatic
		Pod pubescence color	Tawny	Monogenic	
		Flower color	Purple	Monogenic	Epistatic
		Hilum color	Black	Monogenic	
		Leaf Shape	Broad	Monogenic	Epistatic

		Pod pubescence	Pubescent	Monogenic	
		Leaf Shape	Broad	Monogenic	Epistatic
		Pod pubescence color	Tawny	Monogenic	
		Leaf Shape	Broad	Monogenic	Epistatic
		Hilum color	Black	Monogenic	
		Pod pubescence color	Tawny	Monogenic	Epistatic
		Hilum color	Black	Monogenic	
		Flower color	Purple	Monogenic	Epistatic
		Leaf Shape	Broad	Monogenic	
		Flower color	Purple	Monogenic	Epistatic
		Pod pubescence	Pubescent	Monogenic	
		Flower color	Purple	Monogenic	Epistatic
		Pod pubescence color	Tawny	Monogenic	
4	NRC 136 × RVSM 2011-35	Flower color	Purple	Monogenic	Epistatic
		Hilum color	Black	Monogenic	
		Leaf Shape	Broad	Monogenic	Epistatic
		Pod pubescence	Pubescent	Monogenic	
		Leaf Shape	Broad	Monogenic	Epistatic
		Pod pubescence color	Tawny	Monogenic	
		Leaf Shape	Broad	Monogenic	Epistatic
		Hilum color	Black	Monogenic	
		Pod pubescence color	Tawny	Monogenic	Epistatic
		Hilum color	Black	Monogenic	

Linkage Detection between traits

Genes that are linked together always pass along the same combination of alleles for more than two generations. Morgan noticed that when two genes were crossed, they did not divide in accordance with Mendel's law. There is a significantly higher likelihood of a parental combination arising when two genes are on the same chromosome than with non-parental combinations. linkage describes how closely genes or other DNA sequences are located to one another on the same chromosome. The likelihood that two genes or sequences will be passed down from parents to their offspring increases with the proximity of those genes or sequences on a chromosome.

Test of independence among traits in soybean

In the present study, test of independence was calculated for detection of linkage to know its presence or absence. In the cross between JS 95-60 × RVS 2012-11, the segregation ratio in F_2 population for the trait flower color was 3 purple: 1 white, whereas for pod pubescence color was 3 tawny: 1 grey. Considering the proportions of plants with purple flower color and white flower color in each of the two classes, tawny pod pubescence color and grey pod pubescence color (Table 4).

Table 4: Observed frequencies for flower color and pod pubescence color in cross JS 97-52 × RVS 2012-11

Flower color Pod pubescence color	Purple	White	Total
Tawny	234	40	274
Grey	41	52	93
Total	275	92	367

The χ^2 can be calculated from the observed frequencies with help of Panse and Sukhatme (1954) formula. If a. b, c and d denote the frequencies in four cells of the 2×2 table. It can be shown that

$$\chi^2 = \frac{(ad-bc)^2(a+b+c+d)}{(a+b)(a+c)(b+d)(c+d)}$$

For 1 degree of freedom

a = 234 b = 40

c = 41 d = 52

This formula involves apart from factor $(ad\text{-}bc)^2$ the four marginal totals and grand total making use of formula we have.

$$\chi^2 = \frac{(234\times52-40\times41)^2\,(234+40+41+52)}{(234+40)(234+41)(40+52)(41+52)}$$

= 63.09

Since the calculated value of 63.09 is greater than the table value of 3.841, the chi square value is compared against the value at the 5% or 1% level of significance for one degree of freedom. This leads to the conclusion that the two characters, flower color and pod pubescence color, are linked and are not independent. As same like this, test of independence for all the previously mentioned crosses i.e. JS 97-52 × JS 95-60, JS 97-52 × RVSM 2011-35 and NRC 136 × RVSM 2011-35 were calculated. The calculated value was then compared with tabulated value. Significant difference was found only between flower color and pod pubescence color in cross JS 97-52 × RVS 2012-11 and others were found to be non significant (Table 5).

Table 5: Comparison of calculated values with tabulated values of the crosses for various characters from test of independence

Crosses	Characters	Observed proportion		Calculated value	Tabulated value
JS 97-52 × JS 95-60	Flower color	249	96	0.342	3.841
	Leaf shape	101	34		
	Flower color	260	88	0.197	3.841
	Pod pubescence	96	36		
	Flower color	186	64	0.897	3.841
	Pod pubescence color	68	30		
	Flower color	251	99	0.016	3.841
	Hilum color	94	36		
	Leaf Shape	245	103	1.358	3.841
	Pod pubescence	100	32		
	Leaf Shape	182	68	1.719	3.841
	Pod pubescence color	78	20		
	Leaf Shape	250	100	0.127	3.841
	Hilum color	95	35		
	Pod pubescence	249	101	1.194	3.841
	Hilum color	99	31		
JS 97-52 × RVS 2012-11	Flower color	260	93	0.306	3.841
	Leaf shape	108	34		
	Flower color	278	89	1.471	3.841
	Pod pubescence	90	38		
	Flower color	234	40	63.096*	3.841
	Pod pubescence color	41	52		
	Flower color	276	89	1.181	3.841
	Hilum color	92	38		
	Leaf Shape	260	107	0.152	3.841
	Pod pubescence	93	35		
	Leaf Shape	195	78	2.512	3.841
	Pod pubes. color	75	19		
	Leaf Shape	265	100	1.129	3.841
	Hilum color	88	42		
	Pod pubescence	268	97	1.174	3.841
	Hilum color	89	41		
JS 97-52 × RVSM 2011-35	Flower color	266	94	1.625	3.841
	Leaf shape	99	46		
	Flower color	270	98	0.810	3.841
	Pod pubescence	95	42		
	Flower color	179	86	0.065	3.841
	Pod pubescence color	71	32		
	Flower color	266	96	0.924	3.841
	Hilum color	99	44		
	Leaf Shape	264	104	0.043	3.841
	Pod pubescence	97	40		
	Leaf Shape	186	79	2.66	3.841
	Pod pubescence color	81	22		
	Leaf Shape	255	107	0.446	3.841
	Hilum color	105	38		
	Pod pubescence	257	105	2.278	3.841
	Hilum color	111	32		
NRC 136 × RVSM 2011-35	Flower color	304	86	0.243	3.841
	Leaf shape	81	26		
	Flower color	295	92	1.534	3.841
	Pod pubescence	90	20		
	Flower color	234	68	0.039	3.841
	Pod pubescence color	65	20		
	Flower color	293	87	0.119	3.841
	Hilum color	92	25		
	Leaf Shape	302	85	1.357	3.841
	Pod pubescence	80	30		
	Leaf Shape	235	67	0.818	3.841
	Pod pubescence color	70	15		
	Leaf Shape	297	83	0.093	3.841
	Hilum color	93	24		
	Pod pubescence	300	80	1.093	3.841
	Hilum color	87	30		

"*" – significant characters having calculated value greater than tabulated value

In all the four crosses dominant nature of traits were observed for all the characters in F_1 generation. In F_2 segregation was observed in ratio of 3:1. Flower color, Leaf shape, Pod pubescence and Pod pubescence color proved to be monogenic. Trait seed hilum color was found to be governed by supplementary gene action in two crosses whereas in two other crosses it was monogenic. The current work may be essential to crop development programs and considerably contributes to our understanding of the mendelian inheritance of the stated qualitative traits. To investigate related traits in the aforementioned crosses, linkage detection was carried out by using the test of independence. It was found that trait flower color and pod pubescence color was linked in one of the cross.

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