Inheritance of Seed Related Characters in Soybean Crosses

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ABSTRACT: Soybean seed quality and appearance influence the desirability of seed in the consumer market. It has been reported that seed coat colour and hilum colour are affected by the genes controlling pubescence and flower colour. To achieve concomitant improvement of seed quality and yield in practical plant breeding, it is necessary to explore the genetics of inheritance of seed quality characters in soybean. In this study, chi-square test was used to analyse the inheritance of four traits i.e. seed coat colour, hilum colour, pubescence colour and flower colour in the F_2 derived seed of five crosses made using nine soybean genotypes. Seed coat colour depicted simple monogenic inheritance as the ratios 3:1 and 9:3:4 fitted in three crosses i.e. DT-21 × BHATT, AGS-25×JS- 335 and PS-1556× EC- 389148. Hilum colour expressed several phenotypes that obeyed simple monogenic ratio of 3:1 and 9:3:4 in crosses DT-21 × PS-24, PS-1347×PS1241 and DT-21 × BHATT. Purple flower colour dominated over white flower colour and was in concordance with the Mendelian ratio of 3:1 in three crosses i.e. DT-21 × PS-24, DT-21 × BHATT and PS-1556× EC- 389148. Pubescence colour also showed simple monogenic inheritance with tawny colour dominating over gray pubescence in the cross PS-1347×PS1241. Pubescence presence was found to dominate pubescence absence in the cross AGS-25×JS- 335, where JS-335 was the glabrous parent. These results helped in concluding that seed colour and hilum colour are under simple genetic control and thus can be explored further to improve the performance of soybean varieties in the food market.

Keywords: *Glycine max*, seed coat colour, seed morphology, black soybean

INTRODUCTION

Soybean is considered as one of the most desired food crops in the world as it is a potent source of both high quality protein (40%) and oil (20%), a characteristic that distinguishes it from all other crops. It is a rich source of bioactive compounds such as isoflavones, tocopherols, saponins which make it desirable for consumption, as these compounds have proved to be useful in preventing chronic diseases such as Parkinson's, cancer, diabetes and several cardiovascular diseases. Soybean is the largest source of oil in the world and is bound to play a significant role in the health of the human population.

Soybean as a plant shows a wide range of contrasting characters that help in variety identification such as pubescence colour, seed coat colour, hilum colour, flower colour, leaf shape, growth habit, seed shape and seed size. Among these, characters such as seed colour, seed shape, hilum colour and seed lustre decide the desirability of soybean seeds as food by the consumer. The visual appearance of the soybean has been altered as a result of domestication during the transition of wild *Glycine soja* to the current cultivated *Glycine max* (Gillman *et al.,* 2011). Seed coat colour is also considered as an important marker for solicitation of

plant variety protection and in identification of "off types".

For human consumption, the grains must be uniform, the tegument and the hilum must be of the same colour, there must be not be spillage of the hilum, the tegument cannot present imperfections such as splits, stains caused by diseases and pests, mechanical damage; these are characteristics used as parameters of acceptance by consumers (Juhasz *et al.,* 2014). This necessitates the study of inheritance of seed related characters which can contribute in designing breeding programs as well as improve variety performance in the market.

Seed coat and hilum pigmentation are classified as qualitative characters and thus are expected to show simple inheritance. But the genes involved in the inheritance of seed coat and hilum pigmentation show allelic interactions with the genes involved in the control of pubescence and flower colour (Vernetti and Vernetti Junior, 2009; Nogueira *et al.,* 2015). The seed coat and hilum colour in soybean have been reported to result from the interaction and expression of six main genes. The green seed colour phenotype is attributed to G gene in the dominant condition and yellow phenotype is a result of recessive allele g. The R gene confers black pigmentation and (R_) is dominant over the brown pigment (rr). Soybeans with black seed coats do

not differ from the yellow commodity soybeans in seed composition and plant morphology, and the black seed coat is caused by the accumulation of flavonoids and anthocyanins in the epidermal layer of the soybean seed coat (Jo *et al.,* 2021). The inheritance of black and brown pigments is found to be independent of the green and yellow pigments (Yang *et al.,* 2010).

Gene T and gene W have been reported to be involved in the control of pubescence and flower colour respectively. Gene T expresses pubescence colour (T: dark brown, t^r: light brown, and t: gray) and also shows epistatic interaction over the allelic series R (Woodworth, 1921). Thus, R_T_ result in black seeded genotypes while rrT_ gives the brown phenotype. A similar interaction has been observed between the gene W that controls flower color (W: purple, w: white) and gene R.

Several researchers have reported that similar looking varieties may show variation in seed quality traits after hybridization. The inheritance of morphological characters of soybean seed has been studied by several researchers since the early 1900s. Terao (1918) reported the involvement of both nuclear and cytoplasmic genes in the inheritance of green cotyledon phenotype in soybeans. The nuclear genes that control green seed colour (D1 and D2) have been recently cloned (Fang 2014). Seed coat pigmentation is under epistatic control of 5 genetic loci (I, T, W1, R, and O) and 6 genetic loci (W1, W2, W3, W4, Wm, and Wp) control flower pigmentation (Palmer *et al.,* 2004). A total of 14 loci distributed across ten chromosomes have been found to be associated with soybean seed coat colours using coding SNPs among natural populations (Song *et al.* 2016). Environment affects the intensity of pigmentation in seed coat which indicates that seed quality in soybean is a result of several factors apart from the genetics involved (Woodworth and Cole, 1924). Thus, exploring seed quality traits through hybridization will further improve our understanding of the genetics of these traits.

The study of pubescence, flower colour, seed coat colour and hilum colour will help in increasing the incorporation of better seed traits in breeder seed and further improve its acceptance in the market. The objective of this investigation was to examine the inheritance of seed coat colour, hilum colour, pubescence colour and flower colour in five crosses of soybean genotypes.

MATERIAL AND METHODS

Nine parents with contrasting characters were chosen for the present study. The traits of the parents used are enlisted in Table 1. Genetically pure seed of parents to be used as experimental material was obtained from AICRP, soybean breeding Department of Genetics and Plant breeding, College of Agriculture GBPUAT, Pantnagar for making crosses.

In *kharif* 2017-2018, five crosses were made using 10 parents that were planted in the soybean crossing block at Norman E. Borlaug Crop Research Centre, Pantnagar. In the next year i.e. *kharif* 2018-2019, parents along with crossed seed were planted for evaluation and same crosses were also made for F_1 seed to be sown next year. Five crosses including 9 parents, their F_1 and F_2 generations along with the standard check PS-1572 were sown in a randomized block design with three replications in *kharif* 2019-2020 in 4 m rows with row to row distance of 45 cm and plant to plant distance of 10 cm. Each replication had the five crosses sown with 1 row each of the parents and F_1, along with 5 rows of the F_2 generation. The variety PS-1347 was used as a filler and PS-1572 as a standard check was planted at the borders. The crop was managed according to the procedures appropriate for the region.

The plants obtained in the F_2 generation were categorized under different phenotypic classes. The seed coat colour phenotype was expressed as yellow, black and yellow green. Hilum colour had three phenotypic classes i.e. black, dark brown and light brown. Flower colour had only two phenotypic variants i e. purple and white. Pubescence colour was classified into tawny and gray. Pubescence was completely absent in the genotype JS- 335, and thus the cross AGS-25 × JS- 335 was used for the analysis of inheritance of presence and absence of pubescence.

The crosses that were used to study the inheritance of seed colour are DT-21 × BHATT, AGS-25 × JS- 335 and PS-1556× EC- 389148. The inheritance of flower colour was studied in the crosses, DT-21 × PS-24, DT-21 × BHATT and PS-1556× EC- 389148 where only DT-21 and PS-1556 had white flowers. The crosses DT-21 × PS-24, PS-1347 × PS- 1241, DT-21 × BHATT were studied for the analysis of the inheritance of hilum colour. The analysis of inheritance of pubescence colour was done in two crosses i.e. PS-1347 × PS- 1241 and AGS-25 × JS- 335 (Table 1).

Table 1: Parents used for hybridization and their contrasting characters.

Genotypes		Characters			
		Seed Coat Colour	**Hilum Colour**	**Pubescence Colour**	**Flower Colour**
1.	DT-21	Yellow	Dark Brown	Tawny	White
2.	PS-24	Yellow	Dark Brown	Tawny	Purple
3.	PS-1347	Yellow	Dark Brown	Tawny	White
4.	PS-1241	Yellow	Black	Gray	White
5.	BHATT	Black	Black	Tawny	Purple
6.	AGS-25	Yellow-Green	Dark Brown	Tawny	Purple
7.	JS-335	Yellow	Dark Brown	Absent	Purple
8.	PS-1556	Yellow	Dark Brown	Tawny	White
9.	EC-389148	Yellow-Green	Dark Brown	Tawny	Purple

The data obtained in the F_2 generation for 300 plants was used for the calculation of observed and expected values to be used in chi-square test. Chi-square test was used to compare the frequencies of observed and expected values to verify the goodness of fit for the proposed gene models. The Genes software was used for the estimation of x^2 (Cruz, 2013). The acceptance of the null hypothesis implied that the expected segregation ratios were significantly close to the observed values, at 5% probability.

RESULTS AND DISCUSSION

All the seeds in F_1 generation depicted similar dominant phenotypic expression for the various morphological descriptors that were studied. $x^2=0.000$; P=100% indicated that the F_1 showed homogenous expression and no segregation was observed.

In the first cross, DT-21 × PS-24, segregation was observed for the traits hilum colour and flower colour. Dark brown hilum colour was found to be dominant over light brown hilum colour and the trait depicted simple monogenic inheritance ($x^2=0.231$; P=0.631). Purple flower colour showed complete phenotypic dominance over white flower colour and the inheritance was monogenic in nature ($x^2=0.026$; P=0.872). Both the traits complied to the (3:1) Mendelian ratio. Similar results have also been reported by Carpentieri-Pipolo *et al.,* (2007).
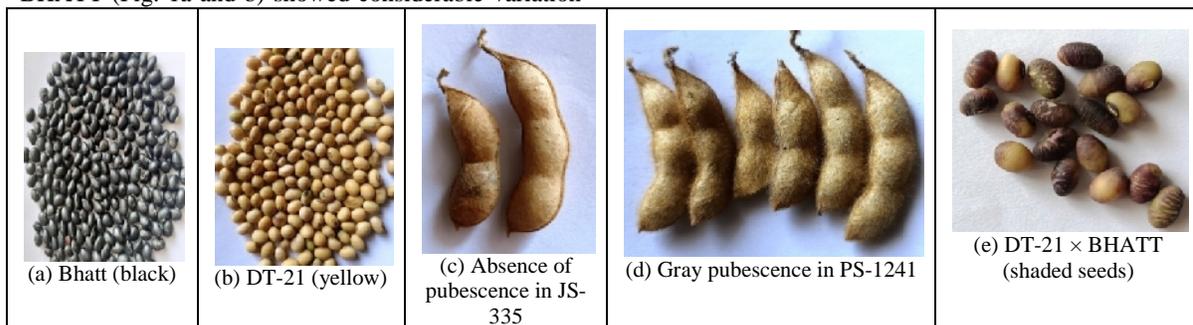
In the second cross that was used for this study, PS-1347 × PS-1241 showed variation for pubescence colour and hilum colour. A chi-square value of ($x^2=0.22$; P=0.637) was obtained for pubescence colour where tawny pubescence colour dominated gray pubescence colour (Figure d) and was found to be in concordance with the monogenic Mendelian ratio of 3:1. Similar results have also been reported by Yan *et al.* (2020). Hilum colour showed goodness of fit for the ratio (9:3:4) with a chi-square value of ($x^2=0.241$; P=0.886). Black hilum colour was found to be dominant over brown hilum colour. These findings are in agreement with the results reported by *Araujo et al.,* (2019).

The third cross that was made between DT-21 and BHATT (Fig. 1a and b) showed considerable variation for seed colour, hilum colour and flower colour. Seed colour with a chi-square value of ($x^2=0.068$; P=0.966), obeyed the segregation ratio of (9:3:4). This cross produced seeds in three colours black, yellow and yellow green. Black seed colour was dominant over both yellow and yellow green as reported by Seo *et al.,* (1993). Black hilum colour dominated over dark brown hilum colour in the F_2 population of this cross that was in accordance with the simple Mendelian ratio of (3:1). A chi-square value of ($x^2=0.051$; P=0.820) was obtained for hilum colour in this cross. The bleeding hilum trait was observed in very few seeds (10 seeds) of this cross (Fig. 1f). Flower colour obeyed the monogenic ratio of (3:1), where purple flower colour was dominant over white flower colour with a chi-square value of ($x^2=0.051$; P=0.820). These results were akin to the findings of Yang *et al.,* (2010) who carried out marker analysis for seed colour and flower colour inheritance in soybean. The inheritance black hilum/black seed coat has been described by Kovinich *et al.* (2011), Carpentieri-Pipolo *et al.* (2015) Yang *et al.* (2010) and Senda *et al.,* (2012).

The fourth cross AGS-25 × JS-335, expressed variation for seed colour and pubescence presence. Yellow seed colour was dominant over yellow green seed colour and showed goodness of fit with a monogenic ratio of 3:1 with a chi-square value of ($x^2=0.545$; P=0.46). JS-335 was the glabrous genotype in this cross with no hair on the plant and pods (Figure c). Presence of pubescence was dominant over absence which was depicted by the segregating F_2 generation of this cross. A chi-square value of ($x^2=0.061$; P=0.805) was obtained for pubescence and this trait followed the simple monogenic ratio of 3:1.

The fifth cross used for this study PS-1556× EC-389148, showed variation for seed colour and flower colour. Yellow seed colour was dominant over yellow green seed colour and was found consistent with the Mendelian ratio of 3:1 with a chi-square value of ($x^2=0.167$; P=0.683). Flower colour had the same chi square value with purple flower colour dominating over white flower colour and fitted the monogenic ratio of 3:1.

(a) Bhatt (black)   (b) DT-21 (yellow)   (c) Absence of pubescence in JS-335   (d) Gray pubescence in PS-1241   (e) DT-21 × BHATT (shaded seeds)

CONCLUSION

These results (Table 2) indicate that artificial hybridization is a way of exploring different morphological variations found in soybean seed. The crosses made in this study displayed significant variation for seed coat, hilum, pubescence and flower colour. The cross DT-21 × BHATT, that was studied for the inheritance of black

and yellow seed coat colour, had several distinctly coloured seeds in its F_2 generation. The simple genetic inheritance of the four traits studied in this investigation depict that the appearance of soybean seeds can be improved using simple breeding procedures so that

good quality seed is made available for human consumption.

Table 2: Chi-square values obtained for various traits in five crosses used in the study.

Cross	Trait	Theoretical Frequencies	χ^2 VALUE	P Value
1. DT-21 × PS-24	Hilum colour	3:1	χ^2=0.231	P=0.631
	Flower colour	3:1	χ^2=0.026	P=0.872
2.PS-1347×PS1241	Pubescence	3:1	χ^2=0.022	P=0.637
	Hilum colour	9:3:4	χ^2=0.241	P=0.886
3. DT-21 × BHATT	Seed colour	9:3:4	χ^2=0.068	P=0.966
	Hilum colour	3:1	χ^2=0.051	P=0.820
	Flower colour	3:1	χ^2=0.051	P=0.820
4. AGS-25×JS- 335	Seed colour	3:1	χ^2=0.545	P=0.460
	Pubescence	3:1	χ^2=0.061	P=0.805
5.PS-1556× EC- 389148	Seed colour	3:1	χ^2=0.167	P=0.683
	Flower colour	3:1	χ^2=0.167	P=0.683

Conflict of interest. The authors declare no conflict of interest.

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