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Genetic Inheritance of Black Seed Coat in Soybean

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ABSTRACT

The standard phenotype of R gene is black hilum on black seed. The genetic control of several soybean (*Glycine max* L. Merr.) natural variants has not been studied. The genetic type T16 is the only occurrence with brown hilum on black seed coat. The aim of this chapter is to further a discussion the genetic control of seed black seed coat in soybean. The commercial cultivars Bragg, BR13 and BR6 present yellow seeds, their natural variants Bragg P and BR13P present black seed color and BR6M, LCV91-26 brown seed color. T16 was combined with these genotypes and it was found that the genetic control of the brown hilum trait in black seed coat of the T16 genotype was controlled by two loci segregating independently and controlling the expression of the color of the hilum and the seed coat color. The expression of the brown hilum trait in black seed coat is dependent on locus *T_*, which controls pubescence color; therefore it occurs only in genotypes with tawny brown pubescence (*T_*). Novel black soybean cultivars, breeding for specific characteristics, selected for direct use could, more suitable for human consumption will increase opportunities for exploring the global market of health and functional food industry and contribute as food source of both calorie and protein to combating hunger in undeveloped countries.

Keywords: Seed coat color; Glycine max; black soybean.

1. INTRODUCTION

Black soybeans containing black seed coats have been a widely used crop for many years due to their dietary health benefits and folk medicine. Consumption of soy foods has been recognized to lower the risk of aging-associated diseases, including cardiovascular disease and cancer, among others [1]. Pigmentation of seed coats is induced via the deposition of a number of flavonoids which derives from an anthocyanin biosynthesis and are secondary metabolites derived from flavonoid pathway such as proanthocyanidins and anthocyanins [1]. Because black soybeans are the only color reported to contain anthocyanins and only brown and black soybeans contain proanthocyanins, this may result in a differing level or type of bioactivity among soybeans with different seed coat colors, moreover these compounds also play a relevant role in plant pathogen defense and protection from UV light exposure [2]. The health-promoting traits of soybean seed are attracting great interest of the market, therefore breeders are working on development of novel soybean lines with a selected seed coat color to be used as bioactive ingredients in functional foods, targeting different healthproblems.

The visual appearance of the soybean seed has been altered as result of domestication during the transition of the wild *Glycine soja*to the current cultivated *Glycine max* [3]. The majority of commercial high yielding soybean cultivars that are grown and consumed throughout the world today exhibit yellow seed coats, whereas the majority of known accessions of the wild progenitor, *Glycine soja*possess black seed coats or, brown seed coats with a range of hilum colors (brown, black, imperfect black, buff, yellow). The genes controlling natural variation of seed coats pigmentation trait in soybean (*Glycine max* (L.) Merr.) are not completely understood. It remains poorly understood mainly because seed coat color of soybean was so far limited as markers to assess hybridity in breeding programs. The color of the seedcoat is important assayed visible marker to detect the

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presence of “off-types” in soybean seed lots of pure strains, is a mandatory descriptor for solicitation of plant variety protection and it has been used to identify mixtures of plantvarieties.

Several natural mutations associated seed coat color have been selected in soybean cultivars. These mutations may represent an important source of genes for the development of soybean food type cultivars in breeding programs.

The study of genetic control of black seed coat trait in T16, and the isolines LC 91-26, BraggP, BR13P and BR6 with brown seeds was investigated by Carpentieri-Pipolo et al. [4] the mainly results are discussed in the presentmanuscript.

2. GENETIC CONTROL OF THE PIGMENTS THATDETERMINE THE SEED COAT COLORS INSOYBEAN

Three independent loci (I, R, and T) mainly control the biosynthesis of the pigments that determine the seed coat colors. The *R* gene produces black hilum and black seed coat. In additional it is found that the locus *R* is also involved in the variegation phenotype affecting seed coat color. The *r-m* allele of this gene produces striped seed coat superimposed on an brown seed color, and the locus *R* and two additional independent loci *I* anthocyanin inhibitor and *T* tawny, control pigmentation and anthocyanin accumulation [5].

The *I* allele inhibits the production and accumulation of pigments over the entire seed coat, resulting in uniformly yellow-colored seeds, whereas the *i*allele leads to completely pigmented seeds by allowing the production and accumulation of pigments over the entire seed coat. The *I* locus controls the presence or absence and spatial distribution of flavonoid pigments and has four alleles (I, i^i, i^k, i). In the presence of *I* the total inhibition of pigmentation occurs; the double recessive allele i^i allows full pigmentation of the seed; with i^iallele, pigmentation is restricted to the hilum; with the allele i^k pigmentation in hilum and in part of the seed coat occurs. Plants carrying the *I* or i^iallele show complete inhibition of pigmentation in the seed coat or pigmentation only in the hilum, respectively, resulting in a yellow seed phenotype. However, there are four main seed coat colors (black, brown, yellow and green), and the inheritance of black and brown pigments is genetically independent from the green and yellow ones [6-9]. The remaining two alleles, i^i and i^k, inhibit pigmentation except in the hilum and a saddle-shaped region, respectively. Yellow soybean cultivars carry the *I* allele for a light (no pigmented) hilum or the i^iallele for a dark (pigmented) hilum [6-9] as observed T16 and Bragg, that carry *i*and i^i, respectively. The dominance relationships among the four alleles are $I > i^i > i^k > i$. Inhibition of seed coat pigmentation by the *I* locus, at least the *I* and i^ialleles, is the result of RNA silencing of chalcone synthase genes, a key enzyme in a flavonoids biosynthesis.

The dominant allele *T* conferred brown (or tawny) and *t* conferred gray pubescence color. Further, *T* generally darkens hilum and/or seed coat color in combination with genotypes at the *I*, W_1, *R* and *O* loci [9,10]. The allelic series *T/t-r/t* produces the seed coat or hilum colors of black, red-buff and buff, respectively [9]. The dominant allele *T* was completely shown to suppress the development of abnormal pigmentation around the hilum region and to partially suppress the cracking of seed coat associated with chilling treatments. One recessive gene conditioning the phenotype spread hilum in the T48 genotype was found. The expression of the spread hilum is dependent of locus *T_*, which controls pubescence color; therefore it occurs only in genotypes with brown pubescence (*T_*), which characterizes pleiotropic effect of this locus on the trait spread hilum [3,10].

The inheritance black hilum/black seed coat was described by Kovinich et al. [6], Carpentieri-Pipolo et al. [4] Yang et al. [8] and Senda et al. [7]. More recently Gillman et al. [3] identified the specific gene and causative basis behind the phenomenon of brown hilum/seed coat coloration in soybean (*R* locus), and discovered an allelic series of four loss of function mutations affecting our *R* locus gene candidate (R_2R_3MYB gene). The presence of each one of these mutations was perfectly correlated with the brown seed coat/hilum phenotype in a broadly distributed survey of soybean cultivars. These findings strongly suggest that loss of function for one particular seed coat-expressed R_2R_3MYB gene is responsible for the brown seed coat/hilum phenotype in soybean.

The allelic sequence *R*, *r-m*, and *r* controls the pigment color and the presence of stripes in the seed coat. The *r-m* allele is dominant over *r* and recessive to *R*. The *r-m* allele exhibits instability in somatic and germinal cell layers and the revertant phenotypic forms are not stable with expression forms of the allele at high frequency [11]. The expression of this not typical phenotypic germinal instability was suggested as transposable elements residing in or near the *r-m* allele. Since the revertants of *r-m* alleles may be not be identical to the standard *R* and *r* forms they were designated by Chandlee and Vodkin [12] as designate *R** and *r** . There is one phenotypic difference between the standart*R* allele, and the *R** carryng brown hilum in black seed and *r* carryng r-m* (striped seed coat); thus the locus *R* is not stable and could produce all three forms *R**, *r-m* and *r* [11].

3. INHERITANCE OF BLACK SEEDS GENETIC TYPE T16, AND IN THE NATURAL VARIANTS BRAGGP, BR 13P, BR 6M, LC91-26

The inheritance of black seeds and brown hilum in genetic type T16, and in the natural variants BraggP, BR 13P, BR 6M, LC 91-26 were reported by Carpentieri-Pipolo et al. [4]. The main results of this study will be followeddiscussed.

The standard phenotype *R* gene is black hilum on black seed [3]. The genetic type T16 is the only occurrence with brown hilum on black seed coat in the *Glycine max* accession of USDA soybean germplasm.

The genetic type T236 is an *Glycine max* accession of USDA Soybean Germplasm Collections with white flower, gray pubescence and a usual red-buff (Rbf) seed coat. LC 91-26 is a T236 isoline, it is homozygous (*r-m*/*r-m*) (brown striped). The standard commercial soybean cultivars Bragg and BR13 present yellow seed coat with a black hilum and the standard BR6 present brown seed coat with a brown hilum. The isolines, natural variants of those cultivars are BraggP and BR13P with black hilum and black seed coat and BR6M with Brown hilum and brown seed coat respectively, the seed coat phenotypes of these soybean access are shown in the Fig. 1.

Table 1 summarize of genotypic of parental used accessing F_1 and F_2 generations resulting from crosses between soybeans genotypes T16 and Bragg, T16 and BraggP, T16 and BR 13P, T16 and BR 6M, T16 and LC 91-26, T16 × T236.

No difference between the original and reciprocal crosses was found, suggesting that there was no effect of maternal control on the characteristic hilum color. Hilum tissue is not maternally-derived, in contrast to the seed coat. Phenotypes of F_1seeds showed that the black hilum was dominant over the brown hilum, a previously expected result as described by Chandlee and Vodkin [11] in their study of the relation between black and brown pigment in soybean hilum and seed coat. Regarding the seed coat, the yellow color was dominant over the black one in the crossing between T16 and Bragg, and over tanned-red in the crossing between T16 and T236, as described by Seo et al. [12].

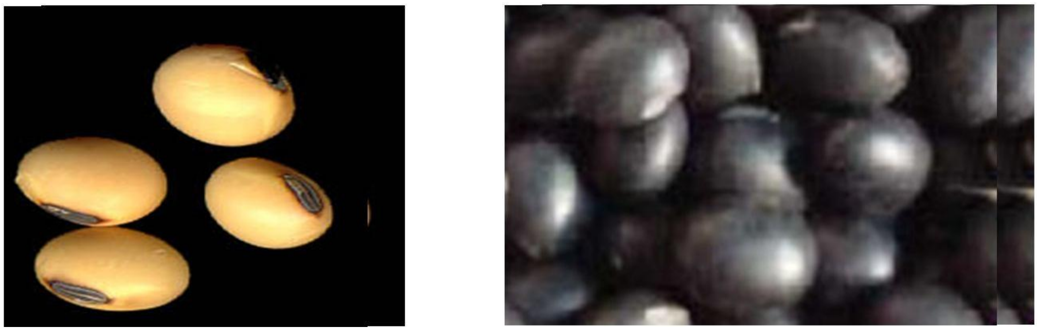
F_2 segregation from T16 and Bragg crossing indicated that the brown hilum pigmentation in black seed coat is controlled by a single allele *R* (Table 2). These results shown that the phenotype brown hilum pigmentation in black seed coat in the genetic type T146 (*R*) is controlled by a singleallele.

In the crosses between T16 and BraggP and T16 and BR 13P, the F_2 segregation presented correspondence to the 3:1 proportion (black hilum: brown hilum). This result was confirmed by the low value observed in the chi-square shows that the inheritance of seed color the hilum color were determined by a single gene in these crosses, and the black hilum dominates over brown hilum.

The dominance relation (3:1) was observed in the crosses between T16 and BR 6M and T16 and LCV 91-26 with dominance of brown striped seed coat color over brown seed coat. However, there was only segregation of seed coat color, and all the seeds showed brown hilum, confirming that all T16 and BR6 Brown genotypes and LCV91-26 (brown striped) have the same genetic constitution for hilum color (Table 2). Chandlee and Vodkin [11] have examined the inheritance of *r- m* allele through several generation and found that it exhibits unusual allele instability which changes in gene expression that can convert to the other expression form of the alleles at high frequencies. This behavior has not been seen in other unstable alleles in soybean and suggests that an insertion sequence or transposable elements reside in or near the *r-m*allele.

The F_2 population from T16 and Bragg demonstrated segregation in four different phenotype classes (yellow seed coat with black hilum, yellow seed coat with brown hilum, black seed coat with black hilum and black seed coat with brown hilum). F_2 showed concordance with the expected one of9:3:3:1 for two loci segregating independently and controlling the expression of the color of the hilum and the seed coat color (Table 2).

LCV91-26:iiR_tr_w_1w_1 **T16: ii__T_w_1w_1**



Bragg:ii_R_T_w_1w_1 **BraggP (with black seeds):iiR_T_w_1w_1**

Fig. 1. Phenotype and genotypes of genetic types LCV 91-26 (red-buff seed coat, white flower and gray pubescence) LCV 91026 is natural variant of T236, T16 (black seed coat, brown hilum, white flower and tawny tawny brown pubescence), commercial cultivar Bragg (yellow seed coat, black hilum, white flower and tawny brown pubescence) and the natural variants BraggP (black seed coat, black hilum white flower and tawny brown pubescence), Bragg P is natural variant of Bragg

Three SSR markers (Satt212, Satt417and Sat_090) linked to the *Lx* genes in the $F_{2:3}$ Segregation analysis of population from the cross between the soybean cultivars BR 36 X BRS 213, and BRS BRS155 X 213.

Table 1. Description of phenotypic effects of four different genetic loci affecting seed coat and hilum colors in soybean

Genotype	**Phenotypes**				**Genotype**
	Seed coat	**Pubescence**	**Flower**	**Hilum**	
T 16	Black	Tawny	white	Brown	$iirrTTw_1w_1$
Bragg	Yellow	Tawny	white	Black	$i^ii^iRRTTw_1w_1$
Bragg P	Black	Tawny	white	Black	$iiRRTT\ w_1w_1$
BR13P	Black	Tawny	white	Black	$iiRRTT\ w_1w_1$
BR6M	Brown	Tawny	white	Brown	$iirrTTw1w1\ w_1w_1$
LC 91-26	Black + striped	Tawny	white	Black	$ii\ r\text{-}m\ r\text{-}m\ TT\ w_1w_1$
T 236	Red-buff	gray	white	Brown	$iirrttoo\ w_1w_1$

Source: Carpentieri-Pipolo et al. [4]

Table 2. Segregation analysis of F_2 population from the cross between the genetic typeT1 and the natural variants Bragg P, BR13 P, BR6 B, T 236 and LCV 91-26

		Number of observed F_2 individuals									
				Seed coat							
Cross	**Yellow**	**Black**	**Brown**	**Brown + striped**		**Red buff**	**Buff**		**Theoretical frequencies**	**χ2**	**P**
				Hilum							
	Black	Brown	Black	Brown	Brown	Brown	Red buff	Buff			
T16 × Bragg P			237	80					03:01	0.009	0.922
T16 × BR13 P			494	160					03:01	0.099	0.751
T16 × BR6 M					98	291			03:01	0.007	0.930
T16 × LCV 91-26					87	276			03:01	0.206	0.649
T16 × Bragg	244	84	72	24					9:3:3:1	1.324	0.723
T16 × T236			146	45			45	15	9:3:3:1	0.160	0.983

X^2 Chi-square value ; P: probability.
Source: Carpentieri-Pipolo et al. [4]

In the cross between T16 and T236, segregation of the F_2 population in four phenotype classes also occurred (black seed coat with black hilum, black seed coat with brown hilum, red-buff seed coat with buff hilum and red-buff seed coat with red-buff hilum) which agreed with the results reported by Seo et al. [12] from the crossing between T236 and Clark isoline L68-2077 (*r-m*). The obtained results indicating two loci segregating independently. The F_2 population have shown two phenotypes for pubescence color: tawny brown and gray pubescence. Plants with gray pubescence (*tt*) did not produce seeds with tawny brown hilum on black seed coat (*T_*), it only was observed in the plants with tawny brown pubescence, therefore the expression of the brown hilum trait on black seed coat is dependent on locus *T_*, which controls pubescence color; therefore it occurs only in genotypes with tawny brown pubescence (*T_*), these phenomenon characterize epistatic effect of the T locus on the hilum brown trait on seeds with black coat. The pubescence color allele, T, is involved in the dihydroxylation of the B ring in the formation of cyandin-3-glucoside that id necessary for function in flavonoids and anthocyanins biosynthesis.

4. CONCLUDING REMARKS AND FUTUREPERSPECTIVES

Our study concerning the analyses of the black seed coat/hilum various genotypes of black soybean. The genetic control of the brown hilum trait in black seed coat in the T16 genotype is controlled by a single recessive. There is no cytoplasmic effect in the expression of this trait and although belonging to the same allelic sequence, the genetic control of the brown hilum in black seed coat is different and independent from the one that determines black hilum in black seeds coats. Moreover the expression of the brown hilum trait on black seed coat is dependent on locus *T_*, which controls pubescence color, its occur only in genotypes with tawny brown pubescence (*T_*). The information of this chapter could have utility in breeding programs for developing novel black soybean varieties to directly use in daily food. The information also could help for development of molecular markers of traits which are linked with seed color to be used in marker-assisted breeding.

Novel black soybean cultivars, breeding for specific characteristics more suitable for human consumption with good acceptability will increase opportunities for exploring value-added processing products, to foster the global market of health and food industry. Moreover considering that soybean is the world's faster expanding crop with potential yield in temperate, subtropical and tropical zones, soybean varieties with black coat, selected for direct use could contribute as food source of both calorie and protein intake to combating hunger in undeveloped countries.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Any other remarkable point(s): Doctor Valeria Carpentieri Pipolo. She is wheat and soybean breeder. Actually DrCarpentieri-Pipolo works at Embrapa-wheat at Rio Grande do Sul State, Brazil where she works at wheat breeding program. She is leading research projects funded by National and International Agencies. She coordinated the International project in collaboration with the International Center for Genetic Engineering and Biotechnology- ICGEB, (Italy) granted by Brazilian National Council for Scientific and Technological Development (Science Without Borders Program and Parana State Araucaria Research Foundation). Advised more than 20 PhD student's thesis at Agronomy Program From State University PhD Course. Professor of the specialization course in Food Science, teaching the Food Safety of GM plants and foods derived from new biotechnologies. As a member of the Brazilian Biosafety Association, ANBIO, she teach capacity building courses for more than 300 professionals all over Brazil in food security and risk assessment of GM plants. Dr Carpentieri-Pipolo was awarded with scholarship for her productivity from the Brazilian National Council for Scientific and Technological Development since from 1989 to 2020.

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