

Inheritance of seed coat color in sesame

Hernán Laurentin⁽¹⁾ and Tonis Benítez⁽¹⁾

⁽¹⁾Universidad Centroccidental Lisandro Alvarado, Decanato de Agronomía, Departamento de Ciencias Biológicas, Vía Agua Viva, Cabudare 3023, Venezuela. E-mail: hlaurentin@ucla.edu.ve, tonisbenitez@hotmail.com

Abstract – The objective of this work was to determine the inheritance mode of seed coat color in sesame. Two crosses and their reciprocals were performed: UCLA37 x UCV3 and UCLA90 x UCV3, of which UCLA37 and UCLA90 are white seed, and UCV3 is brown seed. Results of reciprocal crosses within each cross were identical: F₁ seeds had the same phenotype as the maternal parent, and F₂ resulted in the phenotype brown color. These results are consistent only with the model in which the maternal effect is the responsible for this trait. This model was validated by recording the seed coat color of 100 F₂ plants (F₃ seeds) from each cross with its reciprocal, in which the 3:1 expected ratio for plants producing brown and white seeds was tested with the chi-square test. Sesame seed color is determined by the maternal genotype. Proposed names for the alleles participating in sesame seed coat color are: Sc₁, for brown color; and Sc₂, for white color; Sc₁ is dominant over Sc₂.

Index terms: *Sesamum indicum*, export market, maternal effect, seed color, sesame breeding.

Herança da cor do tegumento em sementes de gergelim

Resumo – O objetivo deste trabalho foi determinar o padrão de herança da cor do tegumento da semente de gergelim. Dois cruzamentos com recíprocos foram feitos: UCLA37 x UCV3 e UCLA90 x UCV3, dos quais UCLA37 e UCLA90 apresentam sementes brancas e UCV3, sementes marrons. Os resultados dos cruzamentos recíprocos, dentro de cada cruzamento, foram idênticos: as sementes F₁ mostraram o mesmo fenótipo que o parental materno, e as F₂ resultaram no fenótipo semente marrom. Esses resultados são consistentes apenas com o modelo no qual o efeito materno é responsável por essa característica. Esse modelo foi validado pelo registro da cor do tegumento da semente de 100 plantas F₂ (sementes F₃) para cada cruzamento com o seu recíproco, em que a proporção esperada de 3:1 para plantas produzindo sementes marrons e brancas foi testada com o teste do qui-quadrado. A cor do tegumento da semente de gergelim é determinada pelo genótipo materno. Os nomes propostos para os alelos participantes na determinação da cor da semente de gergelim são: Sc₁, para a cor marrom; e Sc₂, para a cor branca; Sc₁ é dominante sobre Sc₂.

Termos para indexação: *Sesamum indicum*, mercado de exportação, efeito materno, cor da semente, melhoramento de gergelim.

Introduction

Sesame (*Sesamum indicum* L.) is an important cultivated species in tropical areas. It is mainly used as a source of high-quality edible oil and for different types of direct consumption (Laurentin & Karlosvky, 2006). According to Food and Agriculture Organization of the United Nations (2013), the average annual production in the last ten years has been over 4 million tons. Myanmar, India, China, Ethiopia, and Nigeria are the major producers worldwide. About 25% of their production is export-driven and mainly sent to China, Japan, Turkey, Korea, and Syria. Sesame production in Latin America is also oriented to exportation. Paraguay, Guatemala, Mexico, Bolivia, and Venezuela

are the major exporter countries in the region and are responsible for about 10% of the international market. The most exported agricultural product in Venezuela is represented by sesame grains.

One of the most important attributes for introducing sesame grains in the market is seed color. Although most are light colored (Morris, 2009), there is a wide variability in sesame seed coat color, which varies from white to black. Due to the importance of this trait for the export market, seed color is a central target in sesame breeding programs; however, there are few studies on the inheritance of this essential seed attribute, and determination of genetic factors affecting any trait is necessary to establish useful breeding programs.

The inheritance of traits may be explained in three ways by: nuclear inheritance, attributed to genes in the nucleus; cytoplasmic inheritance, due to genes in the mitochondria or chloroplast; and maternal effect, which is the effect of the mother plant on some progeny phenotypes, especially on seeds (Laurentin, 2011). A comprehensive inheritance study must consider reciprocal crosses, to compare F_1 and F_2 progenies coming from different maternal genotypes.

Previous inheritance studies about seed coat color in sesame report different inheritance mechanisms. Gutiérrez et al. (1994), Falusi (2007), and Zhang et al. (2013) concluded that inheritance was controlled by major genes at the cell nucleus. Shim et al. (2005) and Ashri (2006) reported that minor genes were the ones responsible for this trait; however Ashri (2006) also observed that the white color was a recessive character. None of these investigations used reciprocal crosses. Pandey et al. (2013), using reciprocal crosses, found that the maternal effect is responsible for the inheritance of seed coat color; however, these authors did not compare F_2 and reciprocal F_2 ; therefore, doubt remains if this result is due to maternal effect or cytoplasmic inheritance.

The objective of this work was to determine the inheritance mode of seed coat color in sesame.

Materials and Methods

The experiment was carried out at the Miguel Luna Lugo Experimental Station, at Universidad Centroccidental Lisandro Alvarado, in Cabudare, state of Lara, Venezuela (10°01'25"N, 69°16'44"W, at 510 m above sea level), in 2011 and 2012 (one cycle per year). The region has silty-clay-loam soils and an annual average rainfall of 750 mm.

Two cultivars, UCLA37 and UCLA90 (Laurentin et al., 2004), were used as white-seed genes donors; and the cultivar UCV3 (Laurentin et al., 2004) was used

as a brown-seed gene donor. Individual plants from the two white-seed cultivars, UCLA37 and UCLA90, were crossed reciprocally to individual plants of the brown-seed cultivar, UCV3. Crosses were made at plant maturity by placing fresh pollen on the stigma tip of a flower emasculated 12 hours before. In this way, four F_1 populations were obtained: the first one from UCLA37 as the female parent crossed to UCV3 as the male parent; the second one by the reciprocal cross of UCLA37 as the male parent to UCV3 as the female parent; the third one using UCLA90 as the female parent crossed to UCV3 as the male parent; and the fourth one by the reciprocal cross of UCLA90 as the male parent to UCV3 as the female parent. Five seeds of each F_1 generation were sown to get F_1 plants and F_2 seeds by natural self-pollination.

For each of the four populations, for both generations (F_1 and F_2), color seed was recorded for each individual seed. Observed values within each population in each generation were compared to the expected values for: one-gene nuclear inheritance; cytoplasmic inheritance; and maternal effect by means of a chi-square (χ^2) goodness of fit test. Null hypothesis for F_1 and F_2 must be that all individuals had only one phenotype, and alternative hypothesis, that individuals had different phenotypes; however, because the χ^2 test requires at least two phenotypes, hypothesis was tested arbitrarily with 99% of individuals for one phenotype and with 1% of individuals for another phenotype. This information was complemented with the comparison between F_1 and reciprocal F_1 , and also between F_2 and reciprocal F_2 . Phenotypic designation of each generation and comparison between populations were done for both crosses. The dichotomous key used for determining the inheritance mode of seed color is described in Table 1. To validate the model, 100 F_2 seeds from all crosses were sown, and seed color in each individual F_2 plant (F_3 seeds) was recorded. χ^2 goodness of fit test

Table 1. Dichotomous key used to determine the inheritance mode of seed coat color in sesame (*Sesamum indicum*).

	Alternative results	Inheritance mode
1	100% of individuals with the same phenotype within each F_1 (F_1 and reciprocal F_1), and both F_1 's (F_1 and reciprocal F_1) with the same phenotype.	Nuclear inheritance
	100% of individuals with the same phenotype within each F_1 (F_1 and reciprocal F_1), but both F_1 's (F_1 and reciprocal F_1) with different phenotypes.	
2	100% of individuals with the same phenotype within each F_2 (F_2 and reciprocal F_2), and both F_2 's (F_2 and reciprocal F_2) with the same phenotype.	Maternal effect
	100% of individuals with the same phenotype within each F_2 (F_2 and reciprocal F_2), but both F_2 's (F_2 and reciprocal F_2) with different phenotypes.	
		Cytoplasmic inheritance

was performed for comparison of the observed and expected phenotype ratios according to the determined inheritance mode. All χ^2 tests were performed with the software Genes, version 4.1 (CNPq and UFV, Viçosa, MG, Brazil).

Results and Discussion

F₁ phenotypes were defined by recording coat color of individual seeds and using the χ^2 test (Table 2). For the four F₁ generations, null hypothesis was accepted, since 100% of the individuals within each F₁ had the same phenotype. Crosses ♀UCLA37 x ♂UCV3 and ♀UCLA90 x ♂UCV3 resulted in the phenotype white seed coat; however, crosses ♀UCV3 x ♂UCLA37 and ♀UCV3 x ♂UCLA90 resulted in the phenotype brown seed coats. As for F₁, null hypothesis was accepted for the four F₂, since 100% of the individuals within each F₂ resulted in the same brown seed coat phenotype (Table 2). Comparison between F₁ of UCLA37 x UCV3 and F₁ from its reciprocal crosses resulted in different phenotypes; the same was recorded for F₁ of UCLA90 x UCV3 and its reciprocal. Laurentin (2011) attributes these results to the influence of the maternal genotype. Similar results have been reported in several species and traits; for example, in interspecific hybrids of *Passiflora* for vegetative traits (Primot et al., 2005), or in the composition of fatty acids in soybean (*Glycine max* L.) seeds (Gilsinger et al., 2010).

When UCLA37 was the female parent, F₁ had white seeds, but in the reciprocal cross, when UCV3 acted as the female parent, seed coats were a brown color. Similarly, in the cross UCLA90 x UCV3, F₁ resulted in white seeds when UCLA90 was the female parent; however, when UCV3 was the female parent, the seed coats were brown. For both crosses, the phenotype depended on the female parent. These results discard nuclear inheritance for this trait, and results on F₂ must be analyzed to determine if maternal effect or cytoplasmic inheritance are occurring (Table 1). For both crosses, F₂ resulted in brown seeds, independently of which parent was acting as the female parent in the original cross. When the maternal effect is determining the inheritance of a trait, there is a causal influence of the maternal genotype or phenotype on the offspring phenotype (Wolf & Wade, 2009). A possible explanation is that for both F₂ generations within each cross (the cross and its reciprocal cross), offspring have different cytoplasmic content, but maternal parents have the same nuclear genotype.

The presence of only one phenotype within each generation for both crosses suggests that major genes, located in the mother plant, are determining seed coat color. Therefore, the identification of these genes has been proposed according to seed color (Sc), using numbers to indicate different alleles; the genotype of UCV3 has been identified as Sc₁Sc₁, and the genotypes of UCLA37 and UCLA90 as Sc₂Sc₂. Pandey et al.

Table 2. Phenotypes and seed number obtained for F₁ and F₂ seeds, and for F₂ plants (F₃ seeds) from crosses between UCLA37 and UCV3, and between UCLA90 and UCV3 sesame (*Sesamum indicum*) cultivars.

Crosses	Seed number for F ₁	Number of seeds with color		Calculated chi-square	p-value
		White	Brown		
F ₁ and reciprocal F ₁					
♀UCLA37 x ♂UCV3	182	182	0	0	0.32
♀UCV3 x ♂UCLA37	137	0	137	0	0.32
♀UCLA90 x ♂UCV3	199	197	2	1.00	0.31
♀UCV3 x ♂UCLA90	176	176	0	0	0.32
F ₂ and reciprocal F ₂					
♀UCLA37 x ♂UCV3	217	0	217	0	0.32
♀UCV3 x ♂UCLA37	433	0	433	0	0.32
♀UCLA90 x ♂UCV3	431	0	431	0	0.32
♀UCV3 x ♂UCLA90	411	0	411	0	0.32
F ₂ plants (F ₃ seeds)					
♀UCLA37 x ♂UCV3	100	33	67	3.41	0.06
♀UCV3 x ♂UCLA37	100	31	69	1.92	0.17
♀UCLA90 x ♂UCV3	100	20	80	1.33	0.24
♀UCV3 x ♂UCLA90	100	29	71	0.85	0.35

(2013) suggested names to describe genes that determine seed coat color; however, they did not evaluate phenotypes obtained in reciprocal crosses, or record phenotypes of the two F₂ generations, only of one F₂ per cross. The results obtained by these authors in F₁ and reciprocal F₁ agree with those of the present work, but it is not possible to discern if the trait is under maternal effect or cytoplasmic inheritance because of the lack of results from both F₂.

Seed phenotype was determined by the genotype of the mother plant, not by the cytoplasmic content or the genotype of the seed itself (Figure 1). When the mother plant presented a homozygous Sc₁ allele, F₁ seeds were brown; when the mother plant presented a homozygous Sc₂ allele, F₁ seeds were white. Likewise, for F₂, seed color was determined by the mother plant genotype; and, since both genotypes were the same, both phenotypes were also the same. For F₁ plants (that carried F₂ seeds), the Sc₁ allele was dominant over the Sc₂ allele; therefore, both F₂ resulted in brown coat seeds. To validate the model, 100 F₂ plants were obtained for each of the four populations, and seed coat color was recorded for each individual plant. If the

maternal effect is responsible for seed coat color, 75% of the F₂ plants must produce brown seeds and 25% of the F₂ plants must produce white seeds (Figure 1). The χ^2 test was used to verify this hypothesis (Table 2).

According to most of the previous studies on the inheritance of sesame seed coat color, this trait is determined by major genes at the embryo (Gutiérrez et al., 1994; Ashri, 2006; Falusi, 2007; Zhang et al., 2013). Although these reports did not present results of reciprocal crosses, it is possible to conclude that there was segregation for F₂, which discards the maternal effect. The results obtained in the present study also show that major genes are responsible for sesame seed coat color and for the dominance of dark color over light color, but differ strongly as to the inheritance mode. Only Pandey et al. (2013) indicate that maternal effect controls sesame seed coat color; however, the authors do not discuss this result. That could be explained by the different genetic background of the plant material used, which originates a different inheritance mechanism due to the presence of a single locus controlling the mechanism of this maternal influence, such as the one proposed by Wolf &

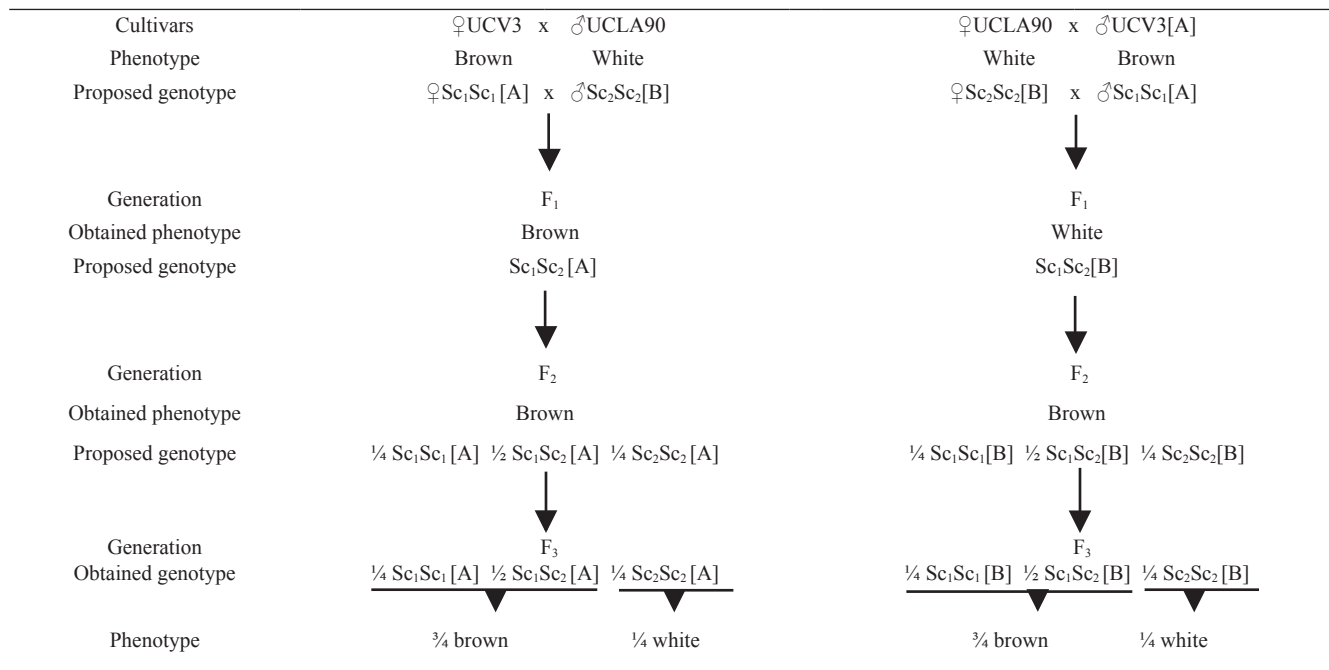


Figure 1. Proposed model to explain the maternal effect on seed coat color when UCLA90 was crossed to UCV3, and when UCLA37 was crossed to UCV3, both reciprocally. Sc₁ and Sc₂ represent nuclear alleles; [A] and [B] represent cytoplasmic content. The model shows that phenotype differences are a consequence of the maternal genotype and are not related to nuclear genotype or to cytoplasmic content.

Wade (2009): pre-loading of messenger RNAs into the unfertilized ovule, or nutritional provision from mother plant to zygote. In a similar way, Gilsinger et al. (2010) explained maternal effect on fatty acid composition of soybean seeds as a consequence of factors translocated from the maternal plant. The presence of this allele in a maternal plant would originate a maternal effect, promoting the transport of proteins or mRNA by another gene (in this case Sc) to the zygote, determining maternally the seed coat color. When the allele is not present in the maternal plant, seed coat color would be determined by the genotype of the own zygote, as is the case in previous studies. Different inheritance modes for seed color have been described for other cultivated species; for instance, in *Brassica campestris*, it has been found that this trait is determined by nuclear genes (Stringam, 1980), but also by the maternal effect (Chen & Heneem, 1992). Rahman et al. (2001) reported inheritance in *B. napus* due to nuclear genes; however, Liu et al. (2005) also observed maternal effect. In soybean, Terao (1918) obtained a result that adjusts to the proposed model of inheritance, in which a major gene is responsible for maternal effect, concluding that a single factor is responsible for green seed coat color when the maternal parent was yellow, but that there was no segregation when the maternal parent was green, since F₁ and F₂ were the same color as the female parent.

The results obtained in the present study, which contradict those of previous reports, indicate that the inheritance pattern for seed coat color in sesame depends on the genetic background of the genotypes acting as parents. Therefore, it is necessary to determine the inheritance mode for this trait for parents, which will be used to obtain segregant populations, when seed coat color is the target in sesame breeding.

Conclusions

1. The inheritance mode of seed coat color in sesame (*Sesamum indicum*) observed for the two crosses evaluated – UCLA37 x UCV3 and UCLA90 x UCV3, both reciprocally –, is the maternal effect.

2. Seed coat color in sesame is determined by the maternal genotype, in which the allele Sc₁ is the code for brown color and Sc₂ for white color, being Sc₁ dominant over Sc₂.

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