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Genetic control of early grain darkening of carioca common bean

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ABSTRACT – *To obtain information on the genetic control of early darkening in common bean with carioca grain and verify the feasibility of selection by means of a grade scale, two contrasting parents were crossed, line VC-3 with cultivar BRSMG Majestoso. The seeds of F_2 plants and $F_{2:3}$ progenies were evaluated 30, 60 and 90 days after harvest for grain darkening, on a 1 - 5 grade scale. Genetic and phenotypic parameters were estimated. The grade scale efficiently discriminated the progenies in terms of grain darkening. The heritability of the trait increases with the storage time and was high enough to ensure success with selection after 30 days. The genetic control seems to be monogenic with dominance of the allele for early darkening.*

Key words: plant breeding, quantitative genetics, grain quality, heritability, *Phaseolus vulgaris* L.

INTRODUCTION

The preference for the consumption of a specific bean type varies widely, according to the region. However, carioca beans, i.e., beige with brown stripes, are preferred in most parts of Brazil. A large number of lines and/or cultivars with this grain type is available which differ mainly in terms of the beige color of the grain tegument.

Among the traits that affect the acceptance of Carioca varieties by bean producers is the early darkening of the grain tegument, since consumers associate a dark grain color with difficult cooking. Therefore, any bean with a darkened base color is considered old and hard to cook. Early darkening, possibly, depends on the genotype and the environment. The influence of the environment is mainly determined by the factors humidity and time of drying after harvest. Apparently, the amount of polyphenols, specifically tannin, may also contribute to a quicker grain darkening (Junk-Knievel et al. 2007).

There are some common bean lines with a light beige color tone that persists for a long time. In the grains of others, however, grain darkening sets in shortly after storing. It would be important to check the possibility of successful selection for this trait and, above all, identify alternatives of a selection that is as efficient as possible.

The purpose of this study was to obtain information about the genetic control of early grain darkening of Carioca beans. A second objective was to determine whether selection based on a grade scale is feasible and which is the shortest possible storage period to initiate selection.

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MATERIAL AND METHODS

The experiments were conducted in a greenhouse on an area of the Universidade Federal de Lavras (UFLA), in the southern state of Minas Gerais (lat 21 ° 58 ‘ S; long 42 ° 22’ W; 918 m asl).

Two parents were used: the line VC-3 from a breeding program of the Universidade Federal de Viçosa, derived from a cross between AN512666-6 x AN7300031; and cultivar BRSMG Majestoso, (line OPNS-331), from a cross between ‘Ouro Negro’ x ‘Pérola’, performed at the Universidade Federal de Lavras. The grain type of line VC-3 is carioca and has a very light beige base color and there is clear evidence that this shade persists for a long time. On the other hand, the grain type of BRSMG Majestoso is also carioca with a light beige color, but darkens more quickly during storage.

The crosses were performed in a greenhouse. In the field, the F_1 and F_2 generations were established to obtain the generation of the seed tegument. These were sown again. 196 plants were used, with tegument in the F_2 generation. After harvest the grain was sun-dried for six hours, to obtain a homogeneous moisture content. A part was stored away in a transparent plastic tube bag (diameter 5 cm and 21 cm long), and the remainder used to establish the $F_{2:3}$ progenies. Grain darkening was evaluated 30 days after harvest on a 1 to 5 grade scale, where: 1 very light and 5, very dark grain base color. The grades were individually assigned by two raters. The process was repeated 60 and 90 days after harvest in both generations.

The seed-coat color was evaluated in the five most light-colored and the five darkest F_2 plants. A colorimeter (Minolta, model CR-310) was used, for color readings in a three-dimensional system, where the color can be evaluated on three axes. In this study however only data from the vertical axis L were used to evaluate color hues from black to white. Each sample was evaluated five times in a recipient (diameter 6 cm), in which the bottom was completely covered by the beans.

The seeds of F_2 plants were sown in a row (1 m long, row spacing 0.50 m) using standard cultural treatments. At harvest, the progenies $F_{2:3}$ were harvested and grains treated as described above for the F_2 generation.

The tannin in ten $F_{2:3}$ progenies, contrasting in darkening, was also quantified. The method described by Porter et al. (1985) was used, where eight grams of each sample were weighed (accuracy 0.1 mg), placed in a polytron with 60 ml of 70% (v / v) acetone: water and ground for 3 minutes. The extract was filtered through quantitative filter paper (Brum et al. 2005). The CT (condensed tannins) was quantified by the butanol-HCl method, described by Porter et al. (1985), modifying only the reaction time. In a 10 mL tube, 1 mL sample extract was added, 6 mL of a butanol reagent solution: 95% (v/v) hydrochloric acid and 0.2 mL of a ferrous sulphate solution (2% ferrous ammonium sulphate in 2 mol L^{-1} HCl). The tubes were shaken and then heated in a thermostatized bath at 95ºC for 10 minutes. The TC in the samples was measured by a spectrophotometer UV/VIS (SP-2000), at a wavelength of 550 nm. For the construction of the analytical curve, the standard TC extracted from the plant Quebracho (*Schinopsis* spp) was used, purified by Sephadex LH-20 and freeze-dried by a technique described by Terrill et al. (1990).

In both generations the darkening grade data were processed by analysis of variance, initially based on the evaluation date, and considering the treatment effect as random (F_2 plants or $F_{2:3}$ progenies plus the two parents) and error using the following model: $Y_{ij} = m + a_j + p_i + e_{ij}$; Where: Y_{ij} is the darkening grade of grains of progeny i, obtained by the evaluator j; m is the overall mean; a_j is the effect of the evaluator j, j = 1.2; p_i is the effect of progeny i, where (i = 1,2, ... 196); e_{ij} is the error associated to the experimental observation ij, where $e_{ij} \cap N(0,\sigma^2_e)$.

Then the joint analysis of variance of postharvest periods was performed in each generation, according to a model similar to that proposed by Stell et al. (1997), followed by the joint analysis involving generations and postharvest periods. The analyses of variance were performed using the program MSTAT-C (1991).

The genetic and phenotypic parameters were inferred from the expectations of the mean squares, through the following estimators: genetic variance in F_2 plants or $F_{2:3}$ progenies in each postharvest period: $\sigma^2_{Pk} = \frac{MS_{pro} - MS_{erro}}{2}$. The confidence intervals (CI) associated with estimates of the variance components were obtained by the following expression of Ramalho et al. (2005): CI: $P\left[\frac{V_P\hat{\sigma}^2_{Pk}}{x^2_{\alpha/2}} < \sigma^2_{pk} < \frac{V_P\hat{\sigma}^2_{Pk}}{x^2_{1-\alpha/2}}\right] = (1-\alpha)\,100\%$; where: α: pre-established level of significance (in this case, 0.05); $\hat{\sigma}^2_{Pk}$: estimate of genetic variance in F_2 plants or $F_{2:3}$ progenies in evaluation k; Vp: number of degrees of freedom associated with the component of genetic variance, which was determined according to Satterthwaite (1946) $x^2_{1-\alpha/2}$ and $x^2_{\alpha/2}$: tabulated values of x^2 (Chi-square) distribution for Vp degrees of freedom.

The broad-sense heritability, among the F_2 plants or $F_{2:3}$ progenies in the postharvest periods k, h^2_k; $h^2_k = \frac{\hat{\sigma}^2_{Pk}}{\hat{\sigma}^2_{Fk}}$; where: $\hat{\sigma}^2_{Pk}$: defined above; $\hat{\sigma}^2_{Fk}$: phenotypic variance in F_2 plants and $F_{2:3}$ progenies. By the equations proposed by Knapp et al. (1985), with confidence of $1-\alpha = 0.95$, the lower (LI) and upper limits (LS) of the heritability estimates were obtained (h^2).

The realized heritability h^2_R was estimated by the following expression: $h^2_R = \frac{SG_{F_{2:3}/\bar{F}_{2:3}}}{ds_{F_2/\bar{F}_2}} x100$; where: ds_{F_2} is the differential of selection considering 10% of the F_2 plants with lower darkening grade in the evaluation after 90 days, $ds_{F_2} = \overline{F_{2S}} - \overline{F_2}$ where $\overline{F_{2s}}$ is the mean of the selected plants minus the general mean of $\overline{F_2}$ plants; $SG_{F_{2:3}/\bar{F}_{2:3}}$, is the selection gain in the $F_{2:3}$ generation from the selection including 10% of the best F_2 plants; $SG_{F_{2:3}} = \overline{F_{2:3*}} - \overline{F_{2:3}}$; where $\overline{F_{2:3*}}$ is the mean of progenies selected in F_2 and $\overline{F_{2:3}}$ is the overall mean of the progenies in the $F_{2:3}$ generation. The genetic correlationsof performance of the F_2 plants and/or $F_{2:3}$ progenies were estimated at the time of evaluation k and k^1 ($r_{G_{kk'}}$) by the expression $r_{G_{kk'}} = \frac{COV_{xy}}{\sqrt{\sigma^2_{P_k}\sigma^2_{P_{k'}}}}$; where: COV_{xy} is the covariance between the means of plants or progenies in times of evaluation k and k'. $\sigma^2_{p_k}$ and $\sigma^2_{p_{k'}}$, are the genetic variances between plants and/or $F_{2:3}$ progenies in the evaluations performed k or k' days after grain harvest.

Analysis of variance was performed with the colorimeter readings and tannin content data based on a randomized block design.

RESULTS AND DISCUSSION

The joint analysis of grain darkening grades indicated significance for the progeny x generation and progeny x postharvest period interactions, indicating that the performance of the progenies did not coincide in the two generations and three postharvest periods. Likewise, the triple interaction progeny x generation x postharvest period was also significant ($P \leq 0.01$).

It must be emphasized that the correlation estimates of the mean progeny performance in the different generations and postharvest periods were relatively high, especially in the evaluations after 60 and 90 days, in both generations (Table 1). When interaction occurs and the genetic correlation estimates are high, it appears that the interaction is predominantly simple (Cruz et al. 2004), i.e., the changes in the progeny ranking are not pronounced. This is inferred from the mean performance of the best progenies in different postharvest periods and generations. Generally, the progenies that stood out in one generation or postharvest period were virtually the same in the other generation or periods. The same applies to the progenies with more rapid darkening (higher grades). Therefore, we will focus on the mean results, independent of the postharvest period.

The contrast progenies vs parents was not significant ($P \leq 0078$) for grain darkening. This result indicates that for this trait, the mean progeny performance in each generation was similar to the mean parent performance, showing that progenies with other grades than the parents can be identified. It was also observed that the mean of VC-3 line was always lower than of BRSMG Majestoso. This difference increased with storage time, showing, as evidenced in the analysis, that the two parents were contrasting for the time of darkening.

Table 1. Estimates of the genetic correlations of the mean performance of the progenies in the different post harvest periods, considering the same or different generations for the grades of grain darkening

Postharvest period (days)		Generations				
		F_2		$F_{2:3}$		
		60	90	30	60	90
F_2	30	0.96	0.79	0.76	0.70	0.70
	60	-	0.99	0.68	0.78	0.81
	90	-	-	0.59	0.74	0.81
$F_{2:3}$	30	-	-	-	0.66	0.54
	60	-	-	-	-	0.92

The differences between the generations were significant. It was found that regardless of the postharvest period and progenies, the mean obtained with the $F_{2:3}$ progenies (2.76) was higher than with the F_2 plants (2.36), ie., 16.9% higher (Table 2). As expected, the increasing grain age resulted in an increase of the mean of grades of the F_2 plants and $F_{2:3}$ progenies. For example, the mean grade in the F_2 generation, 30 days after harvesting, was 71% of that after 90 days. The range of the grades of grain darkening of progenies varied markedly in both generations, mainly with grain aging. After 90 days, in the $F_{2:3}$ generation for example, the grades ranged from 1.0, very light-colored to 5.0 dark grains. The most significant fact is that a large proportion of F_2 plants or $F_{2:3}$ progenies were graded below 2.0, ie, have a very light base color, even in the evaluations 90 days after harvest, indicating that the trait is easy for selection.

The existence of genetic variability in the progenies was also inferred from the estimates of genetic variance. The h^2 estimates were all different from zero. It is noteworthy, however, that h^2 estimates were obtained in the broad sense, because in the F_2 plants the genetic variance contains $\hat{\sigma}^2_{PF2} = 1\hat{\sigma}^2_A + 1\hat{\sigma}^2_D$, but in the $F_{2:3}$ progenies $\hat{\sigma}^2_{PF2:3} = 1\hat{\sigma}^2_A + 1/4\hat{\sigma}^2_D$ (Ramalho et al. 1993). In the case of dominance in the trait control, estimates of the gain expected with selection using h^2 were overestimated. Unfortunately, no reports were found in the literature for a comparison with the estimates obtained here. It is worth mentioning that the realized heritability (h^2_R), was estimated considering the evaluations after 90 days in the F_2 plants and $F_{2:3}$ progenies. The value was very high ($h^2_R = 0.8130$), indicating that success with selection may be expected.

One question is related to the adequate moment of trait evaluation. The differences are not significant soon after harvest. With aging, the differences become more marked, as observed in the two generations. However, this delay causes difficulties in breeding programs, since the waiting period of 90 days for the evaluations of darkening would practically result in the impossibility of planting three generations per year. After 30 days, the heritability estimates were still high (over 70%), but lower than after 60 and 90 days (Table 3). It was concluded that selection can be performed by breeders after 30 days, in this case without delaying the breeding program. Besides, there is the possibility of speeding up the process by environmental factors, as by the use of UV light, as done by Junk-Knievel et al. (2007).

Apparently, early grain darkening is associated with the content of polyphenols, mainly tannin (Beninger et al. 2005, Junk-Knievel et al. 2007). This fact was confirmed here because, by the grades of the two evaluators, the mean tannin content in the five darkest progenies was 0117 mg TC^{-1}, which is 3.42 times higher than in the five lightest colored progenies (0034 mg CT^{-1}) (CT-condensed tannins).

However, the analysis of tannin is slow, expensive and the grain has to be destroyed. Another alternative would be to evaluate the color of the seed-coat by a colorimeter. This procedure has been used relative frequently in research aimed at evaluating the bean color (Brackmann et al. 2002, Junk-Knievel et al. 2007). The grades of the evaluators assigned to the grains of the five lightest and the five darkest F_2 plants coincide with the pattern of light and dark colorimeter (Table 4). It was inferred that the use of color shade grades is feasible. Considering the large number of bean progenies that breeders usually analyze in each generation, a grade scale is a viable alternative.

Table 2. Mean grades of grain darkening of carioca common bean, in the F_2 generations and $F_{2:3}$ progenies in the different evaluation periods

Generations	Evaluation period	Mean grades of grain darkening				
		Parents			Progenies	
		VC-3	Majestoso	Mean	LI[1/]	LS
F_2	30	1.5	3.0	2.02	1.0	3.0
	60	1.75	3.75	2.26	1.0	4.0
	90	2.85	3.0	2.80	1.0	5.0
	Mean	2.36	2.03	3.25		
$F_{2:3}$	30	1.75	3.0	2.12	1.0	3.5
	60	2.25	3.75	2.65	1.0	4.0
	90	2.50	3.0	3.52	1.0	5.0
	Mean	2.76	2.16	3.5		

[1/]Ll and Ul – Lower and upper limits of the confidence interval, at α=0.05% probability

Table 3. Estimates of the genetic variance ($\hat{\sigma}^2_P$), phenotypic variance ($\hat{\sigma}^2_{F}$) and heritability (h^2) in F_2 and $F_{2:3}$ progenies for each period of evaluation of grades of grain darkening of common bean

Estimates	Days after harvest F_2			Days after harvest $F_{2:3}$		
	30	60	90	30	60	90
σ^2_P	0.123	0.3184	0.7348	0.113	0.657	0.958
LI[1/]	0.105	0.269	0.617	0.093	0.552	0.836
LS[1/]	0.142	0.382	0.890	0.133	0.801	1.108
σ^2_F	0.176	0.382	0.821	0.152	0.729	1.051
h^2	77.62	85.60	90.56	74.17	90.13	91.10
LI	70.38	80.96	87.51	65.78	86.93	88.22
LS	83.03	89.12	92.87	80.49	92.55	93.28

[1/]Ll and Ul – Lower and upper limits of the confidence interval, at α=0.05% probability

Table 4. Value of evaluations of the tegument color of F_2 por grains by a colorimeter (Minolta, model CR-310); mean grades of the color evaluation by eye on the L* axis;); mean grades of the color evaluation by eye and quantification of the tannin grain content in $F_{2:3}$ progenies

F_2 plants	Grades	L*[/1]	$F_{2:3}$ progenies	Grades	mg of TC/mg sample
3	1.5	58.42a	35	1.5	0.035
23	1.0	56.05a	41	1.5	0.035
35	1.5	58.75a	59	1.5	0.035
90	1.5	57.29a	106	1.5	0.027
95	1.5	59.37a	177	1	0.039
mean prog. light-colored	1.5				0.034
5	3.0	39.97c	5	5	0.107
6	3.5	41.74c	6	5	0.151
7	3.0	42.30c	120	5	0.108
138	3.0	45.68b	133	5	0.112
142	2.5	40.56c	181	4.5	0.104
Mean prog. dark	3.5				0.117

L*- value varies from black to white, [/1]- means followed by the same letter belong to the same group by Scott-Knott , at α=0.05% probability

With a view to future studies it would be important to verify whether there is a relation between the early darkening of common bean and grain cooking time. Since is known that older beans are darker and harder to cook (Barrón et al. 1996, Ribeiro et al. 2007), a dark color represents a serious market restriction. If the cooking time of light-colored bean lines, even after 90 days of storage, were not extended, this would be a great advantage, besides the aspect of ease of marketing. With such a cultivar, farmers could wait longer until selling the harvested grain, to obtain best prices in the second growing season, without losing product quality.

We also tried to estimate the number of major genes that control the trait grain darkening. The difficulty, in this case, is to establish a grade criterion to define the phenotype as light or dark-colored. It was determined to consider all plants of the F_2 generation grade with less than 1.5 after 60 days as light-colored. It was found that, under these circumstances, the segregation fits the proportion of 3 dark grains: 1 light-colored grain, indicating that a gene with allele dominance is probably involved in the trait control of fast darkening (Table 3). This gene is most likely involved in the control of the grain tannin content, while the dominant allele would be responsible for the higher level.

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Controle genético do escurecimento precoce de grãos de feijão tipo carioca

RESUMO - *Para obter informações sobre o controle genético do escurecimento precoce dos grãos de feijões do tipo carioca e verificar a viabilidade da seleção por meio de uma escala de notas, foi realizado o cruzamento entre dois genitores contrastantes, a linhagem VC-3 e a cultivar BRSMG Majestoso .Os grãos da plantas F_2 e progênies $F_{2:3}$, foram avaliadas para o caráter escurecimento de grãos, por meio de uma escala de notas que variava de 1 a 5 aos 30, 60 e 90 dias após a colheita. Obtiveram-se as estimativas dos parâmetros genéticos e fenotípicos. A escala de notas foi eficiente em discriminar as progênies com relação ao escurecimento dos grãos. A herdabilidade do caráter aumenta com o tempo de armazenamento, contudo, mesmo com 30 dias, ela foi alta suficiente para permitir o sucesso com a seleção. O controle genético, ao que tudo indica, é monogênico com dominância do alelo para o escurecimento precoce.*

Palavras-chave: Melhoramento de plantas, genética quantitativa, qualidade de grãos, herdabilidade, *Phaseolus vulgaris* L.

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