# ESTIMATES OF GENETIC PARAMETERS OF LATE SEED-COAT DARKENING OF CARIOCA TYPE DRY BEANS

## Estimativas de parâmetros genéticos do caráter escurecimento tardio dos grãos de feijão carioca

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**ABSTRACT**

In order to facilitate commercialization of cultivars of carioca type dry beans, the grains must have the lightest possible cream color and this phenotype must be persistent (late seed-coat darkening). There are reports of genetic variability for this trait. The objectives of this study were to obtain information regarding genetic control of the trait, with emphasis on the estimate of heritability and if it varies according to days after harvest, to verify the effect of locations and/or crop season on seed-coat darkening of the grains and to estimate the genetic and phenotypic correlations of the trait with cooking time, tannin content and grain yield. $F_{2:3}$ and $F_{2:4}$ progenies derived from crossing of the cultivar BRSMG Madrepérola (late seed-coat darkening) and the line RP-2 (early seed-coat darkening) were used. It is concluded that seed-coat darkening is influenced by the environment, crop season or locations; nevertheless, the interaction progenies x environments and progenies x locations is predominantly simple, not expressively changing classification of the progenies. Although the heritability of the darkening scores tends to increase with the storage time of the grain, the interaction progenies x time periods of assessment was not observed. Grains with late seed-coat darkening present a lower tannin content and require less cooking time. The genetic correlation between a dark seed-coat and grain yield was practically null.


**Index terms:** *Phaseolus vulgaris* L., genetic plant breeding, quantitative genetics, grain quality.


**RESUMO**

Para facilitar a comercialização das cultivares de feijão carioca, estas devem possuir cor creme dos grãos o mais claro possível e esse fenótipo deve ser persistente (escurecimento tardio). Há relatos de variabilidade genética para esse caráter. Os objetivos deste trabalho foram obter informações sobre o controle genético do caráter, com ênfase na estimativa da herdabilidade e se ela varia com os dias após a colheita, verificar o efeito de locais e/ou safras sobre o escurecimento dos grãos e estimar as correlações genéticas e fenotípicas do caráter com o tempo de cozimento, teor de tanino e produtividade de grãos. Para isso foram utilizadas progênies $F_{2:3}$ e $F_{2:4}$ derivadas do cruzamento entre a cultivar BRSMG Madrepérola (escurecimento tardio) e a linhagem RP-2 (escurecimento precoce). Concluiu-se que o escurecimento é influenciado pelo ambiente, safras ou locais; contudo a interação progênies x ambientes e progênies x gerações é predominantemente simples, não alterando expressivamente a classificação das progênies. Embora a herdabilidade das notas de escurecimento tenha tendência de aumentar com o tempo de armazenagem dos grãos, não foi constatada interação progênies x épocas de avaliação. Grãos com escurecimento tardio possuem menor teor de tanino e exigem menor tempo de cocção. A correlação genética entre escurecimento e produtividade de grãos foi praticamente nula.


**Termos para indexação:** *Phaseolus vulgaris* L., melhoramento genético de plantas, genética quantitativa, qualidade de grãos.

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## INTRODUCTION

Most of the dry beans grown in Brazil are of the carioca type grain, that is, with grain of a cream color and beige streaks. The original Carioca cultivar was obtained in 1969 (ALMEIDA et al., 1971). Since then, the work of dry bean breeders has been concentrated on this type of grain, and innumerable other cultivars have been obtained (ABREU et al., 2004; MATOS et al., 2007; ABREU et al., 2007; MELO et al. 2010; ABREU et al., 2011). In breeding studies, in addition to grain yield, greater yield stability and resistance to pathogens, culinary qualities have received a great attention (FARINELLI; LEMOS, 2010; JACINTO-HERNÁNDEZ et al., 2010; PAULA et al., 2004).

Among the characteristics associated with the culinary qualities of carioca beans, the lightest possible cream color is highly desirable. This is because it was observed that recently harvested grains cook more quickly, and that with the aging of grains, they become darker (JACINTO-HERNÁNDEZ et al., 2011). Dark beans are an indication of old beans and therefore they have lower commercial value.

Some environmental strategies are recommended with a view toward maintaining the light color of the grains,

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such as an earlier harvest and not drying the grains in full sun for a long period of time (RIOS et al., 2002). Recently, a carioca cultivar was identified which has a very light grain color and, in addition, it maintains this phenotype for some time, i.e., even some months after harvest, the grains remain light colored, which leads to greater commercial value (SILVA et al., 2008).

Given the importance of this trait for agribusiness, information was obtained in respect to genetic control of this trait (SILVA et al., 2008). However, there is no information if changes occur in the heritability over time and if the storage environment (local and years) affects the expression of the character. There is also lack of information about the association between grain yield, time of cooking and tannin content with the darkening of the seed coat. The present study was performed for the purpose of estimating genetic and phenotypic parameters of late seed-coat darkening trait of grains, with a view toward directing breeders in regard to selection of individuals and/ or progenies with the desired phenotype.

## MATERIAL AND METHODS

Field experiments were conducted in an experimental area of the Department of Biology in the Plant Genetics and Breeding Sector of the Universidade Federal de Lavras (UFLA) in Lavras, MG, Brazil, at an altitude of 918 meters, 21º14' latitude South and 45º00' longitude West, and at the Sertãozinho Experimental Farm of the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG) in Patos de Minas, MG, Brazil, at an altitude of 815 meters, 18º34' latitude South and 46º31' longitude West. The population of the cross between the cultivar BRSMG Madrepérola and the RP-2 line was used. The cultivar BRSMG-Madrepérola has carioca type grain with a very light cream background and this color persists for a long period of time (ABREU et al., 2011). The RP-2 line has carioca type grain with a cream background that darkens rapidly. $F_1$ generation seeds were obtained in a greenhouse and $F_2$ generation seeds were obtained in field.

The progenies of the $F_{2:3}$ generation were assessed in the "dry" crop season, with sowing in February, 2011 in Lavras. The progenies of the $F_{2:4}$ generation were assessed in the "winter" crop season, with sowing in July, 2011 in Lavras and Patos de Minas. We evaluated 100 progenies in a simple lattice 10x10 experimental design. The plots consisted of single row that were 2 m long for the $F_{2:3}$ generation and two rows that were 2 m long for the $F_{2:4}$ generation. In both generations, a spacing between rows of 0.50 m was used. Management for the experiments was that normally recommended for the crop.

The main trait assessed was grain darkening score at 30, 60 and 90 days after harvest (DAH) for $F_{2:3}$ and at 30 and 60 days for $F_{2:4}$. For that purpose, samples of grains from the progenies, after harvest, were placed in transparent plastic bags. These samples of the progenies were assessed for the grain darkening trait by means of a scale of scores ranging from 1 to 5, with 1 for light colored grains and 5 for dark colored grains, as observed by two evaluators.

Analysis of variance of the grain darkening scores, the average of the two evaluators, was carried out initially per generation, considering the different time periods as fixed effect, using the following model of plots subdivided in time (STEEL et al., 1997):

$$Y_{ij} = m + a_j + e_{1j} + t_i + (ta)_{ij} + e_{2ij}$$

In which: $Y_{ij}$: is the value observed of the progeny i, at time j; m: effect of the overall mean of the experiment; $a_j$: is the effect of the time period of assessment j, with j = 1,2,3 for $F_{2:3}$ generation and j= 1, 2 for $F_{2:4}$ generation; $e_{1j}$: effect of the experimental error associated with the effect of the time period; $t_i$: effect of the progeny i, with i = 1,2,3,...100; $(ta)_{ij}$: effect of the interaction between the progeny i and the time period of assessment j; $e_{2ij}$: effect of the experimental error associated with the observation $Y_{ij}$, assuming independent and normally distributed errors, with zero mean and variance $\sigma^2$.

For joint analysis of the grain darkening scores of beans considering the analyses at 30 and 60 DAH involving generations ($F_{2:3}$ and $F_{2:4}$) or environments (Lavras and Patos de Minas in the $F_{2:4}$ generation) the following model was used, considering generations or environments as fixed effect:

$$Y_{ijk} = m + a_j + e_{lj} + t_i + (ta)_{ij} + l_k + (al)_{jk} + (tl)_{ik} + (tal)_{ijk} + + e_{2ijk}$$

In which: $Y_{ijk}$: is the value observed of the progeny i, at time period j, and environment k; m: effect of the overall mean of the experiment; $a_j$: is the effect of the time period of assessment j, with j = 1,2; $e_{1j}$: effect of the experimental error associated with the effect of the time period; $t_i$: effect of the progeny i, with i = 1,2,3,...100; $(ta)_{ij}$: effect of the interaction between the progeny i and time period of assessment j; $l_k$: effect of the location of assessment k, with k = 1,2; $(al)_{jk}$: effect of the interaction between the time period of assessment j and location of assessment k; $(tl)_{ik}$: effect of the interaction between progenies i and the location of assessment k; $(tal)_{ijk}$: effect of the interaction between the progenies i, time period of assessment j and location k; $e_{2ijk}$: random effect of the experimental error associated with the observation $Y_{ijk}$, assuming independent and normally distributed errors, with zero mean and variance $\sigma^2$.

The following genetic and phenotypic parameters were estimated: heritability, using the expressions presented by Ramalho et al. (2005) and the respective limits of confidence interval (KNAPP et al., 1985); and, realized heritability for the trait of grain darkening scores at 60 DAH considering the selection performed in $F_{2:4}$ in Lavras with response in Patos de Minas and considering the selection performed in $F_{2:4}$ in Patos de Minas with response in Lavras (FEHR, 1987).

Using the expressions presented by Ramalho et al. (2012) and Cruz et al. (2004) were also estimated the genetic, phenotypic and environmental correlation coefficients between the grain darkening score with grain yield, tannin content and cooking time. For this were used data of 20 $F_{2:3}$ progenies, selected at 30 DAH, ten progenies with the greatest average in assessment of grain darkness, and ten with the lowest average. Grain yield, in grams per plot was obtained in the experiment of evaluation the $F_{2:3}$ generation. Cooking time was assessed in the 20 $F_{2:3}$ progenies at 30, 60 and 90 DAH using the equipment Mattson JAB-77 minor type and the methodology described by Baldoni & Santos (2005). Tannin content was also evaluated in the same 20 $F_{2:3}$ progenies at 30, 60 and 90 DAH, using the methodology described by Marinova et al. (2005).

## RESULTS AND DISCUSSION

One of the difficulties in assessment of the late seed-coat darkening trait of the grains is finding a parameter that can assess their speed of darkening. One alternative is by means of a scale of scores. This procedure was successfully used by Silva et al. (2008). For that reason, it was used in this study. Significant difference (P=0.00) was detected for the grain darkening scores among the $F_{2:3}$ and $F_{2:4}$ progenies in both locations, and among the time periods of assessment (ANOVA no presented). The interaction progenies x time periods was not significant in both generations, indicating that the behavior of the progenies coincided in the different time periods of assessment.

Frequency distribution of late darkening scores at 90 DAH show that 23% of the $F_{2:3}$ progenies still had very lightly colored grains; that is, with a score of less than 3.0. As 100 progenies were assessed, it may be inferred that the segregation obtained is near the proportion of 1 light colored grain to 3 dark colored grains (Figure 1). It was also observed that part of the $F_{2:3}$ progenies exhibited within segregation, that is, there were light and dark colored grains in the same sample. These progenies certainly received greater scores, probably above 3.0. These $F_{2:3}$ progenies that segregate must have their origin in an $F_2$ plant heterozygous for the trait. Those that received a score less than 3.0, with a more uniform light color, must have their origin in $F_2$ plants homozygous for the recessive alleles. For the same reason, it may be inferred that the progenies that received a 5.0 score, with a darker and more uniform color, must have their origin in homozygous $F_2$ plants with dominant alleles for the trait. This condition gives the suggestion that the trait is controlled by one gene with dominance of the late seed-coat darkening allele. These results are in agreement with that which was observed by Silva et al. (2008).

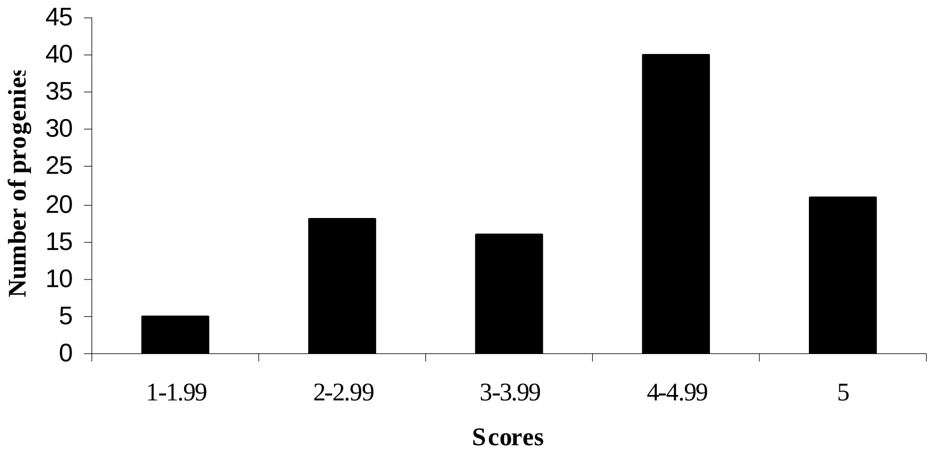


Figure 1 – Frequency distribution of the means of the trait of grain darkening score of the $F_{2:3}$ progenies at 90 days after harvest. Lavras, MG, 2011.

In the $F_{2:3}$ generation, as already mentioned, significant difference was detected for the grain darkening scores among the time periods of assessment. In this generation was observed to the extent that the grains aged, they became darker (Table 1). In the $F_{2:4}$ generation the mean scores for darkening in Patos de Minas were greater than those obtained in Lavras (Table 1). This fact clearly stands out in the frequency distribution of means of the grain darkening scores of the progenies in Lavras and Patos de Minas (Figure 2). Observe that in Lavras, a large part of the progenies obtained scores below 3.0. In contrast, in Patos de Minas, the opposite occurred. The explanation is related to crop management, since in Patos de Minas the bean harvest occurred at a later stage, that is, when the pods were already dry and the plants practically without leaves. The incidence of ultraviolet light in the field contributes to grain darkening even within the pods, before harvest, which was proven by the study of Junk-Knievel et al. (2007), in which ultraviolet light was used to accelerate the darkening of grains in the laboratory.

It was observed that the estimates of heritability for selection at the mean of the progenies, in relation to the grain darkening scores, increased with the age of assessment (Table 1). However, it may be noted that the increase was not very expressive and, in almost all cases, there was overlap in the confidence intervals. The values obtained were similar to those reported by Silva et al. (2008) and shows that that it is possible to successfully perform selection for the trait of late grain seed-coat darkening scores and that this selection may be performed earlier; that is, even at 30 DAH.

Table 1 – Means of the grain darkening scores of beans and estimates of heritability ($h^2$) between the $F_{2:3}$ and $F_{2:4}$ progenies in different time periods of assessment, and of joint analysis of the two environments in different time periods of assessment. Lavras/ Patos de Minas, MG, 2011.

| Generation/ Location / Time period | Mean | $h^2$ |
|---|---|---|
| $F_{2:3}$/ Lavras / 30 DAH[1/] | 3.01 | 72.39 (59.00-81.40)[2/] |
| $F_{2:3}$/ Lavras / 60 DAH | 3.53 | 85.85 (78.99-90.47) |
| $F_{2:3}$/ Lavras / 90 DAH | 3.72 | 87.03 (80.74-91.26) |
| $F_{2:4}$/ Lavras / 30 DAH | 2.27 | 76.13 (64.56-83.92) |
| $F_{2:4}$/ Lavras / 60 DAH | 2.50 | 85.38 (78.28-90.15) |
| $F_{2:4}$/ Patos / 30 DAH | 3.71 | 85.36 (81.23-91.49) |
| $F_{2:4}$/ Patos/ 60 DAH | 3.87 | 87.24 (81.05-91.40) |
| $F_{2:4}$ /Lavras and Patos/30 DAH | 2.99 | 70.33 (55.91-80.04) |
| $F_{2:4}$ /Lavras and Patos/60 DAH | 3.18 | 71.45 (57.56-80.79) |

[1/] Days after harvest. [2/] In brackets, limits of the confidence interval of $h^2$.

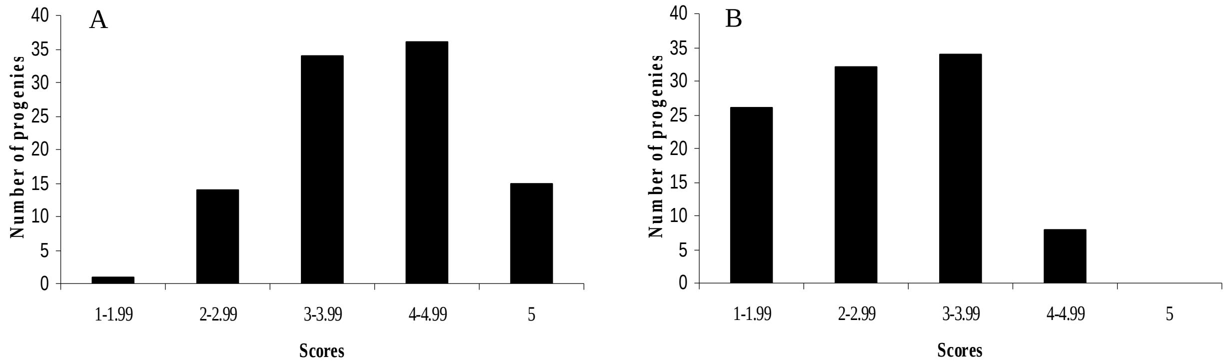


Figure 2 – Frequency distribution of means of the $F_{2:4}$ progeny scores in Patos de Minas (A) and Lavras (B) at 60 days after harvest. Lavras/Patos de Minas, MG, 2011.

In joint analysis to verify the effect of crop season/ generations on the experiments conducted in Lavras, in the $F_{2:3}$ and $F_{2:4}$ generations, it was observed that the source of variation progeny was significant (P=0.00), and the interaction progenies x time period once more was not significant (P=0.082). The effect of generations/ crop season, for its part, was significant (P=0.00), with the same occurring for the interactions progenies x generations and time periods x generations (P=0.00). The lowest mean was obtained in the $F_{2:4}$ generation, with the experiment being performed in the fall/winter crop season and sowing performed in July. The harvest in this crop season was earlier than that of the $F_{2:3}$ generation because it occurs at the end of the month of October, a period in which the harvest is normally performed earlier due to greater probability of the occurrence of rainfall. This probability increases with delay in harvest; therefore, it is performed with some pods on the plant still green. In addition, exposure of pods to ultraviolet rays is less.

Although the interaction progenies x generations was significant, it was observed that the genetic correlation between the two generations was 0.81, which shows that the interaction is predominantly simple, thus not contributing to change in classification of progenies in the different generations. Considering the ten progenies with later seed-coat darkening, i.e., with lower scores in the $F_{2:3}$ and $F_{2:4}$ generations, coincidence is 50%. The same coincidence percentage was also observed considering the ten progenies with more rapid darkening, that is, with a greater score in the $F_{2:3}$ and $F_{2:4}$ generations.

In joint analysis involving the $F_{2:4}$ generation in Lavras and Patos de Minas, significant difference (P=0.00) was also observed among progenies, and the interaction progenies x time periods (P=0.178) was also not significant. Nevertheless, the effect of locations, and all the interactions involving locations were significant (P=0.00). Although the interactions progenies x locations were significant, the genetic correlation between the means of the progenies in the two locations was 74%, allowing to infer that the interaction was predominantly simple because there was no great alteration in classification of the progenies. Reinforcing this estimate, if the ten progenies with the lowest score in Lavras at 60 DAH are considered, seven of them would also be among those with the lowest score in Patos de Minas.

The performed heritability at 60 DAH through selection of the ten best progenies in Lavras and response in Patos de Minas was 61%. When the reverse was carried out, selection in Patos de Minas and the gain in Lavras, realized heritability was 84%. In the mean of the two locations, the realized heritability was 72.5%, a value which is quite similar to the heritability for the selection of progenies in the mean of the two locations (Table 1). This fact is one more indication that the interaction progenies x locations was predominantly simple, as has already been mentioned (CRUZ et al., 2004).

Estimates of phenotypic, genetic and environmental correlations between darkening of grains and the traits of yield, cooking time and tannin content are presented in table 2. The estimate of phenotypic, genetic and environmental correlation between grain darkening and cooking time was of small magnitude, except in the assessment performed at 60 DAH. In this situation, the estimate of genetic and phenotypic correlation was positive and high, that is, according to expectations.

Table 2 – Estimates of genetic ($r_G$), phenotypic ($r_F$) and environmental ($r_E$) correlation between the grain darkening trait with grain yield, tannin content and cooking time in $F_{2:3}$ progenies in different time periods of assessment. Lavras, MG, 2011.

| Days after harvest | | Darkening | | |
|---|---|---|---|---|
| | | $r_G$ | $r_F$ | $r_E$ |
| 30 | Yield | -0.5332 | -0.3797 | -0.0205 |
| | Cooking | 0.001 | 0.0243 | 0.3338 |
| | Tannin content | 0.7935 | 0.6936** | -0.0113 |
| 60 | Yield | -0.4262 | -0.338 | -0.2167 |
| | Cooking | 0.868 | 0.6942** | -0.3121 |
| | Tannin content | 0.7844 | 0.7711** | 0.4634 |
| 90 | Yield | -0.5709 | -0.3993 | 0.1423 |
| | Cooking | -0.1109 | -0.1215 | -0.3646 |
| | Tannin content | 0.8617 | 0.833** | 0.096 |

** Significant a 5% probability by the t test (1974).

Thus, it may be inferred that the lighter colored grains have more favorable cooking characteristics, even when stored for a more prolonged period of time. Estimates of genetic and phenotypic correlation between the grain darkening scores and the tannin content were also all high and positive, proving what was stated earlier. A similar result was reported by Silva et al. (2008). A fact which proves this association is identification of a molecular marker associated with tannin content and the grain darkening scores performed by Couto et al. (2010). Thus, tannin content may be used as a criterion for selection of progenies with late seed-coat darkening of grains. Nevertheless, tannin analysis takes a long time and it is necessary to destroy the grains, increasing the cost of the breeding program. Thus, assessing the degree of darkening by means of a scale of scores appears as a better option.

Another important association is between grain yield and the grain darkening scores. Estimates of the phenotypic and genotypic correlations were negative. However, were all of small magnitude and not significant. Since heritability ($h^2$) for the grain darkening scores is high, it may be inferred that the selection of progenies with lighter colored grains, in a time period of early evaluation, may be efficient and will not affect the selection for grain yield, or if it affected, it will be in the direction desired by breeders.

## CONCLUSIONS

Darkening scores are highly influenced by the environment, crop season or locations; however, the interaction progenies x generations and progenies x locations is predominantly simple, not altering classification of the progenies in an expressive way.

Due to the estimates of heritability obtained, it may be inferred that the late seed-coat darkening trait selection is efficient and may be performed as of 30 days after harvest.

Grain with late seed-coat darkening has a lower level of tannin and requires less cooking time. Genetic correlation between seed-coat darkening and grain yield was practically null.

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