# Induction of seed coat darkening in common beans (*Phaseolus vulgaris L.*) and the association with cooking time after storage

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**Abstract**

Carioca is the most important edible dry bean (*Phaseolus vulgaris* L.) grown in Brazil. It represents the largest dry bean market class in the world. The seed coat of carioca beans will darken under adverse harvest conditions and with the increasing of storage time. In general, darkened seeds are associated with older seeds that suffer from prolonged cooking times. A relatively new 'slow darkening' trait is available in carioca that delays seed coat darkening under storage. However, its effect on cooking time is unknown. The objective of this work was to evaluate two induction methods of seed coat darkening and to examine the effect of slow darkening trait on cooking time after storage. Lines derived from four segregating populations resulting from crossings between cultivar BRSMG Madrepérola with slow seed coat darkening, and the parents BRS Estilo, BRS Cometa, BRS Notável and BRS Sublime with normal darkening were evaluated. An experiment inlcuding 220 lines, 55 per population and the five parents, in a 15x15 triple lattice was conducted in the winter growing season in Brasilia. Seed coat darkening and cooking time traits were evaluated. Two methods for inducing seed coat darkening were compared: an accelerated aging test using UV light for 72 hours, and an extended storage for 90 days under ambient conditions. The correlation between the induction methods ranged from 0.77 to 0.85 for the different populations indicating either method could be used to discriminate lines with normal versus slow darkening trait. The percentage of light-colored grain lines was identified by both induction methods of seed coat darkening ranged from 75 to 85.7% in the populations. The genetic correlation between seed coat darkening and cooking time varied from -0.06 to -0.48, indicating that in some populations there is no significant genetic correlation between seed coat darkening and cooking time. Thus, light colored grains are not indicative of low cooking time, when they are considered genotypes with genetic variation for the seed coat darkening.

**Keywords:** accelerated darkening, extended darkening, genetic correlation, slow grain darkening.
**Abbreviations:** UV light - ultraviolet light; ED - extended darkening; CT - Cooking time; CI - coincidence index; SD - slow darkening; ND - normal darkening; AD - accelerated darkening

## Introduction

The seed (syn. grain) appearance (color, shape, and size) of dry edible beans is very important aspect of the commercialization process. In Brazil, consumer preference is differentiated by region mainly by seed color. By far, Carioca is the most popular dry bean market class in Brazil occupying 70% of consumer demand (Del Peloso and Melo, 2005). The carioca market type is defined by a seed coat with a light cream colored tegument with beige streaks. However, carioca beans will darken easily under delayed harvest conditions or with increasing time in storage (Junk-Knievel et al., 2007; Couto et al., 2010). Pinto bean which is a major market class in Mexico and the United States similarly suffers from seed coat darkening (Junk-Knievel et al., 2007). The environmental, biochemical and genetic factors affect seed coat darkening. Environmental factors include temperature, humidity, and luminosity conditions during

harvest and while in storage (Couto et al., 2010). High levels of polyphenols (tannins) in the seed coat may be associated with increased darkening (Junk-Knievel et al., 2007; Silva et al., 2008). A few carioca cultivars exhibit slower darkening of the seed coat. Among them is BRSMG Madrepérola, which maintains a light cream coloration of the seed coat even after long storage periods (Silva et al., 2008, 2014; Carneiro et al., 2012; Baptista, 2016). Inheritance of the slow seed coat darkening trait in BRSMG Madrepérola varies from a single recessive gene to a few genes depending on the population and environment (Caneiro et al., 2012). This similar slow darkening trait in pinto bean is conditioned by a single recessive gene '*sd*' (Junk-Knievel et al., 2008; Islam et al., 2017).

Fast and reliable techniques have been developed recently for detecting the slow darkening trait (Junk-Knievel et al.,

2007). The accelerated aging method uses ultraviolet light to distinguish between slow and fast darkening lines in as little as three days. Extended storage method allows for gradual monitoring of the color changes of the seed coat, as well as the differentiation of the intensity between the evaluated genotypes.

Breeding programs are now concerned with characterizing any affects that the slow darkening seed coat trait may have on other traits, such as reduced cooking time (Carbonell et al., 2003; Alvares et al., 2016). On the other hands, consumer demand is for faster cooking beans (Corte et al., 2003; Ribeiro et al., 2013). Cooking time of beans is influenced by genetic and environmental factors. The interaction between G x E makes it difficult to select for faster cooking breeding lines (Carbonell et al., 2003; Corte et al., 2003; Rodrigues et al., 2005; Ribeiro et al., 2007; Bertoldo et al., 2009; Perina et al., 2014; Silva et al., 2014; Chiorato et al., 2015). Many studies have shown that cooking time increases with increased storage (Brackmann et al., 2002; Baldoni and Santos, 2005; Ribeiro et al., 2007, 2008; Morais et al., 2010). Moreover, beans with seed coat darkening are assumed to be older beans that will take longer to cook. The main question is: will the storage time have a similar effect on cooking time of beans which do differ in darkening? Our objective was to determine the effect of storage on cooking time of slow darkening carioca beans. We also evaluated two methods for inducing seed coat darkening of carioca beans.

**Results and discussion**

*Induction methods of seed coat darkening*

Cultivar BRSMG Madrepérola exhibited slower seed coat darkening in the accelerated aging (Fig.1) and extended storage (Table 2) tests, as confirmed in previous studies (Silva et al., 2008; Carneiro et al., 2012; Silva et al., 2014, Baptista, 2016). Cultivar BRSMG Madrepérola displayed a visually lighter color pattern after accelerated darkening when compared to the other parents. Cultivars BRS Estilo, BRS Cometa, BRS Notável and BRS Sublime presented darker coloration after the aging test and were classified as normal darkening. Junk-Knievel et al. (2007) reported that this distinction is consistent over locations and years, and does not alter seed germination. Thus, the selection of light grain genotypes using the accelerated aging method can be used to expedite breeding for the slow darkening trait, bypassing the need for extended storage to phenotype the trait.

The cultivars BRS Estilo and BRS Sublime had intermediate seed coat darkening, and BRS Notável and BRS Cometa exhibited the darkest seed coats under extended storage (90 ED) (Table 2). The population BRSMG Madrepérola x BRS Sublime exhibited slower seed coat darkening, followed by BRSMG Madrepérola x BRS Notável population at 90 ED. This range for cultivar and population means indicates the extended storage tests for detecting additional genetic variability and making gains from selection for the slow darkening trait in Carioca beans.

Highly significant phenotypic correlations (Table 3) were obtained for the seed coat darkening assessments between the accelerated aging and extended storage (90 ED) tests, considering the lines of each population separately, which

ranged from 0.77 (BRSMG Madrepérola x BRs Estilo) to 0.85 (BRSMG Madrepérola x BRS Cometa). Further analysis showed the best agreement for classification of slow vs. regular darkening lines by both methods in BRSMG Madrepérola x BRS Estilo population, with a coincidence of 85.7%. The lowest coincidence which was observed in the BRSMG Madrepérola x BRS Sublime population (75%) was still high (Table 4). These results confirm good agreement for seed coat classification between methods, suggesting the accelerated aging test will be useful for routine selection of slow darkening lines in a breeding program.

It should be stressed that the use of the ultraviolet light chamber provides faster results when compared to extended storage induced darkening. Moreover, it has the advantage of not harming seed germination (Junk-Knievel et al., 2007), making selection for the slow seed coat darkening trait possible at the plant level. However, each method should be used in accordance with the needs of the breeding program, given extended storage is able to detect greater variability for the trait than the accelerated aging test.

*Slow darkening and cooking time*

A primary goal of this project was to determine if the slow darkening trait affected cooking time, a trait shown to be variable in previous studies (Carbonell et al., 2003; Corte et al., 2003; Baldoni and Santos, 2005; Bertoldo et al., 2009; Ribeiro et al., 2013). Two populations were evaluated for cooking time. No significant differences were observed for the BRSMG Madrepérola x BRS Estilo population for cooking time after extended storage for 90 d. However, after 180 d of storage, there was a significant difference (P <0.01). There were significant differences detected for cooking time among lines in the BRSMG Madrepérola x BRS Cometa population for both 90 and 180 (P <0.05) days of extended storage (Table 5). Of the two populations, BRSMG Madrepérola x BRS Cometa averaged shorter cooking times for both 90 and 180 d storage periods (Table 6). Conversely, Silva et al. (2014) observed the opposite, whereas BRSMG Madrepérola x BRS Estilo population had a shorter cooking time (29.3 min) than the BRSMG Madrepérola x BRS Cometa population (33.6 min) when evaluating grains stored for 190 days after harvest. Interestingly, the lines overall averaged less cooking time than the parental averages. Moreover, it is evident that cooking time increased with increasing storage time, corroborating results from many studies (Brackmann et al., 2002; Baldoni and Santos, 2005; Ribeiro et al., 2007, 2008; Morais et al., 2010; Arrudda et al., 2012; Siqueira et al., 2014). Possible factors increasing cooking time of stored beans can be the action of polyphenols, by means of their polymerization in the tegument, or by lignification within the cotyledons, which in turn, inhibits water penetration and the hydration capacity of the grains (Moura, 1998).

Regarding the performance of the parents, BRSMG Madrepérola and BRS Estilo presented the lowest values for cooking time in the evaluations, remaining grouped by the means of the comparison test at 90 and 180 days after harvest (Table 6). Cultivar BRS Cometa showed the worst performance, presenting a mean of 36 minutes.

We examined phenotypic, genetic and environmental correlations between cooking time for seeds stored 90 after

**Table 1.** Characteristics of the parents used in the obtain segregant populations.

| Genotype | Maturity Cycle | Plant Architecture | Genealogy |
|---|---|---|---|
| BRSMG Madrepérola | Normal | prostrate | AN 512666-0/AN 730031 |
| BRS Cometa | Semi-early | Upright | A 769 /4/ EMP 250 /// A 429 / XAN 252 // C 8025 / G 4449 /// WAF 2 / A 55 // GN 31 / XAN 170 |
| BRS Estilo | Normal | Upright | EMP 250 /4/ A 769 /// A 429 / XAN 252 // V 8025 / PINTO VI 114 |
| BRS Notável | Semi-early | Upright | A 769 /4/ A 774 /// A 429 / XAN 252 // V 8025 / G 4449 /// WAF 2 / A55 // GN 31 /XAN 170 |
| CNFC Sublime | Normal | Upright | EMP 250 / 4 / A 769 /// A 429 / XAN 252 // V 8025 / PINTO UI 114 |



**Fig 1.** Common beans cultivar grains after 72 hours in grain accelerated darkening test (CNFC 10429 was the code used to identify cultivar BRS Sublime).

**Table 2.** Mean seed-coat darkening (rated 1 to 5) of parents and four segregating populations for seed harvested in Brasilia, 2012 winter crop, and stored for 90 days (90 ED).

| Genotypes | Mean seed-coat darkening |
|---|---|
| BRSMG Madrepérola | 1.1 A |
| BRS Estilo | 2.8 B |
| BRS Sublime | 2.7 B |
| BRS Cometa | 3.5 C |
| BRS Notável | 4.2 D |
| Mean of the parents | 2.9 |
| BRSMG Madrepérola x BRS Sublime | 2.7 a |
| BRSMG Madrepérola x BRS Estilo | 3.0 c |
| BRSMG Madrepérola x BRS Cometa | 3.0 c |
| BRSMG Madrepérola x BRS Notável | 2.9 b |
| Mean of the populations | 2.9 |

Means followed by the same letter (upper case and lower case letter) in the vertical do not differ from each other by the Scott & Knott's test, at 5% of probability.

**Table 3.** Pearson correlation coefficient estimates between seed-coat darkening scores from 90 day extended storage (90 ED) and the accelerated darkening UV light test for 72 h for four populations.

| Genotypes | Correlation |
|---|---|
| BRSMG Madrepérola x BRS Sublime | 0.78** |
| BRSMG Madrepérola x BRS Estilo | 0.77** |
| BRSMG Madrepérola x BRS Cometa | 0.85** |
| BRSMG Madrepérola x BRS Notável | 0.78** |

**significant at 1% of probability by test-t

**Table 4.** Number of lines classified as slow (SD) and normal darkening (ND), according darkening method (accelerated - AD and extended – 90 ED) for each population.

| Genotypes | SD | | C (%)[1] | ND | |
|---|---|---|---|---|---|
| | AD | 90 ED | | AD | 90 ED |
| BRSMG Madrepérola x BRS Sublime | 14 | 16 | 75.0 | 41 | 39 |
| BRSMG Madrepérola x BRS Estilo | 13 | 14 | 85.7 | 41 | 40 |
| BRSMG Madrepérola x BRS Cometa | 24 | 21 | 83.3 | 29 | 34 |
| BRSMG Madrepérola x BRS Notável | 22 | 19 | 77.3 | 33 | 37 |
| Total across populations | 73 | 70 | 83.6 | 144 | 150 |

[1]Number of coincidents: percentage of lines identified as slow darkening by the accelerated and extended darkening test.

**Table 5.** Summary of analysis of variance for cooking time of seeds for 90 and 180 days of extended storage (ED) for two populations grown in Brasilia, 2012 winter crop.

| Source of variation | DF | 90 ED | | 180 ED | |
|---|---|---|---|---|---|
| | | MS | P-value | MS | P-value |
| Blocks | 1 | 182.00 | 0.003 | 269.50 | 0.001 |
| Treatments | 111 | 28.00 | 0.023 | 49.77 | 0.000 |
| Lines | 108 | 23.78 | 0.127 | 49.25 | 0.000 |
| BRSMG Madrepérola x BRS Estilo | 53 | 17.33 | 0.649 | 46.66 | 0.002 |
| BRSMG Madrepérola x BRS Cometa | 54 | 28.87 | 0.035 | 37.28 | 0.034 |
| Between populations | 1 | 90.18 | 0.032 | 833.04 | 0.000 |
| Parents | 2 | 70.15 | 0.029 | 80.95 | 0.041 |
| Pops vs. Parents | 1 | 433.87 | 0.000 | 43.97 | 0.184 |
| Error | 111 | 19.11 | | 24.59 | |

DF = degrees of freedom, MS = mean squares

**Table 6.** Mean cooking time (minutes) at 90 and 180 of extended storage (ED) after harvest for two populations grown in Brasília, 2012 winter crop.

| Genotypes | 90 ED | 180 ED |
|---|---|---|
| BRSMG Madrepérola | 24.2 A | 24.4 A |
| BRS Estilo | 28.7 A | 27.3 A |
| BRS Cometa | 36.1 B | 36.6 B |
| Mean of the parents | 29.7 | 29.4 |
| BRSMG Madrepérola x BRS Estilo | 21.6 b | 28.7 b |
| BRSMG Madrepérola x BRS Cometa | 19.8 a | 24.8 a |
| Mean of the populations | 20.7 | 26.8 |
| CV (%) | 21.1 | 18.5 |
| Selective accuracy (SA) | 0.57 | 0.71 |

Means followed by the same letter (upper case and lower case letters) in the vertical columns do not differ from each other by the means grouping test by Scott & Knott, at 5% of probability.

**Table 7.** Estimates of the phenotypic, genetic and environmental correlation coefficients among cooking time (CT) in minutes at 90 and 180 d after harvest and seed coat darkening score at 90 d obtained with extended storage (ED) for two populations grown in Brasília, during the winter harvest.

| Genotype | Phenotypic correlation | |
|---|---|---|
| | CT 90 d/90 d score | CT 90 d/CT 180 d |
| Population | -0.08 | 0.60** |
| BRSMG Madrepérola x BRS Estilo | 0.01 | 0.68** |
| BRSMG Madrepérola x BRS Cometa | -0.16 | 0.53** |
| | Genetic correlation | |
| Population | -0.13 | 0.98** |
| BRSMG Madrepérola x BRS Estilo | -0.06 | 0.99** |
| BRSMG Madrepérola x BRS Cometa | -0.48* | 0.97** |
| | Environmental correlation | |
| Population | 0.01 | 0.31* |
| BRSMG Madrepérola x BRS Estilo | 0.01 | 0.32* |
| BRSMG Madrepérola x BRS Cometa | 0.01 | 0.18 |

*and **significant at 1 and 5% of probability, respectively by the Student's t-test.

harvest with the seed coat darkening scores (Table 7). Literature provides evidence that the cooking time is related to seed coat darkening. However, such conclusions are based on evaluations of genotypes that only show normal seed coat darkening. No significant phenotypic associations were found between CT90 and darkening (Table 7). Therefore, there is evidence that there is no relationship between the increase in cooking time and dark seed coat when slow-darkening lines are considered, nor that genotypes of light seed coat are indicative of low cooking time. The results also suggest that the selection should be practiced individually for each character since light grain is not an indicative of shorter cooking time. This finding disagrees with those reported in the literature (Brackmann et al., 2002; Baldoni and Santos, 2005; Coelho et al., 2009; Morais et al., 2010; Oliveira et al., 2011; Araújo et al., 2012).

Silva et al. (2014) also did not obtain any significant correlations between darkening and cooking time, concluding that the means for seed coat darkening do not reflect the behavior of the genotypes for a cooking time after grain storage. It is worth mentioning that the author also worked with slow-darkening populations. Oliveira et al. (2011) evaluated the quality of cooking of grains immediately after harvest and after six months of refrigerated storage at 0ºC. Their results showed that for *Carioca* grain genotypes, Pérola, Carioca and LH5, an increase in the darkening of the integument was observed, but only for the cultivar Pérola, a rise in the cooking time was noticed. These results corroborate with those obtained in this study, including Oliveira et al. (2011), who evaluated normal darkening genotypes.

Araújo et al. (2012) obtained estimates of phenotypic, genetic and environmental correlation between darkening, cooking time, yield and tannin content in grains of population obtained from the crossing between BRSMG Madrepérola x RP-2 (normal-darkening line). The evaluations were performed at 30, 60 and 90 days after harvest. The authors found significant and positive phenotypic correlation only between darkening and cooking time at 60 days post-harvest (0.69). The other correlations between these two characters were not significant. Significant correlations were also obtained between darkening at 30, 60 and 90 days after harvest and tannin content (0.69, 0.77 and 0.83, respectively).

The increase in cooking time was significantly correlated with grain storage time in the work of Siqueira et al. (2014) when evaluating five cultivars and one line of the Embrapa Arroz e Feijão breeding program for cooking time, coloring, hardness and lignin contents in six storage conditions. Among the cultivars evaluated, only cultivar BRSMG Madrepérola presented seed coat darkening. The authors also identified that the increases in cooking time with storage are independent of the grain lignification process. Another finding is that darkening and hardening occur during grain storage at different intensities in each genotype and there is not always a correlation between these variables. The cultivar BRSMG Madrepérola did not have its cooking time affected with storage. In contrast, BRS Pontal obtained additions in the cooking time, besides presenting dark seed coat.

All the phenotypic correlation estimates between cooking times for grains stored 90 and 180 d after harvest were positive, significant and of medium magnitude, suggesting that the selection for cooking time among lines can be practiced already at 90 days after harvest.

In the present study, the genetic correlations between darkening and cooking time were negative and of medium magnitude, corroborating with estimates of phenotypic correlation. Araújo et al. (2012) found a negative and low magnitude estimate between the cooking time at 90 days after harvest and seed coat darkening (-0.11). However, the authors obtained a high and positive value between darkening and cooking time at 60 days after harvest (0.87), disagreeing with the results shown in this study.

A genetic correlation occurred between darkening and cooking for the BRSMG Madrepérola x BRS Cometa population (-0.48), indicating that the association between the two characters is inversely proportional, but of medium magnitude. The presence of negative correlations for the breeding is undesirable since the selection of low cooking time may cause undesirable changes for seed coat darkening. However, it is worth mentioning that although significant values had occurred, they were intermediate, indicating a small association.

The results indicate that when observing a cultivar that presents light seed coat even after a long period of storage, its cooking time cannot be inferred. This indicates that light seed coat are not indicative of low cooking time, nor that dark seed coat need more time for cooking. Increases in cooking time after storage have been reported to be associated with changes in grain proteins after storage, which may cause hardening of the integument and, consequently, longer time for its cooking (Rios et al., 2003).

Araújo et al. (2012) reported that slow-darkening seed coat presents smaller amount of tannins and require less time for cooking. The authors obtained positive and high correlation values between tannin content and seed coat darkening, suggesting that the increase in cooking time can be attributed to the increase in the content of tannin.

As a result, the consumer's habit of searching for light-seed coat beans in the market does not necessarily imply in a shorter cooking time in the pan, since no positive and significant correlation between cooking time and seed coat darkening has been identified. This result agrees with that of Siqueira et al. (2014), according to whom the tegument coloration in *Carioca* beans is not an appropriate parameter to predict the cooking time of the beans, as it has been used by traders and consumers.

All the phenotypic correlation estimates between cooking times for grains stored 90 and 180 d after harvest were positive, significant and of medium magnitude, suggesting that the selection for cooking time among lines can be practiced already at 90 days after harvest. Genetic correlations between cooking times were positive and close to the unit, as expected (Table 7). A positive environmental correlation was found between CT90 and CT 180 for populations and for BRSMG Madrepérola x BRS Estilo. The presence of this type of correlation indicates that the variation in the environmental factors affected the cooking time of the lines of this population in the same direction. Therefore, the unfavorable environmental conditions that negatively affect the cooking of grains, the seed coat darkening will be directly influenced, producing darker seed coat. However, Araújo et al. (2012) did not find a significant environmental correlation for cooking time and grain darkning of $F_{2:3}$ progenies.

In 100% of the evaluated pairs of characters, genetic correlations were equal to or greater than phenotypic correlations, an extremely interesting fact, since genetic correlations have a greater practical effect on works of plant breeding. In 100% of the cases, estimates of phenotypic and genetic correlation were higher than the environmental correlations. Although one undesirable correlation had been significant, it did not form a complete association, being possible to obtain recombinants that are promising for both characters.

**Materials and Methods**

***Genetic material, plant grown and experimental design***

Four segregating populations were generated by crossing the slow seed coat darkening parent BRSMG Madrepérola (Carneiro et al., 2012) with four normal seed coat darkening parents: cultivars BRS Estilo (Melo et al., 2010), BRS Cometa (Faria et al., 2008), BRS Notável (Pereira et al., 2012) and BRS Sublime (Table 1). The parents BRS Estilo and BRS Cometa were chosen because they exhibit short and long cooking times, respectfully.

These populations were advanced in bulk to the $F_5$ generation and 55 $F_{5:6}$ lines were randomly selected and multiplied as $F_{5:7}$ lines for evaluation with the five parents in a 15x15 triple lattice design with three replications. Plots consisted of two 3-m rows with 0.45 m row spacing. The field experiment was conducted in the winter growing season in Brasília, DF, Brazil (15º46'47"S, 47º55'47"W and

1171m altitude). Fertility, irrigation, and cultivation were managed for optimal production.

The seed harvested from all plots were evaluated for seed coat darkening. But only the lines derived from the BRSMG Madrepérola x BRS Estilo and BRSMG Madrepérola x BRS Cometa populations and respective parents were evaluated for cooking time.

*Comparison of induction methods*

The seeds of lines were subjected to extended and accelerated post-harvest darkening methods. The extended darkening (ED) method consisted of placing seeds in transparent plastic bags and then storing them for 90 days (90 ED) under ambient environmental conditions (temperature - 24/28ºC and humidity 40/80%). The level of seed coat darkening for this method was rated from 1 (very light seed coat) to 5 (very dark seed coat) (Silva et al., 2008). A line was considered to be slow darkening if its score averaged less than 2.5.

The accelerated aging method followed the procedure by Junk-Knievel et al. (2007), whereby seeds were exposed to ultraviolet light, which simulates extended storage (Junk-Knievel et al., 2007; Elsadr et al., 2011; Felicetti et al., 2012). The seeds were placed in petri dishes and exposed to ultraviolet light in an enclosed light chamber for 72 hours. Seeds that showed an evident darkening of the seed coat were classified as normal darkening, and those that presented a slight darkening of the seed coat were classified as slow darkening. For this method, lines were scored either 1 for slow darkening or 2 for normal darkening.

Cooking time was evaluated for a portion of the seeds that were subjected to the extended 90 day storage test (90 ED) and a remaining portion that was stored for 180 days (180 ED) after harvest. Cooking time was evaluated at 90 and 180 days after harvest because these storage times represent realistic elapsed times between harvest and product placement on supermarket shelves. Often growers or dealers will delay sale of their product in an attempt to achieve higher prices. Cooking time (CT) was measured in minutes using the Mattson Cooker based on the method of Proctor and Watts (1987). Whole seeds were soaked in 100 ml of distilled water for 16 h at room temperature. Twenty-five randomly chosen seeds were placed in the Mattson Cooker which fit inside a glass beaker containing 1,000 ml of boiling water. The cooker is composed of 25 rods, with each grain individualized in a cavity of the apparatus, supported perpendicularly to a metal rod of 90 grams and with 1.0 mm of tip diameter. The time was recorded, in minutes, until the 13th rod of the cooker penetrated a cooked seed. A 60 minute time limit was set for cooking the samples.

*Statistical and genetics analyzes*

Analysis of variance was conducted for seed coat darkening at 90 ED for all four populations, and for cooking times at 90 ED and 180 ED for the two populations and their parents. Blocks and treatment were considered random effects. Mean comparisons were based on Scott-Knott's test (p-value ≤0.05). The phenotypic, genetic and environmental correlation coefficients between the seed coat darkening obtained were estimated with extended storage at 90 days after harvest, and seed coat at CT90 with CT180. As a

measure of the informative qualification of the experiments, the selective accuracy (SA) was calculated, as proposed by Resende and Duarte (2007), who considered the F-test values of the analysis of variance.

Pearson's simple linear correlation coefficient (Steel and Torrie, 1980) was used to compare seed coat darkening for the extended storage (90 ED) and accelerated aging tests. The coincidence index (CI), which represents the percentage of lines were identified as slow darkening by both methods. Genes software (Cruz et al., 2013) was used to conduct the statistical analyses.

**Conclusions**

Accelerated and extended darkening induction methods allowed discriminating lines that have slow and normal seed coat darkening according to the need. No significant genetic correlation between darkening and cooking time of seed coat was found. Therefore, light seed coat is not indicative of a low cooking time when genotypes with genetic variation for seed coat darkening are considered.

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