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Carioca bean genotypes for tolerance to grain darkening by natural and accelerated methods

Genótipos de feijoeiro carioca para tolerância ao escurecimento de grão pelos métodos natural e acelerado

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ABSTRACT

The slow darkening of grains is sought by bean breeders because the consumers consider that darker grains demand more time for cooking. The analysis currently used takes around 90 days to differentiate grain color among genotypes. The objective was to evaluate the color as a function of the value of L* (lightness) of carioca beans, by natural and accelerated methods to verify equivalence between methods, validation of the methodology and identification of genotypes tolerant to the darkening. The grain darkening was compared and evaluated by natural darkening method under shelf conditions, in days storage, and accelerated darkening method under ultraviolet light, in hours. The natural darkening time of 90 days was statistically equal to 24 hours of accelerated darkening, and the difference among the genotypes could be obtained in a shorter time, indicating a correspondence in the methods. The accelerated darkening method can be used to shorten the analysis time in the routine of breeding programs.

Index terms: *Phaseolus vulgaris* L.; coloring; lightness; plant breeding; ultraviolet light.

RESUMO

O escurecimento lento de grãos é almejado por melhoristas de feijoeiro porque o consumidor considera que grãos mais escuros apresentarão maior tempo para cocção. A análise empregada atualmente leva ao redor de 90 dias para diferenciação da cor do grão entre os genótipos. Objetivou-se avaliar a cor em função do valor de L* (luminosidade) de feijoeiro do tipo carioca pelos métodos natural e acelerado para verificar equivalência entre os métodos, validação da metodologia e identificação de genótipos tolerantes ao escurecimento. O escurecimento de grãos foi comparado e avaliado pelo método de escurecimento natural em condições de prateleira, em dias de armazenamento, e pelo método de escurecimento acelerado, em condições de luz ultravioleta, em horas. O escurecimento natural de 90 dias foi estatisticamente igual a 24 horas de escurecimento acelerado, e a diferença entre os genótipos pôde ser obtida em menor tempo, indicando correspondência entre os métodos. O método de escurecimento acelerado pode ser utilizado para encurtar o tempo de análise na rotina dos programas de melhoramento.

Termos para indexação: *Phaseolus vulgaris* L.; coloração; luminosidade; luz ultravioleta; melhoramento de plantas.

INTRODUCTION

The darkening of the tegument of bean grains (*Phaseolus vulgaris* L.) is an undesirable characteristic for the consumers because it is associated to longer cooking time (Silva et al. 2008) and old bean. This association is not correct because the darker grains do not always lead to longer cooking times or are the oldest ones. The darkening of the grains is influenced by the environment (humidity, temperature and harvesting time), seasons and genotype (Araújo; Ramalho; Abreu, 2012; Silva et al. 2008). In the harvest, rain plays an important role, since it affects the quality of the grain, which according to Carbonell, Carvalho and Pereira (2003), causes membrane permeability. Excess moisture accelerates fermentation of the grain, making it dark, but not necessarily old.

Currently, the standard methodology for evaluation of the grain used is the natural darkening, which consists of packing the beans in zip lock bags and storing them under shelf conditions at environmental temperature and humidity, with coloring scouting every

thirty days. The color tone was given as a function of the value of L* (lightness) (Ribeiro et al., 2014), using a colorimeter, but there are also studies that use scale notes (Alvares et al., 2016) by comparison with other control cultivars. The value of L* has often been used for the simplicity of obtaining and interpreting the results compared to a* (red/green) and b* (yellow/blue) coordinates. This natural darkening methodology enables the identification of cultivars with slow darkening and needs approximately 90 days to show significant differences among the genotypes.

Strategies have been adopted for more rapid and equivalent evaluation of darkening of grains. Siqueira et al. (2016) worked with accelerated aging, in which the grains were aged in the hot air furnace dark at 40±5 °C and 75% relative humidity to accelerate the darkening process. Artificial methods with ultraviolet light illumination have emerged as an alternative to decrease the time needed to test bean darkening and to establish a methodology for the routine of breeding programs. Thus, the accelerated darkening method under ultraviolet and fluorescent light exposure developed by Junk-Knievel, Vandenberg and Bett (2007) was adapted in relation to the equipment installations (chamber) and sampling time, measuring the value of L* every 24 hours, at 0 hour, 24 hours, 48, hours and 96 hours, as it provides faster results in comparison to natural darkening.

Thus, this study aimed to evaluate grain darkening of 19 bean genotypes, including lines and cultivars of carioca tegument, by natural and accelerated (with ultraviolet light) methods using ANOVA, MANOVA, canonical discriminant analysis, contrasts, Tukey and correlation to verify equivalence between methods, validation of the methodology and identification of genotypes tolerant to the accelerated grains darkening.

MATERIAL AND METHODS

The genotypes used in the trial were all carioca type, were provided by the main bean breeding programs in Brazil, three cultivars, BRS Pérola (Embrapa), IAC Milênio and IAC Sintonia (IAC) and 16 lines, CHC 01-175-1 (Epagri), CNFC 11-948 and CNFC 11-954 (Embrapa), Gen 4-1F-19P, Gen 12-2F-67, Gen 20-4F-129, Gen 45-2F-293P, Gen 78-1A-59, Gen 86-12A-122, Gen 90-4A-160, Gen 104-1A-291, Gen 106-4A-317, Gen 106-6A-319, Gen 107-14A-336, Gen 125-10A-510 (IAC) and LP 11-363 (IAPAR). The selection of the genotypes was carried out in function of being elite cultivars and lines of these programs.

The trials were carried out in three seasons: "dry" (Campinas and Tatuí), "winter" (Votuporanga and Ribeirão Preto) and the "rainy" (Mococa and Campinas) in the state of São Paulo, Brazil in 2016. The experimental in the six environments was designed for randomized blocks with three replications. Plots consisted of four 4 m length rows, spaced at 0.50 m, with 10 to 12 viable plants per meter, the useful area of the plot being the two center rows.

Traits analyzed

The genotypes were evaluated based on the value of L* (lightness) of grain tegument by the natural darkening method under shelf conditions (Alvares et al., 2016) and accelerated darkening method under ultraviolet and fluorescent light chamber conditions (Junk-Knievel, Vandenberg; Bett 2007). The grains harvested were submitted to the same conditions of temperature, light and humidity before the analyzes. The values of L* by the CIELAB system, which represent the lightness scale from 0 (black) to 100 (white). Data was recorded using a colorimeter (model CR-410, Konica Minolta, Osaka, Japan) and expressed by the mean of five measurement repetitions for each sample. The standard illuminant D65 was used (corresponding to daylight, including ultraviolet light) and 2nd standard observer. This value was presented in the form of a unit of measurement (u.m.) of the parameter.

(a) *Natural Darkening Method of the Grain:* The grains were packed in zip lock bags (8.5 x 12 cm, 80 grams each) and stored under shelf conditions (Alvares et al., 2016) with environmental temperature and humidity, in a room at Santa Elisa Farm - IAC, Campinas (mean annual temperature: 21.4 ºC, mean annual relative humidity: 71%) (Figure 1A). The gradual change in the color of the grains of the genotypes was determined at 0, 30, 60 and 90 days. During storage, the grains were moved within the bag so that all were exposed to the same lighting conditions; and arranged entirely at random on the shelves at a least once a week.

(b) *Accelerated Darkening Method of the Grain:* The methodology was adapted from Junk-Knievel, Vandenberg and Bett (2007). Two ultraviolet lamps (wavelength centered at $\lambda = 253.7$ nm, model TUV 36W/G36 T8, Philips, Holland) and two fluorescent lamps ($\lambda = 480$ nm, model TL 40W/75RS, extra daylight, Philips, U.S.A.) were installed alternately in the Biotronette Mark III environmental chamber, n. 846 (Lab-Line Instruments, Inc., Illinois) (Figure 1B). The grains were placed in high transparency polystyrene Petri dishes, 90 mm in diameter by 15 mm in height (model K30-9015O, Olen, Kasvi, Brazil), eighteen centimeters below the lamps for 96 hours. The irradiance of ultraviolet energy

(0.95±0.1 mW cm^{-2}) in the surface of the samples was measured using a standard photodiode power sensor, ultraviolet extended (Model S120VC, 200-1100 nm, Thorlabs, Inc., USA). Adding ultraviolet and white light (4.0 ± 0.2 mW cm^{-2}), the samples were exposed to a total irradiance of 5.0 mW cm^{-2}. After 24 hours (86400 seconds) of illumination, the total light fluence calculated was approximately 432 J cm^{-2} and in 96 hours it was 1,728 J cm^{-2}. Ultraviolet represents approximately 20% of total light fluence, resulting in 82 J cm^{-2} in 24 hours and 328 J cm^{-2} after 96 hours. The value of L* was measured every 24 hours to evaluate the pattern of grain darkening. The volume of grains corresponded to a non-overlapping layer in a Petri dish (± 25 grams, 90 grains), mixed at every color tone reading and placed back in the chamber completely randomly. The ventilating fan remained on throughout the experiment and the temperature corresponded to ± 37 °C.

Statistical analysis

The values of lightness (L*) were submitted to univariate analysis of variance by environment and a joint analysis of variance was performed using the procedure for general linear models (Proc GLM), after verifying the residual mean square magnitudes. All effects, except the error, were considered as fixed. The means of the genotypes were compared by the Tukey test, at 5% level of significance, Pearson's phenotypic correlation coefficients were estimated among the traits. It was also performed multivariate analysis of variance (MANOVA) and canonical discriminant analysis, in order to compare methods, genotypes and environments, and to show the greatest possible separation among them jointly considering all the traits, namely: the lightness at the darkening times 0, 30, 60 and 90 days; 24, 48, 72 and 96 hours. The analyzes were performed using the SAS statistical software (Version Studio, SAS Institute, Inc. Cary, NC).

RESULTS AND DISCUSSION

In the present work the grain darkening protocol was adapted by ultraviolet light established by Junk-Knievel, Vandenberg and Bett (2007) for carioca bean. Regarding equipment installations and sampling time, the present study aimed to show the advantages in the substitution of the current methodology of evaluation of natural grain darkening by accelerated darkening.

The method introduced for accelerated grain darkening was used by assembling a system with minor modifications of ultraviolet and white fluorescent lamps and conditions, such as distance from the samples to the 10 cm to 18 cm lamps. The irradiance measurement showed a significant difference compared to the 4 mW cm^{-2} of ultraviolet irradiance previously published by Junk-Knievel, Vandenberg and Bett (2007). The total light fluence represents the total amount of photons of ultraviolet and visible light that passed through the area where the beans were placed. In order to calculate the total light fluence (J cm^{-2}), it first measured the power per area unit (mW cm^{-2}) using a power meter (previously described) and multiplied the value obtained by the time of illumination (seconds).

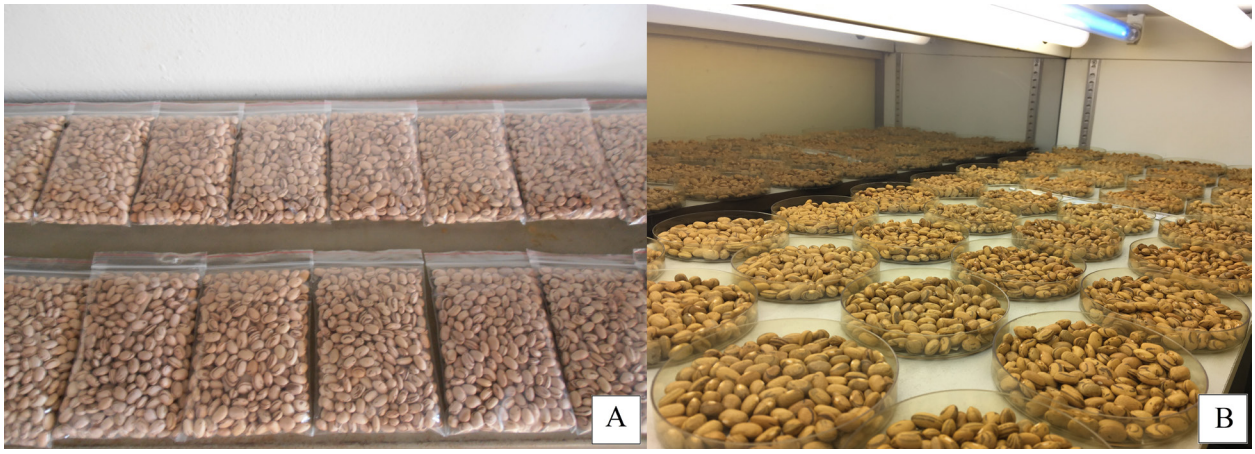

Figure 1: Grains of 19 carioca bean genotypes evaluated in Campinas for natural darkening (A) under shelf conditions at 0, 30, 60 and 90 days, and accelerated (B) in chamber conditions with ultraviolet and fluorescent lamps with colorimetric readings (dry and rainy), Tatuí (dry), Votuporanga (winter), Ribeirão Preto (winter) and Mococa (rainy), state of São Paulo.

Table 1 shows the joint analysis for the value of L* (lightness) in bean genotypes of the carioca type, with the coefficient of variation (CV) and the mean of the traits. The joint analysis was performed after verifying the homogeneity of the variances. In this way, no environment influence was excluded and the data were analyzed together.

The genotypes and environments showed significant effect, indicating that the genotypes present significant differences among them for the value of L* in the darkening times evaluated, as well as significant difference among the evaluated environments. The results of the coefficients of variation (CV) obtained were considered low (<10%) according to Pimentel-Gomes (2009) and ranged from 1.78% (72 hours) to 2.47% (90 days). The CVs indicate good experimental accuracy. The proximity of the mean value of L* for 90 days (48.67) and 24 hours (48.46) is highlighted.

The mean values of L* obtained at 90 days did not differ statistically from the values obtained with 24 hours in the accelerated method by Tukey test (5%), which shows a similar performance of darkening in these two methods (Figure 2). The Pearson correlation estimate based on the residues among traits 90 days and 24 hours was of mean magnitude (r = 0.55), positive and significant at 1%. At 90 days it was possible to verify genetic variability among the genotypes, as they lost color according to the values of L*, which makes the association important in terms of hours for this evaluation. At 120 days the grains were dark, making it difficult to identify genetic differences among the genotypes with the same darkening pattern, as observed by Siqueira et al. (2016). The profile analysis over time was performed between times 1 (0 day / hour), 2 (30 days / 24 hours), 3 (60 days / 48 hours) and 4 (90 days / 72 hours).

According to the multivariate analysis, there is a significant difference at 1% between the profiles in relation to the two levels studied (natural and accelerated darkening). This difference started to be more evident after time 2.

The effect of genotype x environment interaction was significant (Table 1), agreeing with Ribeiro, Jost and Cargnelutti Filho (2004) that evaluated the value of L* in carioca beans. Thus, the performance of each genotype for darkening does not follow the same pattern, depending on the environment in which it was evaluated. This is due to the difference among the environments, with the presence of rain or not at the time of harvest, as well as high temperatures.

Table 1: Joint analysis of variance with mean square and the significance by the test F for the value of L* in carioca beans evaluated by natural darkening (days) and accelerated (hours) methods.

Source	DF	MS	Pr > F	MS	Pr > F	MS	Pr > F	MS	Pr > F
		0 day		30 days		60 days		90 days	
Block	2	1.00	0.4393	2.42	0.1833	1.84	0.2890	2.70	0.1570
Genotype (G)	18	16.81	<0.0001	17.04	<0.0001	15.01	<0.0001	13.63	<0.0001
Environment (E)	5	237.64	<0.0001	168.44	<0.0001	260.05	<0.0001	278.03	<0.0001
G*E	90	3.14	<0.0001	3.63	<0.0001	3.20	<0.0001	3.36	<0.0001
Error	226	1.21		1.42		1.47		1.45	
CV%		2.07		2.32		2.44		2.47	
Mean		53.25		51.37		49.79		48.67	
		24 hours		48 hours		72 hours		96 hours	
Block	2	4.82	0.0104	2.21	0.0684	9.28	<0.0001	3.12	0.0084
Genotype (G)	18	7.97	<0.0001	5.94	<0.0001	5.66	<0.0001	4.62	<0.0001
Environment (E)	5	98.70	<0.0001	74.48	<0.0001	71.07	<0.0001	58.97	<0.0001
G*E	90	1.73	0.0012	1.24	0.0066	1.09	0.0009	0.90	0.0242
Error	226	1.04		0.81		0.64		0.64	
CV%		2.10		1.95		1.78		1.83	
Mean		48.46		46.28		44.85		43.75	

DF: degrees of freedom; MS: mean square; CV%: coefficient of variation.

However, Junk-Knievel, Vandenberg and Bett (2007) reported that they obtained results of the effects of genotype x environment interaction not significant for the beans with *pinto* type. In the present work a greater number of genotypes from different research institutions were used, considering also that the trials were carried out in different environments in regions of tropical climate, contributing to a higher occurrence of genotype interactions per environment. This explains why in Brazil studies of tolerance to grain darkening are important in breeding programs.

The main effects of genotypes and environments were explored considering that the coefficients of variation obtained were low, as well as the presence of residual squares of low magnitude, which leads to a smaller contribution of the error to the differences among the genotypes. The classification of genotypes was similar and the interactions were mainly due to differences in magnitude, where darkening was more pronounced depending on the environment in which the genotypes were cultivated. Thus the contribution to the interaction genotypes by environments was mainly due to genetic rather than environmental effects.

The literature presents the genetic control for grain darkening as monogenic or oligogenic, but without consensus. Junk-Knievel, Vandenberg and Bett (2008) and Silva et al. (2008) suggested that there is a single recessive gene that controls the phenotype of slow darkening. Elsadr et al. (2011) and Silva et al. (2014) presented as oligogenic, with the presence of epistasis, in which the expression of a gene depends on the action of a gene other than one of its alleles. As the genotype x environment interaction was significant in the present study, it is considered that the trait is oligogenic or even polygenic, although this trait was not addressed in any of the analyzes performed.

The Tukey test at 5% of significance (Table 2) was performed based on the joint analysis of the data, to compare the means of the 19 common bean genotypes for value of L* conducted in six environments. On average, the lighter genotypes were Gen 45-2F-293P, Gen 4-1F-19P and Gen 107-14A-336 and the darker BRS Pérola and CHC 01-175-1. The genotype CHC 01-175-1 has in its genealogy the BRS Pérola as one of the parents, which explains the strong influence in the darkening. According to Siqueira et al. (2016), darkening in lighter genotypes seems to be mainly due to the polyphenol oxidase activity, whereas in the dark there is a combination of enzymatic and non-enzymatic oxidation.

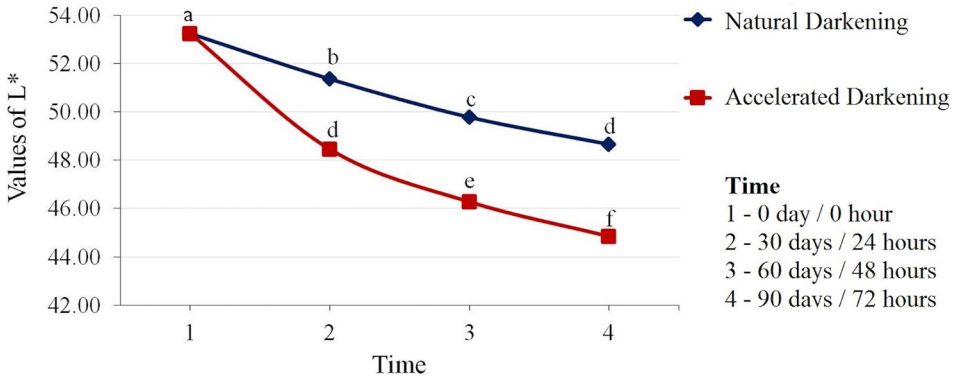

Figure 2: Temporal dynamics of the mean values of L* of carioca bean type submitted to natural darkening and accelerated considering all environments. Means with the same letter in column are not significantly different by the Tukey test (5%).

Table 2: Comparison of the means of 19 carioca bean genotypes for the value of L* cultivated in Campinas (dry and rainy), Tatuí, Votuporanga, Ribeirão Preto and Mococa, evaluated by natural darkening (days) and accelerated (hours) methods.

Genotypes	0 day	30 days	60 days	90 days
Gen 45-2F-293P	54.82±0.48a	53.04±0.46a	51.43±0.62a	50.08±0.50a
Gen 107-14A-336	54.49±0.82ab	52.61±0.84ab	51.07±0.90ab	49.92±0.88ab
Gen 4-1F-19P	54.31±0.48abc	52.66±0.38ab	50.92±0.42ab	49.93±0.36ab

Continue...

Table 2: Continuation.

Genotypes	0 day	30 days	60 days	90 days
Gen 104-1A-291	54.18±0.55abcd	52.41±0.51abc	50.84±0.62ab	49.78±0.69abc
Gen 12-2F-67	53.88±0.50abcde	51.94±0.54abc	49.92±0.59bcde	48.60±0.62bcdef
IAC Milênio	53.87±0.40abcde	51.71±0.46abcd	50.14±0.40abcd	49.06±0.46abcde
Gen 106-6A-319	53.85±0.54abcde	51.94±0.41abc	50.38±0.47abc	49.27±0.50abcd
Gen 125-10A-510	53.80±0.59abcde	51.45±0.53bcd	49.70±0.54bcde	48.52±0.60bcdef
Gen 78-1A-59	53.48±0.38bcdef	51.62±0.34bcd	50.19±0.50abcd	48.83±0.40abcdef
LP 11-363	53.38±0.58bcdefg	51.75±0.53abcd	50.45±0.62abc	49.27±0.71abcd
Gen 106-4A-317	53.27±0.55bcdefgh	51.31±0.60bcde	49.75±0.68bcde	48.53±0.71bcdef
CNFC 11-948	53.03±0.48cdefghi	51.00±0.44cde	49.28±0.50cde	48.36±0.50cdef
Gen 20-4F-129	52.94±0.64defghi	51.04±0.61cde	49.40±0.67cde	48.08±0.62def
Gen 90-4A-160	52.69±0.50efghij	51.06±0.47cde	49.35±0.59cde	48.63±0.62bcdef
IAC Sintonia	52.27±0.55fghij	50.38±0.42def	48.82±0.56de	47.64±0.54ef
Gen 86-12A-122	52.17±0.56ghij	50.48±0.54def	48.89±0.58de	47.68±0.64ef
CNFC 11-954	51.98±0.51hij	50.03±0.49ef	48.54±0.47e	47.60±0.52f
CHC 01-175-1	51.90±0.60ij	50.01±0.43ef	48.52±0.50e	47.60±0.62f
BRS Pérola	51.43±0.55j	49.57±0.42f	48.50±0.49e	47.44±0.51f
MSD	1.31	1.41	1.44	1.43
Genotypes	24 hours	48 hours	72 hours	96 hours
Gen 45-2F-293P	49.20±0.33ab	47.31±0.30a	45.69±0.30a	44.57±0.30a
Gen 107-14A-336	49.34±0.50a	46.66±0.42ab	45.19±0.36abc	43.99±0.27abcd
Gen 4-1F-19P	49.25±0.35a	46.93±0.32ab	45.44±0.27ab	44.31±0.27ab
Gen 104-1A-291	49.03±0.35abc	46.82±0.34ab	45.47±0.31ab	44.25±0.30ab
Gen 12-2F-67	49.21±0.41ab	46.94±0.27ab	45.46±0.32ab	44.30±0.28ab
IAC Milênio	49.06±0.42abc	46.39±0.30abcd	45.08±0.31abc	43.84±0.30abcd
Gen 106-6A-319	48.85±0.33abcd	46.45±0.29abc	45.14±0.29abc	44.02±0.26abc
Gen 125-10A-510	48.63±0.41abcde	46.59±0.36ab	45.28±0.34abc	44.08±0.32abc
Gen 78-1A-59	48.47±0.33abcdef	46.21±0.34bcde	44.68±0.28bcde	43.81±0.26abcde
LP 11-363	49.00±0.42abc	46.74±0.34ab	45.31±0.36abc	44.29±0.33ab
Gen 106-4A-317	48.20±0.37abcdef	46.18±0.33bcde	44.86±0.29abcd	43.67±0.31abcde
CNFC 11-948	48.26±0.31abcdef	46.04±0.30bcde	44.62±0.28bcde	43.43±0.28bcde
Gen 20-4F-129	48.26±0.41abcdef	46.23±0.37bcde	44.70±0.38bcde	43.46±0.31bcde
Gen 90-4A-160	48.00±0.40bcdef	46.04±0.35bcde	44.57±0.34bcde	43.65±0.33abcde
IAC Sintonia	47.40±0.51f	45.41±0.42cde	43.94±0.40de	42.87±0.34e
Gen 86-12A-122	47.92±0.40cdef	45.89±0.35bcde	44.38±0.27cde	43.30±0.26cde
CNFC 11-954	47.76±0.37def	45.90±0.34bcde	44.46±0.33cde	43.43±0.29bcde
CHC 01-175-1	47.33±0.43f	45.38±0.36de	43.94±0.40de	43.06±0.38de
BRS Pérola	47.58±0.33ef	45.17±0.30e	43.85±0.31e	42.86±0.28e
MSD	1.21	1.07	0.95	0.95

Means (± s.e.) with the same letter in column are not significantly different by the Tukey test; s.e.: standard error of the mean; alpha = 0.05; MSD: minimum significant difference; critical value of studentized range: 5.03.

Junk-Knievel, Vandenberg and Bett (2007) suggested that grain darkening is associated with the presence of proanthocyanidins (condensed tannins) present in the tegument. According to Duwadi et al. (2018), most flavonoids, including catechin monomer proanthocyanidins, accumulate at higher levels in regular darkening than slow darkening genotypes. Proanthocyanidins are oligomeric flavonoids composed primarily of catechin and epicatechin units (Duwadi et al., 2018).

Table 3 shows the analysis of the environments, where it was observed that, in general, the environment that presented lighter grains was Campinas, both in the "dry" and "rainy" season. Minimum significant differences ranged from 0.43 (72 and 96 hours) to 0.65 (60 and 90 days). The programs have converged to the improvement to tolerance to darkening of grains and the evaluated genotypes showed low variation, as observed in Tables 2 and 3, where the observed differences are mainly due to genotypes rather than environments.

According to García-Peña and Dias (2009), generally, when there is more than one trait measured per plot in the experimental designs, the multivariate analysis of variance (MANOVA) should be performed. Thus, the analysis was performed and the tests Wilks' Lambda, Pillai's trace, Hotelling-Lawley trace and Roy's greatest root for each of the effects (genotypes, environments and interaction) were calculated. From the results of the four multivariate tests (F = 2.74; F = 2.34; F = 3.27 and F = 17.13 respectively, significance at 1%) the null hypothesis is that there is no difference among the genotypes, H_{0Gen}=Gen_1=Gen_2=…=Gen_g, wherein g=19 was rejected. This indicates that there is a difference among the genotypes considering the eight traits responses of value of L* at 0, 30, 60 and 90 days (natural darkening) and 24, 48, 72 and 96 hours (accelerated darkening) simultaneously.

The null hypothesis is that there is no difference among the environments, $H_{0Environment}$=$Environment_1$=$Environment_2$=⋯=$Environment_e$, wherein e=6. The null hypothesis was reject based on the tests Wilks' Lambda, Pillai's trace, Hotelling-Lawley trace and Roy's greatest root (F = 33.44; F = 23.14; F = 48.52 and F = 170.06 significant at 1%), indicating that there is a significant difference among the environments considering the eight L* responses traits evaluated jointly.

The null hypothesis of no interaction effect was tested $H_{0GenEnvironment}$=Ge_{11}=Ge_{12}=⋯=Ge_{Ge} and rejected by the four tests (F = 1.24; F = 1.20; F = 1.28 and F = 2.99 significant at 1%). It indicates, therefore, that there is a significant interaction effect of the genotypes and the environments on the response traits.

Table 3: Comparison for the value of L* considering the means of the Campinas and Tatuí (dry), Votuporanga and Ribeirão Preto (winter), Mococa and Campinas (rainy) environments.

Environments	0 day	30 days	60 days	90 days
Campinas - dry	55.72±0.19a	53.05±0.17a	51.47±0.18b	51.13±0.17a
Campinas - rainy	55.39±0.24a	53.06±0.25a	52.20±0.24a	50.50±0.19a
Votuporanga	53.49±0.17b	51.53±0.18b	50.10±0.16c	49.20±0.18b
Mococa	52.83±0.23c	51.98±0.23b	50.50±0.23c	49.22±0.23b
Tatuí	51.28±0.22d	49.03±0.23c	47.79±0.23d	46.20±0.24c
Ribeirão Preto	50.79±0.22d	49.57±0.25c	46.69±0.24e	45.79±0.26c
MSD	0.59	0.64	0.65	0.65
Environments	24 hours	48 hours	72 hours	96 hours
Campinas - dry	49.99±0.13a	47.69±0.11a	46.36±0.11a	45.33±0.11a
Campinas - rainy	49.72±0.17a	47.34±0.15a	45.77±0.14b	44.55±0.13b
Votuporanga	48.60±0.16b	46.31±0.13b	45.02±0.13c	43.49±0.11c
Mococa	48.61±0.20b	46.37±0.19b	44.68±0.17c	43.51±0.17c
Tatuí	46.96±0.14c	45.01±0.11c	43.56±0.11d	42.60±0.10d
Ribeirão Preto	46.90±0.20c	44.94±0.17c	43.68±0.16d	42.99±0.15d
MSD	0.55	0.49	0.43	0.43

Means (± s.e.) with the same letter in column are not significantly different by the Tukey test; s.e.: standard error of the mean; alpha: 0.05; MSD: minimum significant difference; critical value of studentized range: 4.06.

From the results of MANOVA that presented significant values for the genotype and environment factors, the contrasts analysis was performed to identify which genotypes and environments present significant differences in the means of the vectors. The effects of genotypes and environments were highly significant for the same contrasts and are presented below for value of L* considering all the darkening times in both natural and accelerated methods.

Contrast was performed among groups of genotypes in which IAC lines (Gen 4-1F-19P, Gen 12-2F-67, Gen 20-4F-129, Gen 45-2F-293P, Gen 78-1A-59, Gen 86-12A-122, Gen 90-4A-160, Gen 104-1A-291, Gen 106-4A-317, Gen 106-6A-319, Gen 107-14A-336, Gen 125-10A-510) presented significative contrast with the cultivar (BRS Pérola) and Embrapa lines (CNFC 11-948, CNFC 11-954) as well as with the Epagri line (CHC 01-175-1). The IAC cultivars (IAC Milênio, IAC Sintonia) showed significant contrast with the cultivar of Embrapa (BRS Pérola).

The canonical discriminant analysis was performed for the contrasts among the genotypes. The square distance of Mahalanobis with the significant probabilities of the values by the Test F are presented in Table 4. The genotypes that presented the greatest distance were BRS Pérola with Gen 45-2F-293P (D^2 = 6.90**) and Gen 12-2F-67 (D^2 = 6.08**). The Gen 90-4A-160 lineage is at a minor distance from the BRS Pérola (D^2 = 1.99*) and further from other IAC lineages such as Gen 12-2F-67 (D^2 = 3.42**), Gen 45-2F-293P (D^2 = 2.48**), Gen 107-14A-336 (D^2 = 2.34**), Gen 125-10A-510 (D^2 = 2.22**).

Considering the results of the comparison contrasts of the mean vectors from MANOVA and canonical discriminant analysis, Gen 45-2F-293P genotype was distinguished with contrasts with dark genotypes, especially with BRS Pérola. This shows the potential of the genotype to maintain the lightness of the tegument over time, an important feature of tolerance to darkening.

The IAC lines presented contrast with the BRS Pérola, which is an old cultivar in the market, which shows that the breeding program is being conducted satisfactorily for the trait. For bean genotypes with carioca tegument, genotypes with a value of L* higher than 55 are desirable, which is associated with lighter grains (Ribeiro et al. 2014; Siqueira et al. 2016) at the time of harvest. On the other hand, lighter grains tend to be more susceptible to the pathogen *Fusarium oxysporum* (Chiorato et al., 2015) and to other diseases and pests. This relationship has been observed in field conditions, however, specific studies are necessary to evaluate the selection threshold between light and dark grains.

Table 4: Square distance of Mahalanobis and probability values (*p*-values) for the contrasts among carioca bean genotypes.

Genotypes	BRS Pérola	CHC 01-175-1	CNFC 11-948	CNFC 11-954	Gen 12-2F-67	Gen 45-2F-293P	Gen 107-14A-336	Gen 125-10A-510	IAC Milênio	IAC Sintonia
CNFC 11-948	2.13*		-							
Gen 4-1F-19P	3.74**	2.23**		1.94*						
Gen 12-2F-67	6.08**	4.30**	1.80*	3.01**	-		1.94*			
Gen 20-4F-129	3.29**	1.80*								
Gen 45-2F-293P	6.90**	3.84**	2.54**	3.07**		-	2.57**			
Gen 78-1A-59	2.41**				1.99*					
Gen 86-12A-122					1.92*	2.33**	1.76*			
Gen 90-4A-160	1.99*				3.42**	2.48**	2.34**	2.22**		
Gen 104-1A-291	3.59**	1.94*								
Gen 106-4A-317	3.06**									
Gen 106-6A-319	2.57**									
Gen 107-14A-336	3.34**	2.83**		3.05***			-			
Gen 125-10A-510	4.92**	2.66**					2.09*	-		
IAC Milênio	2.62**	2.43**		2.32**		3.24**			-	
IAC Sintonia	1.86*				3.11**	2.88**	1.83*	1.82*	1.65*	-
LP 11-363	2.39**				2.34**	1.97*	1.95*	2.04**		2.12*

Probability values for distances by the F test (* 5%; ** 1%).

In the Genetic Bean Breeding Program of the Instituto Agronômico (IAC), the ideal value of L* used has been 53 at the time of harvest as a fast selection criterion, combining market favoring, disease tolerance and broth quality. Below this value the market refutes the product, but there is greater tolerance to diseases and better quality of broth (thick broth). Above this value, the market accepts the product, where, however, a greater number of genotypes susceptible to soil diseases are identified, as well as a lower quality of broth (clear broth).

The polyphenolic compounds are related to the plant defense system and high levels of these compounds are responsible for plants more resistant to pest attack (Islam et al., 2003), however, these genotypes generally present more rapid darkening process of the grain. According to Erfatpour, Navabi and Pauls (2018), polyphenolic compounds have been associated with the color and pattern of grain coverage in common beans. Slow darkening is significantly associated with reduced levels of kaempferol and polyphenol oxidase activity, which is responsible for the oxidation of polyphenols (Beninger et al., 2005; Duwadi et al., 2018).

With the growing appeal for the lower use of pesticides, darker grains that tend to be more tolerant to pests and diseases might respond to market niches focused primarily on organic management. Chen et al. (2015) presents results that confirm the relation of the polyphenolic compounds with darkening and makes a revision on the importance of these and compounds contained in the bean.

The darkening genotypes that slowly presented similar results considering the two methods (Table 2), conferring reliability on the choice of accelerated darkening protocol in relation to natural darkening. Erfatpour, Navabi and Pauls (2018) modified the time of exposure of *pinto beans* to ultraviolet light from 72 hours to 24 hours but did not present the reason for the time reduction. The time correspondence of 24 hours and 90 days, as well as the non-existence of significant differences by the Tukey test (5%) could allow the breeding programs to implement the accelerated darkening method with ultraviolet light as a standard in the routine.

CONCLUSIONS

The evaluation of the accelerated grain darkening method based on the values of L* (light grains) under ultraviolet light chamber conditions can be used to identify bean genotypes tolerant to grain darkening in 24 hours.

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