

ASSOCIATION OF SLOW DARKENING GENE 'SD' WITH GRAIN QUALITY TRAITS IN CARIOCA BEAN AND NEW CANDIDATE MARKER

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INTRODUCTION: The carioca dry bean market class is preferred by most consumers and accounts for 70% of the Brazilian consumer market (Del Peloso & Melo, 2005). Recently, characteristics related to the commercial grain quality such as grain color, cooking time and darkening period have become more important due to an increasingly demanding consumer market (Carbonell et al., 2010). The seed darkens during storage becoming less acceptable to consumers and depreciation of the economic value. Cultivars with delayed grain darkening associated with reduced cooking time after storage will be advantageous for the farmer and seed dealers. Grain storage for longer periods allows flexibility, i.e., the producer can await better prices on the market. The cultivar BRSMG Madrepérola is a slow darkening carioca cultivar with recessive gene inheritance for the trait (Silva et al., 2008).

MATERIALS AND METHODS: Two carioca recombinant inbred populations - BRSMG Madrepérola / BRS Estilo (56 F_{5:7} RILs) and BRSMG Madrepérola / BRS 9435 Cometa (57 F_{5:7} RILs) were planted at three locations in Goiás – Brazil in 2012 using random complete block designs with three replications. BRS Estilo and BRS 9435 Cometa are regular carioca cultivars that rapidly darken during storage. Yield (kg ha⁻¹), plant architecture (scale 1 to 6), weight 100 seeds (g), lodging 1 to 9 score and cooking time (90 and 180 days after harvest – Mattson cooker) were the agronomic traits studied. The populations were exposed to ultraviolet light for 72 hours (Junk-Knievel et al., 2007) and visual dark measurement was obtained at three months post-harvest using the scale 1 (slow dark) to 6 (most dark) (Silva et al., 2008). Means for the traits were obtained using GLM SAS. The molecular marker SSR 1158 (Felicetti et al., 2012) was identified as linked to the *sd* gene and a CAPs (Cleaved amplified polymorphic sequences – digested by enzyme BsiHKAY) marker within a candidate gene were tested across the RILs (Table 1).

Table 1. Primer set used for amplification of CAPs marker.

Seq Name	Seq 5' to 3'
Pv_bHLH-F8	ACTGAGAGAGACACATCATATGTGA
Pv_bHLH-R8	GGATAATCTCATTGTTTGTGATTC

RESULTS AND DISCUSSION: All investigated traits varied significantly ($P < 0.05$) across the carioca bean RILs. SSR 1158 is a co-dominant marker and able to distinguish homozygous and heterozygous RILs in carioca beans (Figure 1). The RILs were separated in two groups – slow and regular – and means of the traits for each group is below (Table 2). Positive correlation between the scale for darkening and the molecular marker SSR 1158 (0.88), the SSR 1158 and UVC light test (0.82) and between darkening scale and UVC light test (0.79) were obtained. Positive correlations were observed between darkening scale (0.84) and UVC light test (0.76) with the CAPs marker. There were no correlations between cooking time, plant architecture and yield with grain darkening.

Table 2. Traits means in the two RIL populations separated by slow and regular darkening.

UV test	n°	Yield ¹	Arch ²	Lodg ³	W100S ⁴	Scale ⁵	CT ⁶ 180	CT ⁶ 90
BRSMG Madrepérola x BRS 9435 Cometa								
Sd	24	3183	4.9	4.9	24.9	2.0	25.6	21.0
Rd	33	3422	4.8	4.7	25.0	3.7	24.7	19.6
BRSMG Madrepérola x BRS Estilo								
Sd	15	3614	5.1	5.1	24.8	1.9	28.4	21.0
Rd	41	3639	4.8	4.7	26.2	3.3	28.6	21.4
For both populations								
Sd	38	3336	5.0	5.0	24.9	1.9	26.7	20.9
Rd	74	3542	4.8	4.7	25.7	3.5	26.8	20.6

¹ kg ha⁻¹, ² plant architecture, ³lodging, ⁴g 100 seeds, ⁵scale post-harvest, ⁶cooking time (min.)

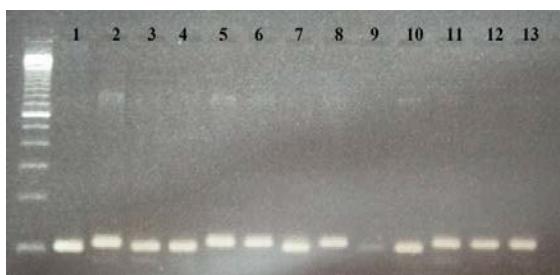


Figure 1. Co-dominant marker SSR 1158 assayed across a set of slow dark (lower band) and dark (upper band) genotypes: 1 SDP, 2 Stampede, 3 BRSMG Madrepérola, 4 CNFC 16688, 5 CNFC 16689, 6 BRS Estilo, 7 CNFC 16697, 8 BRS Cometa, 9 CNFC 16702, 10 CNFC 16709, 11CNFC 16714, 12 CNFC 16718 and 13 CNFC 16736.

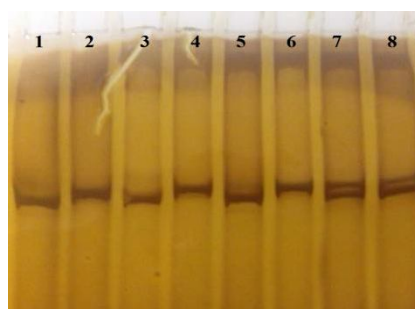


Figure 2. CAPs marker run on a 8% non-denaturing acrylamide gel and visualized with silver staining (1 – SDP, 2 – Stampede, 3 – BRSMG Madrepérola, 4 – BRS Estilo, 5 to 8 - RILs).

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