Genetic diversity of promising 'conilon' coffee clones based on morpho-agronomic variables

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ABSTRACT

Knowledge of the genetic variability of a population is essential to guide its preservation and maintenance in addition to increasing the efficiency of genetic breeding programs. On this basis, this study was conducted to evaluate the genetic diversity of *Coffea canephora* genotypes using multivariate statistical procedures applied to a set of morpho-agronomic variables. The materials employed in this study constitute a crop located in Vila Valério - ES, Brazil, where the genotypes are arranged in a randomized-blocks experimental design with four replicates. Significant differences were detected by the F test at the 1% or 5% probability levels among the genotypes for all evaluated traits, demonstrating heterogeneity of genetic constitution in the studied population, which is favorable to breeding, as it indicates the possibility to identify superior and divergent individuals. Based on the generalized Mahalanobis distance, the most divergent combinations were obtained between genotypes 23 and 10 (256.43) and 23 and 17 (250.09). The clusters formed by Tocher's optimization and the UPGMA hierarchical method agreed, both similarly grouping the genotypes into three clusters. Of the analyzed traits, mature fruit weight (19.08%), yield (15.50%), plant diameter (12.42%), and orthotropic-shoot internode length (10.94%) were the most efficient to explain the dissimilarity among the genotypes.

Key words: *Coffea canephora*, dissimilarity, multivariate analysis, plant breeding.

INTRODUCTION

The coffee crop occupies over 11 million hectares, and its product is one of the most valuable of the world economy, with a production sector involving more than 80 countries (Denoeud et al. 2014,

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Mohanan et al. 2014). The plant that originates this commodity belongs to the family Rubiaceae, which in turn comprises over 11,000 species, ordered into 550 genera (Robbrecht and Manen 2006), one of which is *Coffea*. According to Davis et al. (2011), this genus has at least 124 species, wherein *C. arabica* Linnaeus and *C. canephora* Pierre ex A. Froehner are the two of highest commercial prominence, accounting for approximately 58%

and 42% of the 148 bags of 60 kg produced in 2016 across the globe (ICO 2017).

Today, the species *C. canephora*, also known as 'conilon' or 'robusta' coffee, is cultivated at low to medium elevations in the intertropical regions of Africa, America, and Asia. It adapts well to warmer climates without major variations in temperature, with precipitation greater than 2,000 mm yr^{-1} (Montagnon et al. 2012, Cubry et al. 2013).

The species *C. canephora* is diploid (2n = 2x = 22) and mostly allogamous, of gametophytic self-incompatibility (Carvalho et al. 1991) of monogenic nature; i.e., it is bound to a single "S" locus, with at least three alleles interacting (Conagin and Mendes 1961). This mechanism prevents the pollen grain carrying a certain allele from fertilizing any ovule carrying the same allele, thereby preventing self-pollination and/or cross of individuals with similar alleles.

Therefore, the natural reproduction of the species generates highly heterozygous individuals and populations with high genetic variability. In this regard, the characterization and exploitation of genetic variability within the species *C. canephora* may reveal genetic resources of great value both for production systems and for its use in genetic breeding programs. Rodrigues et al. (2014) reported that the coffee breeding programs aiming at productivity, aggregating agronomic traits for resistance to pests and diseases, and plants of short height and adapted to local climate and soil conditions.

Genetic variability, the raw material of breeding programs, can be estimated by multivariate analyses, which are based on differences between accessions, simultaneously integrating multiple pieces of information from a set of traits. In this sense, the analysis of genetic diversity is important in that it provides parameters for the identification of the parents whose cross produces a greater heterotic effect, to heighten the possibilities of obtaining offspring with superior features (Cruz et al. 2014).

Studies on the genetic diversity of coffee adopting multivariate analyses have been carried out by many researchers, both for *C. canephora* (Fonseca et al. 2006, Ivoglo et al. 2008, Rocha et al. 2013, Dalcomo et al. 2015, Silva et al. 2015, Covre et al. 2016), and *C. arabica* (Dias et al. 2005, Guedes et al. 2013, Silva et al. 2013, Teixeira et al. 2013, Moura et al. 2015, Rodrigues et al. 2016). Information in this regard provides bases to be used in breeding programs for the species.

Given the above-described scenario, this study was carried out to investigate the genetic diversity of promising registered *C. canephora* genotypes using multivariate statistical procedures employed on a set of morpho-agronomic traits.

MATERIALS AND METHODS

STUDIED POPULATION AND DESCRIPTION OF THE EXPERIMENT

We evaluated 30 *C. canephora* genotypes, 29 of which were propagated vegetatively via cuttings and one via seminiferous tubules (Table I).

The crop was implemented in May 2012 in the municipality of Vila Valério - ES, Brazil (18°58'05" S latitude and 40°20'02" W longitude; 150 m asl), with an average annual temperature of 23 ºC. The climate in the region is tropical, characterized by hot and wet summers and dry winters, classified as an Aw type, according to the Köppen classification (Alvares et al. 2013), The average annual precipitation in 2015 was 1,200 mm (ANA 2015).

The genotypes were arranged in randomized-blocks experimental design with four replicates in which the treatments corresponded to the different genotypes and each experimental unit contained four plants. The plants were planted under a spacing of 2.2 m between rows and 1.2 m between plants, establishing a density of 3,086 plants per hectare, with four stems per plant. The sprinkler irrigation system was adopted.

TABLE I
Coffea canephora genotypes evaluated in Vila Valério - ES, Brazil. Promising registered genotypes.

Number	Genotype	Number	Genotype	Number	Genotype
1	Vitória V1	11	Beira Rio 6	21	18a
2	Vitória V2	12	Beira Rio 8	22	Baiano 4a
3	Vitória V4	13	Beira Rio 9	23	Pirata
4	Vitória V5	14	Beira Rio 10	24	Cownga
5	Vitória V7	15	Bamburral	25	Verdim 1
6	Vitória V8	16	Clementino	26	Verdim R
7	Vitória V9	17	P.Seminífera	27	P2
8	Vitória V10	18	Emcapa 153	28	P1
9	Beira Rio 3	19	Emcapa 143	29	LB1
10	Beira Rio 5	20	22a	30	A1

Note: Genotypes from 1 to 8 belong to variety "Vitória Incaper 8142"; Genotypes 18 and 19 belong to variety Emcapa 8131.

MORPHO-AGRONOMIC CHARACTERIZATION

The following 15 morpho-agronomic traits were evaluated: height (PH) and diameter (PD) of the plant at 477 days after planting (DAP); orthotropic-shoot internode length (OSI), measured after the plant reached its adult stage, in full production; weight (WMF), volume (VMF), and density (DMF) of the mature fruit, measured in fruits collected in the second harvest; grain processing rate (PR), corresponding to the ratio between mature fruit and processed grain (% w/w); average yield of the 2014 and 2015 crops (YLD), expressed in bags (60 kg) per hectare; total chlorophyll index (CI), obtained using a manual chlorophyll meter (clorofiLOG®); leaf length (LL), width (LW), and area (LA), the last of which was estimated by the methodology proposed by Partelli et al. (2006); leaf dry mass (LDM); and leaf specific mass (LSM), calculated as the ratio between dry mass and leaf area. For these leaf morphology assessments, we collected ten leaves per plot from the third and/or fourth pair of newly developed leaves from plagiotropic shoots located at the middle third of the plants. Collections took place when the plants were at approximately two years and eight months of age.

STATISTICAL ANALYSIS

The data were subjected to analysis of variance, and the existence of variability was tested by the F test. For the study of genetic diversity, we determined the generalized Mahalanobis (D^2) distance matrix, which was used as a dissimilarity measure for the cluster analysis of the genotypes by Tocher's optimization method and the UPGMA (Unweighted Pair Group Method using Arithmetic Averages) hierarchical method. We also obtained the relative contribution of the traits for the genetic diversity among the *C. canephora* genotypes by Singh (1981)'s method. All of the aforementioned analyses were performed using the resources of Genes software (Cruz 2013).

RESULTS AND DISCUSSION

According to the analysis of variance, significant differences were detected by the F test at the 1% or 5% probability levels among the *C. canephora* genotypes for all the morpho-agronomic traits assessed (Table II).

This fact reveals heterogeneity in the genetic composition of the studied population, which is important for analyses of genetic diversity and also

TABLE II
Summary of analysis of variance for 15 morpho-agronomic traits evaluated in 30 promising registered *Coffea canephora* genotypes Vila Valério - ES, Brazil.

Traits	Mean Square		Average	CV (%)
	Genotype	Residual error		
PH	177.1452	28.1079**	78.95	6.72
PD	574.8038	76.6649**	107.67	8.13
OSI	0.5013	0.0633**	2.81	8.96
PSI	0.5795	0.0611**	3.06	8.07
MFW	0.0838	0.0055**	0.95	7.79
MFV	0.0823	0.0067**	0.91	9.00
MFD	0.0124	0.0057**	1.06	7.14
PR	15.9533	2.4831**	24.84	6.34
YLD	893.9544	79.3112**	64.35	13.84
CI	46.5976	22.6726**	70.93	6.71
LL	1.9338	0.6492**	14.71	5.48
LW	0.3855	0.2236*	5.69	8.31
LA	118.4598	38.9397**	56.96	10.95
LDM	17494.4300	6071.7240**	700.77	11.12
LSM	4.1057	1.7074**	12.40	10.54

** and *, significant at the 1 and 5% probability levels, respectively, by the F test. PH: plant height at 477 DAP (cm); PD: plant diameter at 477 DAP (cm); OSI: orthotropic-shoot internode length (cm); PSI: plagiotropic-shoot internode length (cm); MFW: mature fruit weight (g); MFV: mature fruit volume (mL); MFD: mature fruit density; PR: grain processing rate (% w/w); YLD: average yield of the 2014 and 2015 crops (bags ha^{-1}); CI: chlorophyll index; LL: leaf length (cm); LW: leaf width (cm); LA: leaf area (cm^2); LDM: leaf dry mass (mg); LSM: leaf specific mass (mg cm^{-2}).

favorable to breeding, as it indicates the possibility of discriminating superior and divergent genotypes.

The coefficient of variation (CV), which expresses the residual standard deviation as a percentage of the overall mean, is frequently used to estimate the precision of experiments. In this study, values between 5.48 and 13.84% were found, with an average of 8.61% (Table II). According to Pimentel-Gomes (2009), all of these are classified as low (CV_e < 10%) or medium (CV_e < 20%), demonstrating good experimental precision in the evaluations performed, as stated by Cruz et al. (2014).

The maximum and minimum dissimilarity values for each one of the 30 *C. canephora* genotypes, obtained based on the generalized Mahalanobis (D^2) distance, showed varied magnitude, with values ranging between 5.003 and 256.433, indicating broad genetic diversity in the studied population (Table III).

Dalcomo et al. (2015) evaluated 22 genotypes of conilon coffee from the breeding program of Incaper and also found elevated magnitude for genetic dissimilarity, with values between 7.32 and 168.37.

The highest average dissimilarity (145.72) was observed in genotype 23 (Pirata), which was present in 93.3% of the most dissimilar pairs; i.e., it was the genotype most distant from 28 of the total 30 under evaluation. The most divergent combinations were obtained between genotypes 23 and 10 (256.43) and between 23 and 17 (250.09). Genotypes 6 and 15, on the other hand, were the most similar, as they exhibited the shortest distance (5.00) between the least dissimilar pairs.

TABLE III
Average, maximum, and minimum dissimilarity for 30 promising registered *Coffea canephora* genotypes based on generalized Mahalanobis distance (D²), considering 15 morpho-agronomic traits[1]. Vila Valério - ES, Brazil.

Genotype	Average dissimilarity	Maximum dissimilarity		Minimum dissimilarity	
		Genotype	Distance	Genotype	Distance
1	44.56455	23	159.139842	11	16.338563
2	42.37869	23	171.486173	6	8.380784
3	42.86718	23	160.405796	6	5.309058
4	45.72179	23	138.149287	7	15.940578
5	44.36534	23	121.645149	19	18.294495
6	34.70369	23	136.208307	15	5.003676
7	27.95849	23	108.498885	19	8.787921
8	62.69844	23	156.749164	5	32.325622
9	54.94184	23	152.761368	14	25.273979
10	66.19208	23	256.432611	17	25.995628
11	42.73313	23	156.990554	21	7.673506
12	85.16450	17	125.760347	22	31.701214
13	35.69210	23	82.246938	7	12.512651
14	29.94187	23	131.795703	7	8.899016
15	39.34052	23	131.736855	6	5.003676
16	43.51350	23	115.003605	7	16.578355
17	59.97461	23	250.088603	20	22.282136
18	44.66841	23	172.942595	25	10.809870
19	34.89041	23	125.698329	7	8.787921
20	48.43194	23	224.798217	2	21.668399
21	36.51560	23	144.725625	11	7.673506
22	43.97228	23	128.370045	7	16.221329
23	145.71600	10	256.432611	30	76.313264
24	44.18330	23	118.254270	14	15.618320
25	47.43553	23	187.401413	18	10.809870
26	73.29632	23	126.203228	5	34.716500
27	41.08602	23	150.359086	14	14.049508
28	37.21266	23	93.131116	7	12.989153
29	45.66103	23	142.243507	18	13.493561
30	49.40958	10	89.263453	28	15.767228

[1]Plant height and diameter at 477 DAP; length of the orthotropic - and plagiotropic- shoot internodes; weight, volume, and density of mature fruit; grain processing rate (% w/w); average yield of the 2014 and 2015 crops; chlorophyll index; leaf length, width, and area; leaf dry mass; and leaf specific mass.

It should be stressed that the genotypes belonging to variety Vitoria (V1, V2, V4, V5, V7, V8, V9, V10, and Emcapa 153) did not necessarily show the lowest distances, combining with each other, which corroborates the results obtained by Fonseca et al. (2006) in an evaluation of 32 genotypes of conilon coffee belonging to three clonal varieties. These authors remarked that crosses of greater interest are not always found among germplasms of distinct varieties.

Clustering by Tocher's optimization method, using the generalized Mahalanobis distance (D^2) as the genetic dissimilarity measure, for 15 morpho-agronomic traits, separated the 30 genotypes into three groups (Table IV).

The first group consisted of 28 genotypes, including approximately 93% of the total genotypes studied. This fact is related to the high dissimilarity presented by genotype 23, which was higher by the order of 192.4% as compared with the overall mean of the average dissimilarity (Table III). Similar results were found by Silva et al. (2015), who evaluated 56 genotypes of the genus Robusta and also found 91% of the individuals associated with the first group.

Genotypes 12 (Beira Rio 8) and 23 (Pirata) were, separately, the second and the third groups, respectively, indicating that these are the most divergent. Tocher's optimization method minimizes the intra-group distance and maximizes the inter-group distance (Cruz et al. 2014); in this way, more divergent combinations should be obtained with genotypes allocated in distinct groups.

Studies reporting the use of Tocher's method for clustering *C. canephora* genotypes were also conducted by Fonseca et al. (2006), who worked with 32 clones of three clonal varieties; by Ivoglo et al. (2008), with 21 progenies of half-siblings; and by Covre et al. (2016), with 34 promising genotypes, who reported the formation of three, four, and eight groups, respectively.
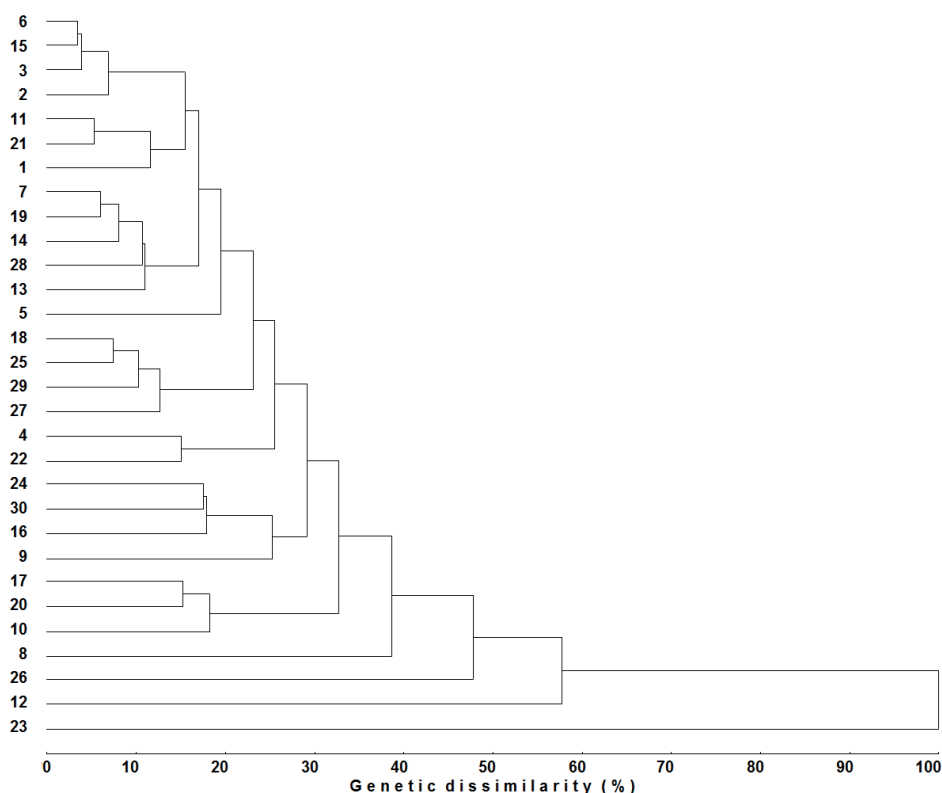
Clustering by the UPGMA hierarchical method, using the generalized Mahalanobis distance (D^2) as a measure of genetic dissimilarity, enabled the generation of the dendrogram illustrating the genetic distance among the studied genotypes. When the maximum dissimilarity limit of 50% was established among genotypes for them to be included in the same group, three groups were formed (Figure 1).

The groups formed by the UPGMA method, assuming a maximum dissimilarity limit of 50% among the genotypes, were formed similarly to those generated by Tocher's method, increasing the consistency between the clustering methods. Similarities between Tocher's and UPGMA hierarchical method were also observed by Ivoglo et al. (2008), Guedes et al. (2013), and Covre et al. (2016), who established the maximum limits of dissimilarity among genotypes as 15%, 45%, and 50%, respectively

Except for genotypes 30 and 10, all the others that represented the most dissimilar parents based on the generalized Mahalanobis distance (D^2) (Table III) were allocated in distinct groups by both Tocher's optimization (Table IV) and the UPGMA hierarchical method (Figure 1), increasing the reliability in the use of clusters to establish divergent combinations. Motta et al. (2014) evaluated *C. arabica* and *C. canephora* genotypes and also observed that the most dissimilar genotypes based on Jaccard's index were mostly allocated in distinct groups by UPGMA.

The study of the relative contribution of the 15 morpho-agronomic traits for genetic diversity among the 30 *C. canephora* genotypes, according to Singh (1981)'s method, showed values between 0.015 and 19.080% (Table V).

The traits that most contributed were mature fruit weight (19.08%), average yield (15.50%), plant diameter (12.42%), and orthotropic-shoot internode length (10.94%); these four variables accounted for 58% of the genetic divergence

TABLE IV

Clusters formed from 30 promising registered *Coffea canephora* genotypes, obtained by Tocher's method, based on generalized Mahalanobis distance (D^2), considering 15 morpho-agronomic traits. Vila Valério - ES, Brazil.

Groups	Genotype
1	6 15 3 2 21 14 7 19 13 11 28 5 1 20 27 18 25 29 24 4 16 22 30 17 9 10 8 26
2	12
3	23

Figure 1 - Dendrogram representing the genetic dissimilarity among 30 promising registered *Coffea canephora* genotypes, obtained by the UPGMA cluster method, using generalized Mahalanobis distance (D^2), considering 15 morpho-agronomic traits. Note: cophenetic correlation coefficient (CCC): 87.27%.

among the studied genotypes. Guedes et al. (2013) evaluated 27 traits in 12 accessions of coffee (*C. arabica* L.) var. Maragogipe and Ivoglo et al. (2008) evaluated 14 traits in 21 progenies of *C. canephora* half-siblings and observed yield as one of the traits that most contributed to divergence among the studied genotypes, with 22.7 and 10.5%, respectively.

Fruit density (0.015), in turn, was the trait that least contributed to genetic diversity, and when it was discarded, it did not lead to significant alterations in the clusters obtained by Tocher's method; therefore, in subsequent evaluations, less importance can be assigned to this trait.

Figure 2 shows the standardized means for morpho-agronomic traits in each group formed by Tocher's optimization method. This graph allows us to know the traits that differentiate each group.

It is clear that among the traits of greatest relative contribution (Table V), those which characterize group 2 are fruits of larger weight and volume and lower grain processing rate. Group 3, in turn is characterized by plants with larger diameter and higher yield (Figure 2).

Despite the genetic diversity detected among the genotypes, breeders are usually interested only in the superior individuals with respect to some traits, with sufficient diversity to generate variability in the segregating population (Guedes et al. 2013). In *C. canephora* breeding programs, breeders aim at genotypes that, among other features, provide higher yields and produce larger grains (Ferreira et al. 2005). In this scenario, the genotypes from groups 2 and 3 showed to be promising, because in addition to their genetic diversity, they exhibit traits of agronomic interest (Figure 2).

TABLE V
**Relative contribution of 15 morpho-agronomic traits
for genetic diversity in 30 promising registered *Coffea
canephora* genotypes based on Singh (1981)'s method,
using generalized Mahalanobis distance (D^2). Vila Valério
- ES, Brazil.**

Traits	S.j	Value (%)
MFW	4125.755056	19.080
YLD	3352.028483	15.502
PD	2685.260696	12.418
OSI	2366.094264	10.942
PSI	1893.711330	8.758
PR	1728.743733	7.995
LDM	1542.608442	7.134
PH	1299.638652	6.010
MFV	968.399258	4.478
CI	602.932788	2.788
LA	382.716226	1.770
LW	272.011552	1.258
LSM	211.840563	0.980
LL	188.668397	0.872
MFD	3.284616	0.015

PH: plant height at 477 DAP; PD: plant diameter at 477 DAP;
OSI: orthotropic-shoot internode length; PSI: plagiotropic-
shoot internode length; MFW: mature fruit weight; MFV:
mature fruit volume; MFD: mature fruit density; PR: grain
processing rate; YLD: average yield of the 2014 and 2015
crops; CI: chlorophyll index; LL: leaf length; LW: leaf width;
LA: leaf area; LDM: leaf dry mass; LSM: leaf specific mass.

After the evaluations, the materials called
Beira Rio 8, Bamburral, Clementino, Pirata, Verdin
R and A1 constituted a new variety called Tributum,
which is registered by UFES. The average of four
harvests of the six genotypes was 90.87 bags
benefited (60 kg) per hectare.

CONCLUSIONS

There is genetic diversity among the 30 promising
registered *C. canephora* evaluated here, indicating
high potential for use in future breeding actions;
genotypes Pirata (promising) and Beira Rio 8
(promising) were the most divergent. Tocher's and
UPGMA hierarchical methods showed consistency
in the grouping of genotypes, separating them
similarly into two groups; of the analyzed traits,
mature fruit weight, average productivity,
plant diameter, and orthotropic-shoot internode
length were the most efficient in explaining the
dissimilarity among the genotypes.
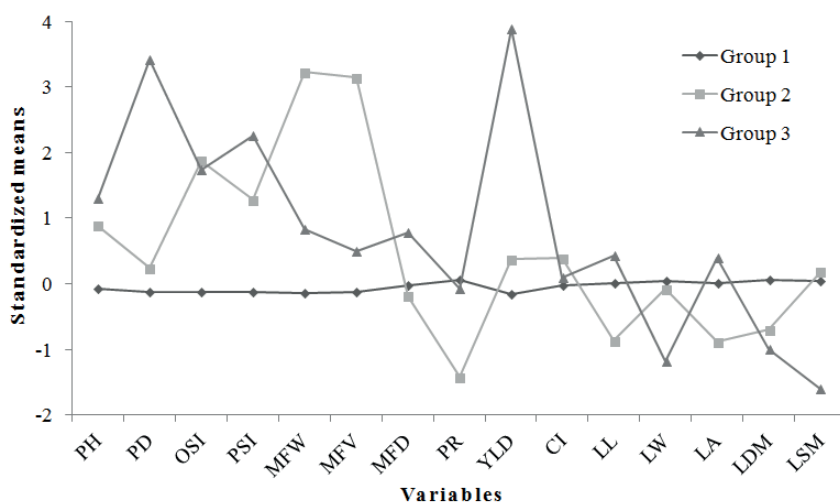
Figure 2 - Standardized means of the 15 morpho-agronomic traits for each group
formed by Tocher's optimization method. Vila Valério - ES, Brazil. Promising registered
genotypes.

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