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Genetic polymorphism among natural populations of *Anacardium humile* A. ST-HIL

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Abstract - The genus *Anacardium* presents nine species, of these, three have sub-bush size, common in the Cerrado of the Center-West of Brazil. The objective of this work was to evaluate the genetic variability of the species, collected in eleven provenances, using RAPD markers. Genomic DNA from 122 accessions was extracted and amplified with 25 decamer primers. The results indicated polymorphism, ranging from 77.71% to 96.18%. The distribution of genetic diversity among and within populations shows that 27.14% of the variability is found between populations and 37.44% within the populations, suggesting the existence of genetic variability that may be related to the reproductive strategies adopted by the species throughout its evolution. The index of variation within the provenances (93.36%) was higher than the index found among populations (6.64%). Molecular analysis indicated that there is genetic divergence between and within the studied populations of Anacardium humile A. St. - Hill. The origin of Itajá-GO presented the highest genetic diversity, presenting the highest values of genetic diversity index, phenotypic diversity and higher percentage of polymorphic loci.
Index terms: genetic variability, diversity, native species, Cerrado.

Polimorfismo genético entre populações naturais de *Anacardium humile* A. ST-HIL

Resumo - O gênero *Anacardium* apresenta nove espécies, das quais, três possuem porte subarbustivo, comuns no Cerrado do Centro-Oeste Brasileiro (*A. humile, A. nanum e A. corymbosum*). Nos últimos anos, com a expansão da agricultura, observa-se diminuição das áreas de ocorrência, e além disso encontram-se poucos trabalhos visando à domesticação da espécie. O objetivo deste trabalho foi avaliar a variabilidade genética da espécie, coletadas em onze procedências, utilizando marcadores RAPD. O DNA genômico de 122 acessos foi extraído e amplificado com 25 iniciadores decâmeros. Os resultados indicaram polimorfismo, que variou de 77,71% a 96,18%. A distribuição da diversidade genética entre e dentro das populações demonstra que 27,14% da variabilidade encontram-se entre populações e 37,44% dentro das populações, sugerindo a existência de variabilidade genética que pode estar relacionada às estratégias reprodutivas adotadas pela espécie ao longo de sua evolução. O índice de variação dentro das procedências (93,36%) foi superior ao índice encontrado entre populações (6,64%). A análise molecular indicou que há divergência genética entre e dentro das populações estudadas de Anacardium humile A. St. - Hill. A origem de Itajá-GO, apresentou a maior diversidade genética, apresentando os maiores valores de índice de diversidade genética, diversidade fenotípica e maior porcentagem de locos polimórficos.
Termos para indexação: variabilidade genética, diversidade, espécies nativas, Cerrado.

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In Brazil, the genus Anacardium presents nine species, and of these, three are sub-shrub, common in Cerrado areas of the Brazilian Center-West (*A. humile, A. nanum* and *A. corymbosum*) (SILVA-LUZ and PIRANI, 2010). O *Anacardium humile* is popularly known as cajuzinho-do-cerrado or cajuí, and stands out due to the exotic flavor of its false fruit, presenting medicinal and alimentary potential, being able to be included in the group of tropical fruit trees and antioxidant (ROCHA et al. 2013). The species occurs frequently in a dirty field and Cerrado stricto sensu (SILVA-LUZ and PIRANI, 2010); RESSEL et al., 2015).

The cajuzinho-do-cerrado presents high genetic divergence, ranging from the size to the fruit (Brown and Wier, 1983). Studies aiming at the knowledge of genetic variability, seeking to identify provenances and/ or environments that have accesses with high genetic divergence may generate information for future work on their commercial potential.

According to Lacerda and Kageyama (2003), the establishment of strategies for genetic conservation of a species includes the study of the distribution of genetic variability among and within their populations.

To assist in the characterization of genetic variations of populations there are molecular techniques, which associated with statistical analyzes can provide important information, such as genetic similarity and dissimilarity. Among these, the molecular markers that provide a more specific approach to each individual and are much more efficient for the determination of genetic variability, by directly analyzing the genetic material, when compared to the use of the morphological characteristics (YANAKA et al., 2005).

In recent studies on fruit species and have generated interesting results with: cagaita, fava d'anta (OLIVEIRA, 2008), pitayas (JUNQUEIRA et al., 2010), and coconut (RAJESH et al., 2014). Knowledge of the genetic parameters of a species assists in the targeting of breeding, management and genetic conservation programs.

Considering the importance of knowing the genetic diversity of this fruit tree of the Cerrado biome, the present study evaluated the genetic variability of the species *Anacardium humile* A. ST-HIL. in eleven provenances.

Newly mature leaves were collected from 122 accessions from 10 sources from the state of Goiás (Aporé, Caiapônia, Chapadão do Céu, Doverlândia, Itajá, Itarumã, Jataí, Mineiros, Santo Antônio do Descoberto and Serranópolis) and 01 from the state of Mato Grosso (Barra do Garça), Brazil (Table 1). The leaves were stored in freezer (-20°C) for better preservation. The genomic DNA was extracted using the protocol of Faleiro et al. (2003), with modifications in the concentration of betamercaptanol, with 10 times more and with the time of immersion in water bath reduced to half. After extraction, the total DNA of each plant was quantified by spectrophotometry. Then the stock solution of the DNA was diluted to the final working concentration of approximately 5ng µL^{-1}.

The amplifications were done in a thermal cycler programmed according to Barros et. al. (2005), for 40 cycles, each consisting of the following sequence: 15 seconds at 94ºC, 30 seconds at 35ºC and 90 seconds at 72ºC. After completion of the 40 cycles, a six minute extension step was performed at 72ºC and reduction to 4ºC. After the PCR reaction, 3 µL of the mixture of bromophenol blue (0.25% w / v), glycerol (60% v / v) in water was added to each sample (with the purpose of assisting the visualization of the Progress of the DNA race in electrophoresis). The total volume of each sample was applied on agarose gel (1.2%), stained with ethidium bromide, submerged in TBE buffer (90 mM Tris-Borate, 1 mM EDTA, and ethidium bromide). The electrophoretic separation was carried out for 3 hours at 90 volts. At the end of each electrophoresis, the gels were photographed under ultraviolet light.

The generated RAPD markers were converted into a binary data matrix, from which the genetic distances between the 122 accessions were estimated based on the complement of the Nei e Li similarity coefficient (NEI e LI, 1979). The genetic distance matrix was used for cluster analysis by the Tocher method, and with the help of the GENES program (CRUZ, 2008).

The characterization of the genetic variability was done with the help of the Popgene software (Young et al. 2000), being carried out the analysis between and inside provenances of cajuzinho-do-cerrado. The number of alleles observed (n), number of effective alleles (ne), Shannon index (Ĥ0), Nei (1973) gene diversity (Ĥe) and percentage of polymorphic loci (P95% = number Of polymorphic loci / total number of loci). For the P95% values, a 95% probability criterion was adopted where a locus is considered polymorphic if the frequency of the most common allele did not exceed 95% (MARTINS-CORDER et al., 2009). The total heterozygosity (Ht), mean heterozygosity within (Hs), population differentiation coefficient (Gst) and allelic flux (Nm) were also estimated (OLIVEIRA et al., 2008).

Genetic variability between and within populations was estimated using the GENES program (Cruz, 2008) using the genetic variance analysis (AMOVA). The AMOVA calculates the value Fst, which according to Excoffier et al. (1992) equals the proportion of the total genetic variation fractional among the provenances. Of the 25 primers (Table 2), 10 were selected for characterization of the genetic polymorphism between and within the provenances under study. A total of 146 bands with a mean of 14.6 bands per primer were obtained. The values estimated in this study (Table 2) ranged from 0.4135 to 0.5497, indicating that the provenances present moderate to low diversity.

Table 1. Accesses, provenances, and georeferenced data (DG) of the 122 accessions of *Anacardium humile* A. ST-HIL.

ACCESSES	PROVENANCES	DG
01, 02, 03, 04, 05, 06, 07, 08, 09 e 10 (10 accesses);	Mineiros-GO	Altitude: 704m S: 17. 0,66. 0,130 W: 52. 0,5. 0,271
11,12, 13,14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 e 25 (15 accesses);	Barra da Garça-MT	Altitude: 570m S: 15. 0,85. 0,072 W: 52. 0,25. 0,048
26, 27, 28, 29,30, 31,32, 33, 34, 35, 36, 37, 38, 39 e 40 (15 accesses);	Doverlândia-GO	Altitude: 618m S: 16.0,71.0,09 W: 52. 0,15.0,0125
41, 42, 43, 44, 45, 46, 47, 48, 49 e 50 (10 accesses);	Itarumã-GO	Altitude: 637m S: 18. 0,6. 0,0272 W: 51. 0,76. 0,093
51, 52, 53, 54, 55, 56, 57, 58 e 59 (10 accesses);	Itajá-GO	Altitude: 484m S: 18. 0,85. 0,225 W: 51. 0,53. 0,228
60, 61, 62, 63, 64, 65, 66, 67, 68 e 70 (10 accesses);	Chapadão do Céu-GO	Altitude: 809m S: 18. 0,216. 0,112 W: 52. 0,86.0,165
71, 72, 73, 74, 75, 76, 77, 78 e 79 (09 accesses);	Aporé-GO	Altitude: 681m S: 18. 0,783. 0,147 W: 52. 0,08. 0,182
80, 81a, 81b, 82, 83, 84, 85, 86, 87, 88, 89 (11 acessos);	Serranópolis-GO	Altitude: 577m S: 18. 0,35. 0,147 W: 52. 0,93. 0,075
91, 92, 93, 94, 95, 96, 97, 98, 99 e 100 (10 accesses);	Jataí-GO	Altitude:708m S: 17. 0,90. 0,0163 W: 51. 0,68. 0,256
101, 102, 103, 104, 105, 107, 108, 109 e 110 (09 accesses);	Caiapônia-GO	Altitude: 599m S: 16. 0,7. 0,0165 W: 52. 0,11. 0,0066
111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123 e 124 (14 accesses);	Santo Antônio do Descoberto-GO	Altitude: 1050m S: 15. 0,98. 0,00438 W: 48. 0,21.0,0150

Table 2. Number of total bands, number of polymorphic bands, percentage of polymorphism, average of bands per primers, expressive primers, less expressive primers, bootstrap and measure of heterogeneity between bands, using RAPD marker in the cajuzinho-do-cerrado, by provenances collection.

PROVENANCES	Ntb****	Npb	%P	Abp	EP	LEP	BStrap	B
Aporé-GO*	154	140	91	14	OPA11 e OPA08	OPM03	65	0,48
Barra do Garça-MT*	155	146	94	14,6	OPA11	OPA04	50	0,47
Caiapônia-GO*	136	132	97	13,2	OPA08 e OPA09	OPA04	45	0,46
Chapadão do Céu-GO*	155	144	93	14,4	OPA11	OPA04	70	0,47
Santo Antonio do Descoberto-GO*	148	146	99	14,6	OPA11	OPM03	140	0,43
Doverlândia-GO*	156	146	93	14,6	OPA11	OPA04	40	0,47
Itajá-GO*	153	141	92	14,1	OPA11 e OPA08	OPB05	70	0,47
Itarumã-GO*	153	143	93	14,3	OPA- 11	OPA04	130	0,43
Jataí-GO*	138	115	83	11,5	OPM02	OPA04	35	0,48
Mineiros-GO*	156	141	90	14,1	OPA08	OPM03	58	0,48
Serranópolis-GO*	153	148	97	14,8	OPA11	OPA04	118	0,45
Municípios**	146	134	92	13,4	OPA11 e OPA08	OPN05	85	0,46
All accesses***	157	156	99	15,6	OPA11	OPA18	90	0,37

* Analysis performed with access within each municipality; ** analysis performed among municipalities; *** analysis performed with all 122 accesses; **** Ntb = number of total bands; Npb = number of polymorphic bands; % P = percentage of polymorphism (or polymorphism rate); EP = expressive primers; LEP = less expressive primers; Abp = Average bands per primers, BStrap = bootstrap and b = measure of heterogeneity between bands.

The percentage of polymorphic loci (P 95%) (Table 3) varied from 77.71% in the Jataí origin to 96.18% in the Itajá source. It can be noted that only the origin of Jataí and Caiapônia did not present P95% higher than 90%. The distribution of the genetic diversity of Nei (Ĥe) between and within the provenances (Table3) ranged from 0.2714 (source of Caiapônia) to 0.3744 (source of Itajá). The Shannon index (Ĥ0) is estimated from the presence frequencies and band absences. The total genetic heterozygosity (HT) obtained can be considered to be relatively high (Table 4), presenting a small deviation, indicating value very close to the mean (0.3821 ± 0.0115) for most loci.The GST value found in this study among the provenances was 0.1167 (Table 4), indicating that 11.67% of the genetic divergence is between provenances and 88.33% within provenances.

The results of the analysis of molecular variance - AMOVA (Table 5) show the variation index within and among the populations of *Anacardium humile*. The index of variation within the provenances (93.36%) was higher than the index found among populations (6.64%), which although low, was highly significant. The results are in agreement with the expected ones in cerrado woody species, that is, considerable levels of genetic variability exist within their populations and with high levels of divergence. The Fst value (0.0664) observed (Table 6) shows moderate genetic differentiation.

In the cluster analysis between the 122 accessions of cajuzinho-do-cerrado, from the dissimilarity index of Nei e Li and the criterion of Tocher grouping (Table 6), five groups were obtained, four of which are constituted by only one access and a group with the other accesses.

Knowledge of the degree of genetic variability, through divergence studies, is fundamental in the process of identifying new sources of genes of interest. One of the ways to evaluate diversity is molecular characterization, which allows, from genetic marks, indifferent to environmental interference, to infer about the degree of diversity between individuals and between populations.

In the work of Gomes Filho et al. (2010), the RAPD markers were effective in investigating the diversity among guava tree accesses, indicating that the studied population has a wide genetic variability. This result is indicative of the lower genetic variability in these origins. For the others, the percentage of polymorphic loci (P95%) was close to the values found by Zimback et al. (2004). The number of alleles observed (na) varied from 1.7771 to 1.9618, while the number of effective alleles (ne) ranged from 1.4511 to 1.6615 among provenances.

The values of (Ĥe) found in this study can be considered superior to those found by Oliveira et al. (2008) and close to those of Zimback et al. (2004). Despite being a species with populations threatened by intensive agriculture, landscape fragmentation and extractivism, there is genetic variability, probably due to the reproductive strategies adopted by the species throughout its evolution. However, studies with reproduction biology of *A. humile* should be carried out in order to better understand the reproductive strategies, which allow for new genotypic combinations in the offspring.

The provenance that presented the greatest diversity among all the studied origins was that of Itajá. This

result was superior to that found in other species, which used similar methodologies (TOREZAN et al., 2005; OLIVEIRA et al., 2008).

Genetic heterozygosity is defined by Brown e Weir (1983) as being the most important measure to estimate genetic variability, which is less influenced by variations in sample size when compared to other variables. Which according to Zimback et al (2004), is indicative that the species presents a reasonable reserve of genetic variability. Similar values of HT were found by Zimbacka et al (2004) and Oliveira et al. (2008).

Genetic divergence (GST) is a statistical data that measures the genetic differentiation between populations, and has been widely used since it is not affected by the reproductive system of the species, by the number of alleles per locus and by the evolutionary action (Moura, 2005). Their values vary from 0 to 1, and according to Yeh (2000) values lower than 0.050 indicate low levels of genetic differentiation, in contrast values above 0.151, are an indication of great genetic differentiation This result is lower than that reported in the literature for other tropical allogamous species, which presented GST values of 0.125, 0.270, 0.281 and 0.285, respectively: Zimback et al. (2004), Wadt (2001) and Sales et al. (2001).

The value of the gene flow (Nm) observed was 3.78, which is considered moderate according to Wrigth (1931), who stated that values of gene flow less than 1 indicate genetic isolation. This same author states that gene flow value greater than 1 is sufficient to prevent random losses of alleles within populations (drift effects). According to Wright (1978), which indicates this classification to values

between 0.05 and 0.15. However, it can be inferred that the Fst value was not higher, due to the possible occurrence of pollination of pollen accesses of genetically close individuals (FRANZON et al., 2010).

In the cluster analysis between the 122 accessions of cajuzinho-do-cerrado, from the dissimilarity index of Nei and Li and the criterion of Tocher grouping, five groups were obtained, four of which are constituted by only one access And a group with the other accesses. This structure of grouping of accesses confirms the result of the analysis, which showed a greater genetic divergence within the provenances than among provenances, due to the fact that there was no formation of groups with accesses distributed by source. This result is similar to those obtained by Oliveira et al. (2008) and Franzon et al. (2010).

The accessions from Itajá origin were distributed in 60% of the groups formed, this can be justified because this provenance presented the highest values for the percentage of polymorphic loci (P 95%), for genetic diversity of Nei (Ĥe) and for the index Of Shannom of phenotypic diversity (Ĥ0), showing the provenance presents diversity and alleles that should be better studied. Molecular analysis indicated that there is genetic divergence between and within the studied populations of Anacardium humile A. St. - Hill (Figure 1). The origin of Itajá-GO presented the highest genetic diversity, presenting the highest values of genetic diversity index, phenotypic diversity and higher percentage of polymorphic loci.

Table 3. Genetic diversity of natural populations of *Anacardium humile* A. St.-Hil.

Prov*	P	na**	ne**	$\hat{H}_e$ **	$\hat{H}_0$ **
AP	90,45	1,9045 (0,2949)	1,5847 (0,3300)	0,3365 (0,1598)	0,4979 (0,213)
BG	92,99	1,9299 (0,2561)	1,6469 (0,3114)	0,3658 (0,1467)	0,5353 (0,1939)
C	84,08	1,8408 (0,3671)	1,4511 (0,3394)	0,2714 (0,1726)	0,4135 (0,2356)
CC	92,36	1,9236 (0,2665)	1,5857 (0,3187)	0,3400 (0,1501)	0,5052 (0,1973)
D	91,08	1,9108 (0,2859)	1,6615 (0,316)	0,3701 (0,1509)	0,5384 (0,2022)
Itj	96,18	1,9618 (0,1923)	1,6579 (0,2859)	0,3747 (0,1314)	0,5497 (0,1687)
Ita	93,63	1,9363 (0,245)	1,5656 (0,329)	0,3299 (0,1529)	0,4937 (0,1977)
J	77,71	1,7771 (0,4175)	1,5431 (0,3827)	0,3046 (0,1947)	0,4452 (0,2696)
M	91,08	1,9108 (0,2859)	1,6067 (0,3256)	0,3468 (0,1558)	0,5111 (0,207)
SAD	92,99	1,9299 (0,2561)	1,5183 (0,2952)	0,3147 (0,1412)	0,4776 (0,1862)
S	95,54	1,9554 (0,2071)	1,6192 (0,2977)	0,3581 (0,1358)	0,5301 (0,1741)
Total	100	2 (0)	1,6674 (0,2573)	0,3842 (0,1065)	0,5662 (0,1245)

* Prov. = Provenance: Aporé (Ap), Barra do Garça (BG), Caiapônia (C), Chapadão do Céu (CC), Doverlândia (D), Itajá (Itj), Itarumã (Ita), Jataí (J), Mineiros (M), Santo Antônio do Descoberto (SAD), Serranópolis (S). (): standard deviation; P: percentage of polymorphic loci; Na **: number of alleles observed; Ne **: number of effective alleles; Ĥe **: genetic diversity of Nei; Ĥ0 **: Shannon index of phenotypic diversity.

Table 4. Population genetic parameters of *Anacardium humile* A. St.-Hill.

	H_T	H_S	G_{ST}	Nm*
Average	0,3821	0,3375	0,1167	3,7852
Standard deviation	0,0115	0,0093		

HT: total heterozygosity; HS: mean genetic diversity within; GST: gene divergence among populations; and Nm: gene flow.

Table 5. Molecular variance analysis (AMOVA) of populations of *A. humile* A. St.-Hill.

SV	DF	SS	MS	Porcentage	P
Entre	10	498.9744	49,8974	6.639	<0,001
Dentro	121	3101.0082	27,9372	93.360	<0,001
Total	131	3600.0082	29,7521		
F_{st}	0,0664**				

* SV = source of variation; DF = degree of freedom; SS = sum of squares; MS = middle-square; Fst = proportion of fractional total genetic variation among populations; ** significant at the 1% probability of error level.
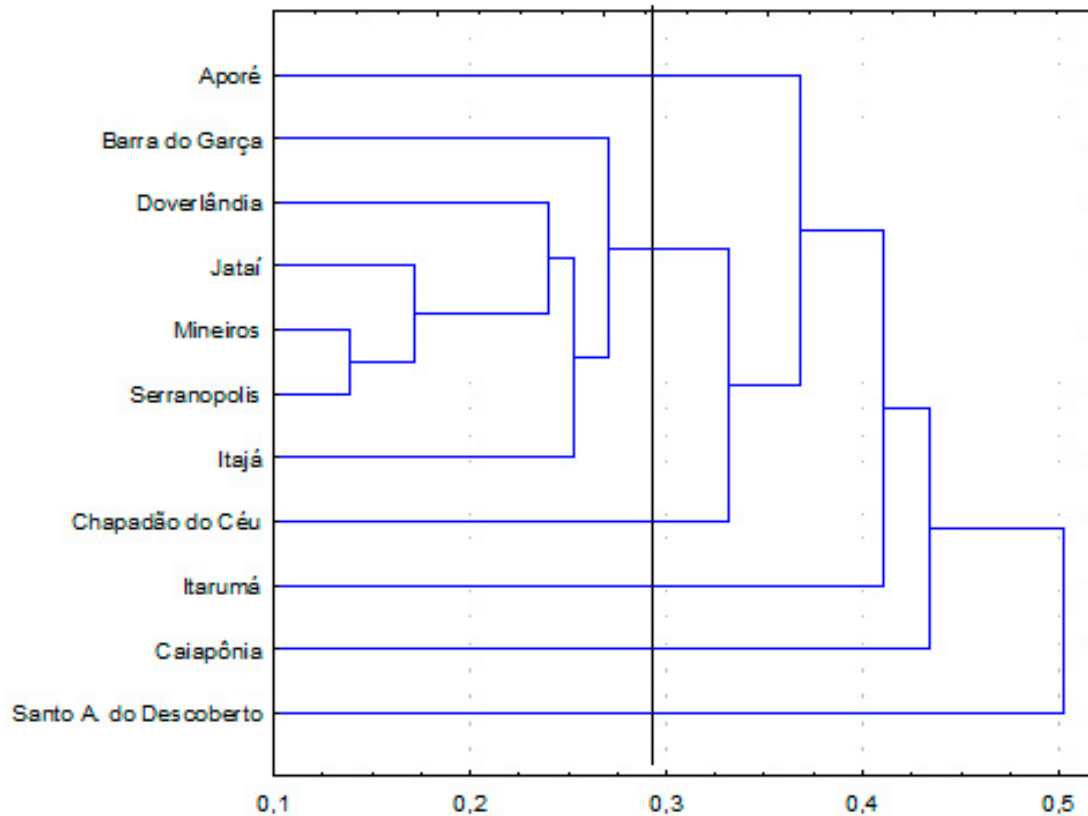

Figura 1. Dendrogram of genetic dissimilarities, from RAPD, among 11 provenances of Cajuzinho-do-Cerrado obtained by UPGMA method based on DNA marker matrix (dg = 0.37).

Table 6. Grouping by the Tocher method between accesses of *A. humile* A. St.-Hill. based on the distance matrix generated from molecular data analysis.

Group	Accesses
I	2_{Ap*} 113_S 34_{CC} 11_{BG} 60_D 103_M 10_{BG} 102_M 112_M 3_{Ap} 35_{CC} 1_{Ap} 74_{Itj} 104_M 61_D 75_{Itj} 58_D 22_{BG} 63_D 70_D 59_D 13_{BG} 85_{Ita} 14_{BG} 73_{Itj} 84_{Itj} 116_S 105_M 86_{Ita} 62_D 68_D 119_S 23_{BG} 71_D 41_{CC} 40_{CC} 88_{Ita} 19_{BG} 20_{BG} 21_{BG} 46_{SAD} 107_M 82_{Ita} 4_{Ap} 64_D 91_{Ita} 66_D 101_J 5_{Ap} 7_{Ap} 111_M 108_M 24_{BG} 48_{SAD} 26_C 100_J 97_J 95_J 93_J 79_{Itj} 81_{Itj} 89_{Ita} 69_D 80_{Itj} 44_{SAD} 12_{BG} 78_{Itj} 57_{SAD} 9_{Ap} 122_S 110_M 92_J 94_J 67_D $115_{}$ 36_{CC} 65_D 37_{CC} 47_{SAD} 98_J 109_M 50_{SAD} 72_D 39_{CC} 29_C 76_{Itj} 15_{BG} 18_{BG} 106_M 96_J 120_S 28_C 38_{CC} 42_{CC} 17_{BG} 77_{Itj} 31_C 53_{SAD} 6_{Ap} 8_{Ap} 99_J 121_S 52_{SAD} 30_C 43_{CC} 25_C 118_S 56_{SAD} 90_{Ita} 27_C 51_{SAD} 45_{SAD} 33_C 16_{BG} 117_S 32_C 49_{SAD} 55_{SAD}
II	114_S
III	87_{Ita}
IV	83_{Ita}
V	54_{SAD}

*Provenance: Aporé (Ap), Barra do Garça (BG), Caiapônia (C), Chapadão do Céu (CC), Doverlândia (D), Itajá (Itj), Itarumã (Ita), Jataí (J), Mineiros (M), Santo Antônio do Descoberto (SAD), Serranópolis (S).

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