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Genetic variability among cashew hybrids and prediction of superior combinations based on agronomic performance

Abstract – The objective of this work was to evaluate the use of RAPD and ISSR molecular markers to determine the genetic variability among cashew (*Anacardium* spp.) genotypes, and to indicate possible promising crosses based on cashew genetic variability and phenotypic performance. Ten hybrids – derived from the crosses CCP 76 x BGC 589, CCP 76 x BRS 226, CCP 76 x HAC 276-1, CCP 76 x Embrapa 51, CCP 76 x BRS 253, CCP 76 x HAC222-4, and BRS226 x Embrapa 51 – and their parents were assessed at the molecular level. The hybrids were evaluated for nut yield, mean nut weight, bored nuts, and powdery mildew on nuts (scale 0–4). The RAPD and ISSR markers were efficient in the determinaton of the genetic variability among cashew genotypes, allowing of the grouping of 21 clusters. Associated with the phenotypic characterization of cashew nut for yield, weight, and health, the used markers can efficiently identify possible combinations with higher genetic variability and higher probability of developing transgressive genotypes in segregating populations.

Index terms: *Anacardium*, cashew breeding program, ISSR markers, nut health, RAPD markers.

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Variabilidade genética entre híbridos de caju e predição de combinações superiores com base no desempenho agronômico

Resumo – O objetivo deste trabalho foi avaliar o uso de marcadores moleculares RAPD e ISSR para determinar a variabilidade genética entre genótipos de cajueiro (*Anacardium* spp.) e indicar possíveis cruzamentos promissores com base na variabilidade genética e no desempenho fenotípico do cajueiro. Foram avaliados, em nível molecular, dez híbridos – originados dos cruzamentos CCP 76 x BGC 589, CCP 76 x BRS 226, CCP 76 x HAC 276-1, CCP 76 x Embrapa 51, CCP 76 x BRS 253, CCP 76 x HAC222-4 e BRS226 x Embrapa 51 – e seus respectivos genitores. Os híbridos foram avaliados quanto à produção de castanha, à massa média da castanha, à incidência de castanhas furadas e à incidência de oídio na castanha (escala 0–4). Os marcadores RAPD e ISSR foram eficientes em determinar a variabilidade genética entre esses genótipos de cajueiro, tendo permitido o agrupamento de 21 grupos. Associados à caracterização fenotípica da castanha de caju quanto à produtividade, à massa e à sanidade da castanha, os marcadores utilizados são eficientes para identificar possíveis combinações capazes de proporcionar maior variabilidade genética e maior probabilidade de obtenção de genótipos transgressivos em populações segregantes.

Termos para indexação: *Anacardium*, programa de melhoramento do cajueiro, marcadores ISSR, sanidade da castanha, marcadores RAPD.

Introduction

The importance of planting early dwarf cashew clones for commercial production is their yield potential – exceeding 1,300 kg ha^{-1} cashew nut under rainfed cultivation – which is far higher than the 250 kg ha^{-1} produced by common cashew trees (Martins Junior et al., 2008). The genetically superior dwarf clones are planted to facilitate an optimized crop management, which results in significant increases of nut and pseudofruit yield and quality, increasing the profitability of the activity.

As described by Vidal Neto et al. (2013), breeding populations that initially gave rise to the first early dwarf commercial clones in Brazil derived from crosses among a small number of introduced plants. However, to broaden the genetic base, many other genotypes were introduced, which currently constitute the germplasm base of the Cashew Breeding Program of Embrapa. The expansion of the genetic base is a constant concern in plant breeding, since a narrow genetic base can threaten future genetic gains by selection and increase the potential risk of genetic vulnerability of crops (Carvalho et al., 2008). Thus, the evaluation of genetic distances among elite genotypes, based on the detection of polymorphisms by molecular markers, can generate important information with a view to the broadening of genetic variability by crosses (Colombari Filho et al., 2010). This information is useful for the choice of parents and their combinations to increase heterozygosity and heterotic effect on progenies and, consequently, raise the probability of identifying transgressive genotypes (Dutra Filho et al., 2013).

For molecular-level studies of species such as cashew, for which less information is available than for other model species, the entire plant genome is usually assessed with random markers such as the random amplified polymorphic DNA (RAPD), and inter simple sequence repeat (ISSR) markers. These techniques increase the chances of identifying polymorphisms and, thus, enable the quantification of genetic variability in improved populations.

In cashew, RAPD and ISSR markers have been used mainly for the evaluation of genetic diversity among accessions in germplasm banks and breeding programs (Thimmappaiah et al., 2009; Asolkar et al., 2011; Shobha & Thimmappaiah, 2011; Dasmohapatra et al., 2014; Sethi et al., 2016; Thimmappaiah; et al., 2016). Nevertheless, the assessment of variability at the DNA level by random molecular markers is not a guarantee that crosses between genetically distinct plants will produce superior progenies, since the per se performance of the parents is not taken into account.

Cashew nut yield and mean cashew nut weight are primary targets of selection in cashew nut breeding, while nut health is essential as well (Melo et al., 2018). In this sense, since 2012, powdery mildew (*Pseudoidium anacardii*) is being regarded as a primary disease in commercial cashew orchards in Brazil, as this microorganism significantly damages the reproductive structures of commercial value (Freire, 2012). Therefore, the phenotypic characterization for the main traits of agronomic importance, including the reaction to the main diseases and pests, is essential to support decision-making, underlying the determination of combinations between genetically distinct and phenotypically superior plants, to increase the chances of developing transgressive genotypes.

The objective of this work was to evaluate the use of RAPD and ISSR molecular markers to determine the genetic variability among cashew genotypes, and to indicate possible promising crosses based on cashew genetic variability and phenotypic performance.

Materials and Methods

The experiment was installed in 2007 in the experimental field of Pacajus, of Embrapa Agroindústria Tropical, in the municipality of Pacajus (4º11'07"S, 38º30'07"W, at 70 m altitude), in the state of Ceará, Brazil. The experiment was arranged in a randomized complete block design with four replicates of four plants each. According to Santos et al. (2018), the soil is predominantly an Argissolo Vermelho-Amarelo, dystrophic, moderate A (Ultisol). The regional climate is sub-humid (dry), with annual means of 737 mm rainfall (Funceme, 2017) and between 26 and 28ºC temperature (Ipece, 2017).

Cashew (*Anacardium* spp.) plants were grown without irrigation in rows spaced at 8 m, and plants spaced 6 m apart, at a density of 208 plants per hectare. The analysis prior to planting indicated the following composition of the soil at 0–20 and 20–40 cm soil depths, respectively: 1.9 and 1.5 mg kg^{-1} P; 1.9 and 1.4 $mmol_c$ kg^{-1} K; 4.4 and 4.9 $mmol_c$ kg^{-1} Ca; 3.5 and 5.2 $mmol_c$ kg^{-1} Mg; 2.9 and 2.9 g kg^{-1} organic matter; and 1.0 and 2.6 $mmol_c$ kg^{-1} Al. The soil was fertilized

based on a yield expectation between 1,200 and 3,000 kg ha^{-1} (production stage), according to technical recommendations for cashew cultivation described by Oliveira & Costa (2005) and Oliveira et al. (2002). Other measures related to crop protection and cultivation were also applied as proposed by these authors.

The evaluated F_1 cashew hybrids derived from the seven following crosses: cultivar CCP 76 (*Anacardium occidentale*) and accession BGC 589 (*Anacardium microcarpum*); cultivars CCP 76 and BRS 226 (*A. occidentale*); cultivar CCP 76 and selection HAC 276-1 (*A. occidentale*); cultivars CCP 76 and Embrapa 51 (*A. occidentale*); cultivars CCP 76 and BRS 253 (*A. occidentale*); cultivar CCP 76 and genotype HAC 222-4 (*A. occidentale*), and between cultivars BRS 226 and Embrapa 51. From a total of 16 hybrids, the 10 most productive ones were pre-selected in the 2012 growing season, when plants reached the biological stage of production. The resulting 70 genotypes, represented by 10 F_1 hybrids per progeny, were evaluated based on the phenotypic performance and molecular analysis. These hybrids were identified based on their genealogy, listed from 1 to 10 (Table 1).

Cashew nut yield (in kg ha^{-1}) of each hybrid was evaluated in six growing seasons, from 2010 to 2015, based on the total cashew nut yield per growing season. In each growing season, the cashew harvest period, that lasts from the beginning of September to the first half of January, was structured in three sub-periods of around 50 days; and the cashew nut yield of each plant was calculated as the sum of the three partial yields. Mean nut weight (in g) was determined by weighing three samples of 20 healthy nuts per hybrid taken from the total amount produced per plant, and then calculating the arithmetic mean. Additionally, at the end of the harvest period, attacked nuts (%) by *Anacampsis phytomiella* was determined by counting the nuts with holes in a sample of 100 nuts randomly collected from the total amount of harvested ones. Based on the total amount of nuts harvested per hybrid, the incidence of powdery mildew (*Pseudoidium anacardii*) was evaluated on a descriptive scale of disease severity (scores from 0 to 4) adapted from Cardoso et al. (1999). Scores 0–4 were attributed as follows: 0, absence of symptoms; 1, presence of lesions covering up to 25% of cashew nut surface; 2, lesions covering 25 to 50%; 3, to lesions covering 50 to 75%; and 4, lesions covering 75 to 100% of the nut surface. The incidence of powdery mildew on nuts was evaluated once at the end of four growing seasons, covering the period from 2012 to 2015.

The performance data of each hybrid for each variable, throughout the growing seasons, were subjected to descriptive statistics, considering the arithmetic mean and standard deviation. Based on this, the genotypes were classified as superior or inferior in relation to the overall trait mean, plus or minus the standard deviation, respectively. These statistical

Table 1. Cashew (*Anacardium* spp.) crosses of parents and respective F_1 hybrids evaluated by molecular analyses.

Cross	Hybrid	Cross	Hybrid
Cultivar CCP 76 X Accession BGC 589	CCP76xBGC589_1	Cultivar CCP 76 X Cultivar BRS 253	CCP76xBRS253_1
	CCP76xBGC589_2		CCP76xBRS253_2
	CCP76xBGC589_3		CCP76xBRS253_3
	CCP76xBGC589_4		CCP76xBRS253_4
	CCP76xBGC589_5		CCP76xBRS253_5
	CCP76xBGC589_6		CCP76xBRS253_6
	CCP76xBGC589_7		CCP76xBRS253_7
	CCP76xBGC589_8		CCP76xBRS253_8
	CCP76xBGC589_9		CCP76xBRS253_9
	CCP76xBGC589_10		CCP76xBRS253_10
Cultivar CCP 76 X Cultivar BRS 226	CCP76xBRS226_1	Cultivar BRS 226 X Cultivar Embrapa 51	BRS226xEmbrapa51_1
	CCP76xBRS226_2		BRS226xEmbrapa51_2
	CCP76xBRS226_3		BRS226xEmbrapa51_3
	CCP76xBRS226_4		BRS226xEmbrapa51_4
	CCP76xBRS226_5		BRS226xEmbrapa51_5
	CCP76xBRS226_6		BRS226xEmbrapa51_6
	CCP76xBRS226_7		BRS226xEmbrapa51_7
	CCP76xBRS226_8		BRS226xEmbrapa51_8
	CCP76xBRS226_9		BRS226xEmbrapa51_9
	CCP76xBRS226_10		BRS226xEmbrapa51_10
Cultivar CCP 76 X Selection HAC 276/1	CCP76xHAC276/1_1	Cultivar CCP 76 X Genotype HAC 222/4	CCP76xHAC222/4_1
	CCP76xHAC276/1_2		CCP76xHAC222/4_2
	CCP76xHAC276/1_3		CCP76xHAC222/4_3
	CCP76xHAC276/1_4		CCP76xHAC222/4_4
	CCP76xHAC276/1_5		CCP76xHAC222/4_5
	CCP76xHAC276/1_6		CCP76xHAC222/4_6
	CCP76xHAC276/1_7		CCP76xHAC222/4_7
	CCP76xHAC276/1_8		CCP76xHAC222/4_8
	CCP76xHAC276/1_9		CCP76xHAC222/4_9
	CCP76xHAC276/1_10		CCP76xHAC222/4_10
Cultivar CCP 76 X Cultivar Embrapa 51	CCP76xEmbrapa51_1		
	CCP76xEmbrapa51_2		
	CCP76xEmbrapa51_3		
	CCP76xEmbrapa51_4		
	CCP76xEmbrapa51_5		
	CCP76xEmbrapa51_6		
	CCP76xEmbrapa51_7		
	CCP76xEmbrapa51_8		
	CCP76xEmbrapa51_9		
	CCP76xEmbrapa51_10		

analyses evaluated the data for the cashew nut traits yield, mean weight, and bored nuts of six growing seasons (from 2010 to 2015), and for powdery mildew on nuts of four growing seasons (from 2012 to 2015).

Molecular analyses were carried out at the molecular biology laboratory of Embrapa Agroindústria Tropical, in the municipality of Fortaleza, in the state of Ceará, Brazil. Hybrid parents were included in these analyses, resulting in a total of 77 genotypes (Table 1). The DNA of healthy young leaves was extracted by the CTAB method modified by Cavalcanti & Wilkinson (2007). The genotypes were evaluated for genetic variability based on RAPD and ISSR primers.

Reactions with the RAPD primers were performed using 1X reaction buffer; 2.0 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} dNTPs, 0.3 µmol L^{-1} RAPD primer, 1 U Taq DNA polymerase, 20 ng DNA, and ultrapure water, to complete 13 uL. Fifty RAPD primers were tested. The amplification reactions occurred in a thermal cycler (Techne TC 512, Burlington, NJ, USA), under the following conditions: 5 min at 94ºC (initial denaturation), followed by 40 cycles with a denaturation step (94ºC for 1 min), annealing (35ºC, 1 min), extension (72°C, 1 min), and a final extension step (72°C, 5 min), maintaining 4°C at the end of the amplification. The amplified products were separated on 1.5% agarose gel with ethidium bromide-stained TBE 1X buffer (0.5 µg mL^{-1} gel). Electrophoresis was performed in a horizontal apparatus. The gels were visualized under UV light, and photographed for digital photo documentation (Loccus L-PIX CHEM, Cotia, SP, Brazil).

Primer reactions by ISSR were performed using 1X reaction buffer, 2.0 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} dNTPs, 0.8 µmol L^{-1} ISSR primer, 1 U Taq DNA polymerase, 20 ng DNA, and ultrapure water to complete 16 uL. Thirty ISSR primers were tested to optimize the annealing temperature. The base annealing temperature (Ta) was calculated based on the content of nitrogenous bases of each primer (adenine, A; thymine, T; cytosine, C; and guanine, G): $Ta_{calculated} = [2\ (A+T) + 4\ (C+G)]$ (Kibbe, 2007). Five variations around the calculated annealing temperature were adopted: 2ºC; -1ºC; $Ta_{calculated}$; +1ºC; and +2ºC. The amplification reactions were performed in a thermal cycler (Applied Biosystems Veriti, Foster City, CA, USA) under the following conditions: 5 min at 94°C (initial denaturation), followed by 40 cycles of denaturation (94°C for 1 min); annealing (42-60°C, 1 min); and extension (72°C, 1 min), followed by a final extension step (72°C, 5 min), maintaining 4°C at the end of the amplification. The amplified products were separated on 1.8% agarose gel with ethidium bromide-stained TBE 1X (0.5 µg mL^{-1} gel) buffer. Electrophoresis was performed in a horizontal apparatus. Gels were visualized under UV light and photographed for digital photo documentation (Loccus L-PIX CHEM, Cotia, SP, Brazil).

The best polymorphic bands generated by the primers were rigorously selected for analysis (Table 2). The genetic matrix was constructed based on the markers generated by the set of RAPD and ISSR primers, by assigning 1 to bands present in the plants, and 0 to missing bands. Then, the genetic similarity matrix (S) was constructed, using the Jaccard coefficient (J), calculated by $S_J = a/(a + b + c)$, in which: 'a' corresponds to the presence of the same band in both plants 1 and 2; 'b' to the presence of the band in plant 1, and absence in plant 2; and 'c' to the absence of the band in plant 1, and presence in plant 2.

In order to assess the genetic variability among plants, the optimal number of required markers was calculated to check the suitability of the set of markers (RAPD + ISSR). The correlation, sum of squared deviations, and the stress value between the original matrix and samples were used to evaluate the optimal number of markers (Dias, 1998). Likewise, the binary matrix generated by RAPD and ISSR markers was used to partition the variance in components among and within populations (hierarchical levels), considering each of the 10 hybrids per progeny as a distinct population. Analysis of molecular variance was performed according to the methodology of Excoffier et al. (1992), with the software Genes (Cruz, 2013). Based on the genetic similarity matrix, the dendrogram was constructed using UPGMA grouping and the clusters grouped based on mean similarity, validated by the cophenetic correlation (Sokal & Rohlf, 1962) and examined for significance by the Mantel test (Mantel, 1967). These analyses were performed using the software NTSYS (Rohlf, 2000).

Results and Discussion

The tests were initially performed for 50 RAPD and 30 ISSR primers, out of which 21 RAPD and 20 ISSR

Table 2. Characteristics and efficiency of 21 RAPD primers (random amplified polymorphic DNA) and 20 ISSR primers (inter simple sequence repeat) in the analysis of cashew (*Anacardium* spp.) genotypes.

Identification of primer	Sequence 5' – 3'	Annealing temperature (°C)	Total markers	Total polymorphic markers	Percentage of polymorphism[1] (%)
			RAPD primers		
OPA-02	TGCCGAGCTG	35.0	18	8	44.44
OPA-07	GAAACGGGTG	35.0	21	8	38.10
OPA-08	GTGACGTAGG	35.0	9	2	22.22
OPA-09	GGG TAA CGCC	35.0	15	7	46.67
OPB-10	CTGCTGGGAC	35.0	13	5	38.46
OPB-20	GGACCCTTAC	35.0	16	7	43.75
OPC-20	ACTTCGCCAC	35.0	14	4	28.57
OPD-02	GGACCCAACC	35.0	17	6	35.29
OPD-20	ACCCGGTCAC	35.0	12	4	33.33
OPE-07	AGATGCAGCC	35.0	9	3	33.33
OPF-12	ACGGTACCAG	35.0	17	8	47.06
OPF-15	CCAGTACTCC	35.0	18	6	33.33
OPG-02	GGCACTGAGG	35.0	7	2	28.57
OPN-05	ACTGAACGCC	35.0	18	5	27.78
OPN-06	GAGACGCACA	35.0	12	4	33.33
OPS-11	AGTCGGGTGG	35.0	13	4	30.77
UBC-305	GCTGGTACCC	35.0	10	3	30.00
UBC-308	AGCGGCTAGG	35.0	10	3	30.00
UBC-318	CGGAGAGCGA	35.0	13	3	23.08
UBC-322	GCCGCTACTA	35.0	17	5	29.41
UBC-341	CTGGGGCCGT	35.0	16	6	37.50
Total (joint performance for RAPD primers)		-	295	103	34.04
			ISSR primer		
I01-$(GACA)_4$	GACAGACAGACAGACA	45.0	19	11	57.89
I02-$(GAAGTGGG)_2$	GAAGTGGGGAAGTGGG	47.0	16	7	43.75
I03-$(GTG)_6$	GTGGTGGTGGTGGTGGTG	60.0	14	5	35.71
I04-$(GTG)_4$	GTGGTGGTGGTG	40.0	16	7	43.75
I05-$(TCC)_5$	TCCTCCTCCTCCTCC	46.5	8	3	37.50
I08-$(AGG)_6$	AGGAGGAGGAGGAGGAGG	56.0	15	5	33.33
I811-$(GA)_8$C	GAGAGAGAGAGAGAGAC	42.0	16	5	31.25
I816-$(CA)_8$T	CACACACACACACACAT	48.0	18	6	33.33
I818-$(CA)_8$G	CACACACACACACACAG	51.0	16	6	37.50
I825-$(AC)_8$T	ACACACACACACACACT	51.0	12	5	41.67
I826-$(AC)_8$C	ACACACACACACACACC	50.0	12	5	41.67
I834-$(AG)_8$YT	AGAGAGAGAGAGAGAGYT	49.0	18	5	27.78
I835-$(AG)_8$YC	AGAGAGAGAGAGAGAGYC	49.0	16	5	31.25
I840-$(GA)_8$YT	GAGAGAGAGAGAGAGAYT	44.0	22	9	40.91
I841-$(GA)_8$YC	GAGAGAGAGAGAGAGAYC	48.0	16	6	37.50
I842-$(GA)_8$YG	GAGAGAGAGAGAGAGAYG	49.0	10	3	30.00
I846-$(CA)_8$RT	CACACACACACACACART	49.0	16	6	37.50
I847-$(CA)_8$RC	CACACACACACACACARC	52.0	12	4	33.33
I848-$(CA)_8$RG	CACACACACACACACARG	52.0	18	8	44.44
I849-$(GT)_8$YA	GTGTGTGTGTGTGTGTYA	46.5	10	2	20.00
Total (combined performance for ISSR primers)		-	300	113	37.00

[1]Relation between the total number of clearly and continuously amplified bands with the primer, and respective number of the polymorphic bands considered. Y = C, or T; V = A, C, or G; R = A, or G; D = A, G, or T.

primers were considered in the evaluations (Table 2). The other primers were disregarded due to inconsistent reproducibility, or inefficient formation of fragments with adequate quantity, intensity, and clarity. Out of 295 RAPD-amplified fragments, 103 had satisfactory quality and polymorphism (34.91%), whereas 300 fragments were amplified by the 20 ISSR primers, from which 113 polymorphic bands were considered in the evaluation (37.00%). The RAPD and ISSR markers were similarly efficient to detect polymorphism in the studied cashew genotypes.

The number of markers used was considered appropriate to determine the genetic diversity, since the analysis indicated that using 196 polymorphic markers, the correlation with the genetic distance matrix of all bands was 0.950, the sum of squared deviations was 0.043, and the stress value was 0.034, suggesting that the series of markers used (295) was sufficient to determine stable associations among the sampled plants (Silveira et al., 2003).

Considering the genotyping of the 70 F_1 cashew hybrids, less than 10% of the detected genetic variability was due to genetic differences between populations (9.22% by RAPD, 8.08% by ISSR, and 8.62 by RAPD + ISSR) (Table 3), since more than 90% of the variability was detected within populations (90.78% by RAPD, 91.92% by ISSR, and 91.38% by RAPD + ISSR). The Fst values of 0.092 (RAPD), 0.081 (ISSR), and 0.0862 (RAPD + ISSR), respectively, suggested a moderate genetic divergence among the studied populations (Mwase et al., 2006). This pattern was possibly due to the fact that six of the seven populations had parent CCP 76 in common, and the parents that constituted the other population (BRS 226 and Embrapa 51) were also used before as parents in the crosses with CCP 76, increasing the genetic similarity among populations and narrowing the genetic base.

Moreover, since cashew (*Anacardium* spp.) has highly heterozygous plants for being predominantly allogamous (Asolkar et al., 2011), segregation in the first generation of hybrids is high, increasing the variability among plants and, consequently, the possibility of selection gains by breeding. This variability was observed in the field, since the hybrids differed widely for fruit characteristics (color, size, flavor, etc.), phenology, plant architecture (Vale et al., 2014), disease susceptibility (Hawerroth et al., 2017), and for other traits of interest (Tables 4 and 5). In this sense, the reproductive biology was suggested as one of the most important factors to determine the genetic structure of plant populations, and can explain the reported results. A similar performance was also observed in other allogamous species. For instance, in an evaluation of the genetic variability of allogamous sugarcane progenies, based on RAPD and EST-SSR markers, Dutra Filho et al. (2013) observed a higher variability within progenies (RAPD=87.75%, SSR=85.19%) than among ones (RAPD=12.25%, SSR=14.81%). Likewise, when assessing the genetic variability in the allogamous native crucifer species *Orychophragmus violaceus* based on ISSR markers, Zhang & Dai (2010) reported a genetic variance of 80.80% within and 16.43% among populations.

The similarity index for the set of 216 (RAPD + ISSR) markers varied between 0.37 and 0.79. Based on the mean similarity (sm=0.49), the 77 analyzed genotypes were separated in 21 distinct clusters, with a cophenetic coefficient of 0.87 (Figure 1). In an evaluation of 100 cashew accessions of the National Bank of Germplasm of India, Thimmappaiah et

Table 3. Analysis of molecular variance, considering 70 cashew (*Anacardium* spp.) genotypes (F_1 hybrids), evaluated on the basis of 103 molecular markers generated by 21 RAPD primers, and based on 113 molecular markers obtained from 20 ISSR primers.

Source of variation	DF	Mean square	Total variation (%)	F_{st}
RAPD markers				
Among populations	6	33.02	9.22	0.092
Within populations	63	16.38	90.78	
Total	69	17.82	100	
ISSR markers				
Among populations	6	34.96	8.08	0.081
Within populations	63	18.61	91.92	
Total	69	20.03	100	
Combined primers (RAPD + ISSR)				
Among populations	6	67.98	8.62	0.0862
Within populations	63	34.99	91.38	
Total	69	37.86	100	

F_{st}: genetic divergence between populations.

Table 4. Mean performance of cashew (*Anacardium* spp.) hybrids in relation to cashew nut yield (kg ha^{-1}) and mean nut weight (g), in the growing seasons from 2010 to 2015 (mean±standard deviation).

Hybrid (population)	Yield (kg ha^{-1})	Mean weight (g)	Hybrid (population)	Yield (kg ha^{-1})	Mean weight (g)
CCP76xBGC589_1	704.77±389.07	7.25±0.48 (I)	CCP76xBRS253_1	695.24±564.76	9.06±0.86 (S)
CCP76xBGC589_2	548.46±258.60	5.12±0.60 (I)	CCP76xBRS253_2	699.71±755.53	9.17±0.66 (S)
CCP76xBGC589_3	587.63±264.20	5.66±0.46 (I)	CCP76xBRS253_3	564.48±179.80	7.82±0.62
CCP76xBGC589_4	414.65±229.89	5.68±0.30 (I)	CCP76xBRS253_4	927.02±893.03	9.59±0.48 (S)
CCP76xBGC589_5	1,477.15±1,465.90	7.01±1.05 (I)	CCP76xBRS253_5	1,447.13±1,450.79	9.51±0.81 (S)
CCP76xBGC589_6	656.59±418.77	7.10±0.55 (I)	CCP76xBRS253_6	662.55±303.18	8.71±0.58
CCP76xBGC589_7	738.09±248.53	5.48±0.70 (I)	CCP76xBRS253_7	1,075.29±746.50	10.50±0.45 (S)
CCP76xBGC589_8	605.14±232.72	5.67±0.58 (I)	CCP76xBRS253_8	806.17±495.42	7.44±0.64 (I)
CCP76xBGC589_9	962.10±410.39	5.96±0.42 (I)	CCP76xBRS253_9	469.84±334.79	11.10±0.99 (S)
CCP76xBGC589_10	600.29±302.37	7.86±0.59	CCP76xBRS253_10	1,015.56±731.10	10.15±0.77 (S)
Population mean CCP76 x BGC589	729.49±422.05	6.28±0.58 (I)	Population mean CCP 76 x BRS 253	836.30±645.49	9.30±0.69 (S)
CCP76xBRS226_1	692.64±414.95	8.94±0.50	BRS226xEmbrapa51_1	1,259.47±684.89	7.82±0.43
CCP76xBRS226_2	1,228.90±856.27	9.05±0.89 (S)	BRS226xEmbrapa51_2	1,352.66±923.01	8.39±0.23
CCP76xBRS226_3	828.43±450.62	7.87±0.53	BRS226xEmbrapa51_3	1,151.42±655.40	9.30±0.43 (S)
CCP76xBRS226_4	671.46±333.39	7.92±1.07	BRS226xEmbrapa51_4	1,533.55±612.06	6.69±0.43 (I)
CCP76xBRS226_5	710.84±310.12	7.98±1.00	BRS226xEmbrapa51_5	1,006.69±445.79	9.00±0.81 (S)
CCP76xBRS226_6	960.47±379.85	5.89±0.57 (I)	BRS226xEmbrapa51_6	1,205.71±693.69	10.58±0.61 (S)
CCP76xBRS226_7	853.70±378.87	7.36±0.72 (I)	BRS226xEmbrapa51_7	1,017.02±888.34	7.30±0.78 (I)
CCP76xBRS226_8	778.41±349.82	9.74±0.83 (S)	BRS226xEmbrapa51_8	1,238.12±1,170.02	11.70±1.26 (S)
CCP76xBRS226_9	792.79±328.06	6.60±0.37 (I)	BRS226xEmbrapa51_9	955.73±736.30	13.85±0.91 (S)
CCP76xBRS226_10	631.38±248.47	7.45±0.55 (I)	BRS226xEmbrapa51_10	1,240.75±624.39	10.08±0.49 (S)
Population mean CCP 76 x BRS 226	814.90±405.04	7.88±0.70	Population mean BRS 226 x Embrapa 51	1,196.11±743.39	9.47±0.64 (S)
CCP76xHAC276/1_1	1,215.83±582.20	7.79±0.46	CCP76xHAC222/4_1	1,842.88±1,695.00 (S)	10.77±0.26 (S)
CCP76xHAC276/1_2	1,105.90±463.56	11.02±0.64 (S)	CCP76xHAC222/4_2	898.66±703.38	8.48±0.72
CCP76xHAC276/1_3	1,507.03±1,106.66	8.72±1.25	CCP76xHAC222/4_3	587.67±356.60	9.38±0.45 (S)
CCP76xHAC276/1_4	1,103.82±910.32	8.02±0.73	CCP76xHAC222/4_4	691.29±300.34	6.55±0.59 (I)
CCP76xHAC276/1_5	2,010.56±1,159.13(S)	9.51±0.77 (S)	CCP76xHAC222/4_5	1,359.14±836.19	10.12±0.42 (S)
CCP76xHAC276/1_6	1,511.71±633.78	7.99±0.72	CCP76xHAC222/4_6	472.13±225.41	7.20±0.75 (I)
CCP76xHAC276/1_7	819.59±412.96	8.94±0.60 (S)	CCP76xHAC222/4_7	689.83±414.29	9.36±0.28 (S)
CCP76xHAC276/1_8	607.46±269.36	6.68±0.25 (I)	CCP76xHAC222/4_8	823.51±313.57	6.19±0.60 (I)
CCP76xHAC276/1_9	617.97±330.07	7.99±0.82	CCP76xHAC222/4_9	435.10±193.07	7.32±0.35 (I)
CCP76xHAC276/1_10	1,411.94±778.12	10.33±1.19 (S)	CCP76xHAC222/4_10	698.46±537.32	11.11±0.42 (S)
Population mean CCP 76 x HAC 276/1	1,191.18±664.61	8.70±0.74	Population mean CCP 76 x HAC 222/4	849.87±557.52	8.65±0.48
CCP76xEmbrapa51_1	1,455.41±865.99	7.71±0.77 (I)	CCP76xEmbrapa51_6	1,744.43±983.12 (S)	9.05±0.56 (S)
CCP76xEmbrapa51_2	1,594.29±723.98	5.47±0.19 (I)	CCP76xEmbrapa51_7	2,502.93±1,685.29 (S)	7.03±0.28 (I)
CCP76xEmbrapa51_3	1,136.06±407.95	7.98±0.50	CCP76xEmbrapa51_8	1,636.89±953.40 (S)	6.49±0.39 (I)
CCP76xEmbrapa51_4	1,134.02±364.96	6.92±0.42 (I)	CCP76xEmbrapa51_9	1,048.74±420.30	10.89±0.82 (S)
CCP76xEmbrapa51_5	1,303.67±559.74	12.14±0.82 (S)	CCP76xEmbrapa51_10	1,171.59±662.61	8.33±0.21
Population mean CCP 76 x Embrapa 51	1,472.80±762.73	8.20±0.50	-	-	-
Mean ± SD	1,012.95±600.12	8.35±0.62	-	-	-

SD, standard deviation; S, higher than the overall mean plus one standard deviation; I, below the overall mean minus one standard deviation.

Table 5. Mean performance of cashew (*Anacardium* spp.) hybrids for percentage of bored nuts (%) in the growing seasons of 2010 to 2015, and occurrence of powdery mildew on harvested cashew nuts (scores 0–4) in the growing seasons of 2012 to 2015 (mean± standard deviation).

Hybrid (population)	Bored nuts (%)	Powdery mildew on nuts (score 1-4)	Hybrid (population)	Bored nuts (%)	Powdery mildew on nuts (score 0–4)
CCP76xBGC589_1	6.52±5.24	2.21±0.25 (I)	CCP76xBRS253_1	7.30±7.03	3.00±0.72
CCP76xBGC589_2	2.46±3.99	2.54±0.42	CCP76xBRS253_2	3.49±3.21	2.42±0.50
CCP76xBGC589_3	2.94±3.07	2.38±0.55	CCP76xBRS253_3	7.03±10.48	2.79±0.63
CCP76xBGC589_4	8.38±7.87	2.25±0.29	CCP76xBRS253_4	5.87±4.24	2.58±0.32
CCP76xBGC589_5	7.92±8.24	3.89±0.19 (S)	CCP76xBRS253_5	2.83±4.43	3.22±0.69 (S)
CCP76xBGC589_6	10.96±10.70	1.75±0.32 (I)	CCP76xBRS253_6	13.44±12.07	2.33±0.47
CCP76xBGC589_7	7.79±13.93	2.50±0.43	CCP76xBRS253_7	9.81±10.84	2.04±0.34 (I)
CCP76xBGC589_8	2.79±3.44	2.29±0.82	CCP76xBRS253_8	12.45±11.23	2.08±0.73 (I)
CCP76xBGC589_9	2.51±1.77	2.75±0.50	CCP76xBRS253_9	4.07±9.02	2.04±0.67 (I)
CCP76xBGC589_10	6.87±6.95	1.88±0.63 (I)	CCP76xBRS253_10	9.91±9.94	2.38±0.48
Population mean CCP76 x BGC589	5.92±6.52	2.44±0.44	Population mean CCP 76 x BRS 253	7.62±8.25	2.49±0.56
CCP76xBRS226_1	5.43±6.77	2.63±0.08	BRS226xEmbrapa51_1	9.40±9.42	2.83±0.19
CCP76xBRS226_2	15.11±9.28(S)	2.58±0.32	BRS226xEmbrapa51_2	2.78±3.84	2.63±0.64
CCP76xBRS226_3	10.94±11.06	2.50±0.43	BRS226xEmbrapa51_3	8.05±12.39	2.79±0.57
CCP76xBRS226_4	9.32±14.60	2.88±0.50	BRS226xEmbrapa51_4	7.23±6.19	1.71±0.21 (I)
CCP76xBRS226_5	6.43±5.06	1.83±0.19 (I)	BRS226xEmbrapa51_5	5.94±5.57	2.17±0.19 (I)
CCP76xBRS226_6	6.41±5.45	1.67±0.27 (I)	BRS226xEmbrapa51_6	2.39±3.58	1.50±0.58 (I)
CCP76xBRS226_7	3.42±3.31	2.54±0.71	BRS226xEmbrapa51_7	12.13±18.08	3.00±0.67
CCP76xBRS226_8	9.26±10.12	2.67±0.47	BRS226xEmbrapa51_8	6.93±11.71	2.96±0.67
CCP76xBRS226_9	8.05±9.87	2.42±0.50	BRS226xEmbrapa51_9	1.11±1.66	3.08±0.42
CCP76xBRS226_10	10.71±11.13	2.25±0.50	BRS226xEmbrapa51_10	1.36±2.23	2.88±0.50
Population mean CCP 76 x BRS 226	8.51±8.67	2.40±0.40	Population mean BRS 226 x Embrapa 51	5.73±7.47	2.55±0.46
CCP76xHAC276/1_1	10.21±13.72	3.04±0.75	CCP76xHAC222/4_1	12.28±10.25	3.04±0.52
CCP76xHAC276/1_2	13.48±13.88	2.50±0.43	CCP76xHAC222/4_2	18.02±10.60 (S)	3.54±0.42 (S)
CCP76xHAC276/1_3	13.14±17.04	2.00±0.72 (I)	CCP76xHAC222/4_3	6.16±6.60	3.25±0.69 (S)
CCP76xHAC276/1_4	8.43±8.90	2.71±0.86	CCP76xHAC222/4_4	4.62±5.78	3.58±0.29 (S)
CCP76xHAC276/1_5	11.60±7.99	3.13±0.37	CCP76xHAC222/4_5	2.69±2.84	3.58±0.50 (S)
CCP76xHAC276/1_6	7.26±8.90	3.38±0.28 (S)	CCP76xHAC222/4_6	1.50±2.21	3.54±0.42 (S)
CCP76xHAC276/1_7	12.33±10.62	3.21±0.60 (S)	CCP76xHAC222/4_7	6.46±6.23	2.92±0.17
CCP76xHAC276/1_8	3.11±4.80	2.92±0.44	CCP76xHAC222/4_8	6.84±6.33	2.00±0.41 (I)
CCP76xHAC276/1_9	8.63±11.07	1.63±0.28 (I)	CCP76xHAC222/4_9	3.98±4.57	3.08±0.63
CCP76xHAC276/1_10	7.22±9.23	3.33±0.62 (S)	CCP76xHAC222/4_10	2.10±3.04	2.50±0.58
Population mean CCP 76 x HAC 276/1	9.54±10.61	2.78±0.54	Population mean CCP 76 x HAC 222/4	6.46±5.85	3.10±0.46
CCP76xEmbrapa51_1	7.01±11.42	2.71±0.21	CCP76xEmbrapa51_6	7.15±5.61	3.29±0.34 (S)
CCP76xEmbrapa51_2	3.07±3.21	2.96±0.39	CCP76xEmbrapa51_7	3.93±4.47	2.79±0.50
CCP76xEmbrapa51_3	7.11±9.14	3.29±0.34 (S)	CCP76xEmbrapa51_8	3.86±4.21	2.83±0.43
CCP76xEmbrapa51_4	3.30±2.95	2.88±0.50	CCP76xEmbrapa51_9	2.68±3.59	3.13±0.25
CCP76xEmbrapa51_5	2.79±3.25	3.08±0.69	CCP76xEmbrapa51_10	1.22±1.88	2.83±0.45
Population mean CCP 76 x Embrapa 51	4.21±4.97	2.98±0.41	-	-	-
Mean ± SD	6.86±7.48	2.68±0.47	-	-	-

SD, standard deviation; S, higher than the overall mean plus one standard deviation; I, below the overall mean minus one standard deviation.

al. (2009) identified 13 clusters based on a set of 51 RAPD markers and 58 ISSR markers, with 0.43 to 0.87 similarity, and 0.66 mean similarity. In general, the performed crosses effectively amplified the genetic variability, since the 77 genotypes were allocated in 21 different groups. This performance was expected due to the peculiar reproductive characteristic of *Anacardium* (Asolkar et al., 2011), and the potential genetic gains by breeding, in view of the significant phenotypic variability in both parents and hybrids (Vale et al., 2014; Hawerroth et al., 2017).

Fifteen groups were formed with a single genotype: parent CCP 76, parent BRS 253, CCP76 x BRS226_1, CCP76 x BRS226_4, CCP76 x BRS226_6, CCP76 x HAC276-1_1, CCP76 x HAC276-1_8, CCP76 x HAC276-1_9, BRS226 x Embrapa51_9, CCP76 x Embrapa51_5, CCP76 x Embrapa51_9, CCP76 x HAC222-4_2, CCP76 x HAC222-4_7, CCP76 x BGC589_4, and CCP76 x BGC589_8. The hybrids BRS226 x Embrapa51_4, BRS226 x Embrapa51_5, and BRS226 x Embrapa51_8 formed one group, and CCP76 x BGC589_3, CCP76 x HAC222-4_3, and CCP76 x

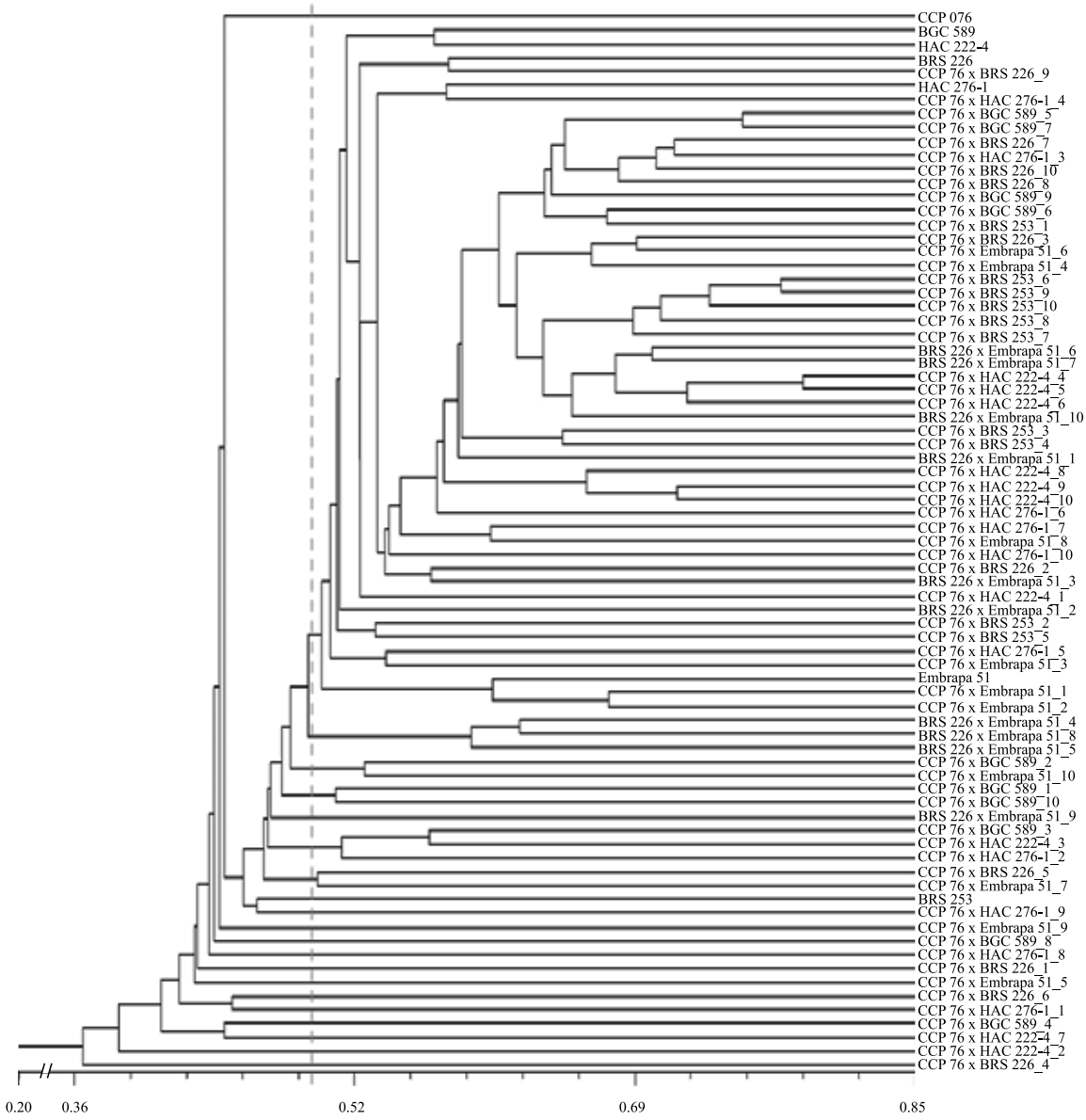

Figure 1. Similarity among 77 cashew (*Anacardium* spp.) genotypes, based on the Jaccard coefficient (J), considering the genetic variability identified by 21 RAPD primers, and by 20 ISSR primers. Twenty-one clusters were formed based on the mean similarity (sm = 0.49; r=0.87).

HAC276-1_2 formed another group. Three groups with two hybrids were observed (CCP76 x BGC589_2 and CCP76 x Embrapa51_10; CCP76 x BGC589_1 and CPP76 x BGC589_10; CCP76 x BRS226_5 and CCP76 x Embrapa51_7). The 17th group consisted of the parents BGC 589, BRS 226, HAC 276-1, Embrapa 51, and HAC 222-4, and of 45 hybrids resulting from the seven crosses.

Establishing a satisfactory performance of all main traits considered in the selection process together for a single hybrid is difficult (Tables 4 and 5), but the hybrids with the best overall performance for the evaluated traits are listed in Table 6. For mean nut weight, the populations derived from the crosses BRS 226 x EMBRAPA 51, and CCP 76 x BRS 253 had a high overall mean, 9.30 g and 9.47 g, respectively, exceeding the mean + 1 SD (Table 4). They are potentially promising for the development of hybrids with higher nut weight, evidenced by the number of hybrids with high performance per population (7 and 6, respectively). In the other populations evaluated, the per se performance of hybrids was superior as well. However, some hybrids performed poorly for nut weight in all crosses. Cross CCP 76 x BGC 589 gave rise to a population with a mean nut weight of 6.28 g, with under-average performance of all hybrids in relation to the overall experimental mean (8.35 g). This can possibly be explained by the characteristics of BGC 589 (*A. microcarpum*), which shows significantly smaller fruit (nuts) and respective pseudofruit (peduncles) than the other parents (Agostini-Costa et al., 2004; Vieira et al., 2014). BGC 589 transmits genes of lower nut weight to the progeny. Nut weight is fundamental in cashew breeding, as a target of indirect selection with a view to genetic gains in kernel weight, a product of great economic importance in cashew cultivation (Vale et al., 2014). Although not warranting superior segregating populations, it is very important that the parents of the hybridizations have a high-per-se performance for the traits of interest, to raise the likelihood of more frequent favorable recombinants and transgressive plants (Carvalho et al., 2008).

In the evaluation of Vale et al. (2014), nut weight was correlated with nut yield (genetic correlation=0.55), proving to be an important component in the definition of the yield potential of the evaluated cashew hybrids. In the present study, the hybrids CCP76 x HAC276/1_5 (2,010.56 kg ha^{-1}), CCP76 x HAC222/4_1 (1,842.88 kg ha^{-1}), CCP76 x Embrapa51_6 (1,744.43 kg ha^{-1}), CCP76 x Embrapa51_7 (2,502.93 kg ha^{-1}), and CCP76

Table 6. Best cashew (*Anacardium* spp.) hybrids in this evaluation, and summary of their characteristics (average performance).

Hybrid	Cashew nut yield (kg ha^{-1})	Mean nut weight (g)	Nuts damaged by *Anacampsis phytomiella* (%)	Powdery mildew on nuts (score 0-4)
CCP76xHAC276/1_2	1,105.90	11.02*	13.48	2.50
CCP76xHAC276/1_5	2,010.56*	9.51*	11.60	3.13
CCP76xHAC276/1_9	617.97	7.99	8.63	1.63*
CCP76xHAC222/4_1	1,842.88*	10.77*	12.28	3.04
CCP76xEmbrapa51_5	1,303.67*	12.14*	2.79	3.08
CCP76xEmbrapa51_6	1,744.43*	9.05*	7.15	3.29
CCP76xEmbrapa51_7	2,502.93*	7.03	3.93	2.79
CCP76xEmbrapa51_8	1,636.89*	6.49	3.86	2.83
CCP76xEmbrapa51_9	1,048.74	10.89*	2.68	3.13
CCP76xEmbrapa51_10	1,171.59	8.33	1.22*	2.83
BRS226xEmbrapa51_5	1,006.69	9.00*	5.94	2.17*
BRS226xEmbrapa51_6	1,205.71*	10.58*	2.39	1.50*
BRS226xEmbrapa51_8	1,238.12*	11.70*	6.93	2.96
BRS226xEmbrapa51_9	955.73	13.85*	1.11*	3.08
BRS226xEmbrapa51_10	1,240.75*	10.08*	1.36*	2.88
CCP76xBRS226_6	960.47	5.89	6.41	1.67*

*Outstanding performance of the genotype for this trait.

x Embrapa51_8 (1,636.89 kg ha^{-1}) showed a superior performance for nut yield (Table 4). The combination between 'CCP 76' and 'Embrapa 51' was the most efficient to generate hybrids with higher nut yield, and with the highest population mean between the tested combinations (1,472.80 kg ha^{-1}). Only the hybrids CCP76 x HAC276/1_5, CCP76 x HAC222/4_1, and CCP76 x Embrapa51_6 showed simultaneously a better performance for mean nut yield and nut weight. As they belong to the same group (Figure 1), crosses between these hybrids might result in populations with plants more similar to each other, due to the lower potential of broadening the genetic variability. A careful and efficient selection will therefore be required, however, with chances of developing transgressive plants for nut yield and weight, even in smaller populations. The nut yield and weight of the hybrids CCP76 x HAC276/1_2, CCP76 x Embrapa51_5, CCP76 x Embrapa51_9, CCP76 x Embrapa51_10, BRS226 x Embrapa51_5, and BRS226 x Embrapa51_8 was high, close to or above the overall mean. Thus, as they belong to distinct clusters, their potential genetic variability is greater, and can be exploited in crosses with each other or with the hybrids CCP76 x HAC276/1_5, CCP76 x HAC222/4_1, and CCP76 x Embrapa51_6.

The hybrid performance for number of nuts attacked by *A. phytomiella* was unstable throughout the study period (Table 5), which can be attributed to biotic factors affecting the insect-plant relationships (DeLucia et al., 2012). However, the population derived from the combination of 'CCP 76' with 'Embrapa 51' showed the lowest mean incidence of bored nuts (4.21%) of the sampled populations. In general, the incidence of bored nuts was lowest for the hybrids CCP76 x Embrapa51_10 (1.22%), BRS226 x Embrapa51_9 (1.11%), BRS226 x Embrapa51_10 (1.36%), and CCP76 x HAC222/4_6 (1.50%). Among these, BRS226 x Embrapa51_10 and CCP76 x Embrapa51_10 stood out with a concomitantly satisfactory performance for nut yield and mean weight (Table 4), aside from having a greater potential of broadening the genetic variability when crossed with each other, for they belong to different similarity groups. However, since the hybrids performance was close to the overall mean for powdery mildew severity (score 2.88 and 2.83, respectively), segregating populations with a high number of plants should be formed to increase the possibility of identifying superior plants for nut yield, as well as nut weight, with low incidence of bored nuts.

Lower powdery mildew severity on nuts was observed in some hybrids, below the mean performance of the evaluated plants (mean - 1 SD) (Table 5), which may represent possible sources of resistance genes to this disease. Among these, the hybrids BRS226 x Embrapa51_6 (score 1.50), CCP76 x HAC276/1_9 (score 1.63), and CCP76 x BRS226_6 (score 1.67) stood out, grouped in distinct clusters of genetic similarity (Figure 1). However, despite their potential variability by inter-crosses, these hybrids should be combined with gene donor parents that confer higher nut weight and yield, as well as reduced susceptibility to *A. phytomiella* attack, associated with the generation of large segregating populations, to raise the chances of selecting transgressive plants (Carvalho et al., 2008).

Conclusions

1. The genetic variability among cashew hybrids are efficiently evaluated by RAPD and ISSR markers, resulting in the grouping of 21 clusters.

2. The hybrids CCP76 x HAC276/1_5, CCP76 x HAC222/4_1, and CCP76 x Embrapa51_6 show high nut yield and mean nut weight, and can be crossed to form superior segregating populations, although with lower potential variability between plants.

3. The combinations between the hybrids BRS226 x Embrapa51_10 and CCP76 x Embrapa51_10 are promising to generate segregating populations with high nut yield and weight.

4. The hybrids BRS226 x Embrapa51_6, CCP76 x HAC276/1_9, and CCP76 x BRS226_6 can be used in combinations to establish less susceptible populations to powdery mildew.

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