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Genetic similarity between coriander genotypes using ISSR markers

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ABSTRACT

With the development of new cultivars, a precise genetic characterization is essential for improvement programs or for cultivar registration and protection. Molecular markers have been complementing the traditional morphological and agronomic characterization techniques because they are virtually unlimited, cover the whole genome and are not environmentally influenced. Genetic characterization constitutes the basis for studies involving estimates of genetic similarity. Therefore, the objective of the present study was to evaluate the genetic similarity between ten coriander genotypes (nine cultivars and one line) using ISSR markers. The cultivars used were: Americano, Asteca, Palmeira, Português, Santo, Supéria, Tabocas, Tapacurá, Verdão and the experimental line HTV-9299. The genetic similarity between the cultivars was estimated using 227 banded regions of ISSR molecular markers. The UBC 897 oligonucleotide generated the highest number of fragments (16), resulting in a higher polymorphism. The results indicate that the twenty-nine oligonucleotides chosen were satisfactory for detecting polymorphism. Based on the grouping analysis determined from the similarity data, there were two groups and two sub-groups. The calculated similarity for the genotypes varied from 52 to 75%. The lowest similarity was observed between Português and Verdão, at 52%. The highest similarity was found between Português and Palmeira, at 75%. The ISSR is efficient for identifying DNA polymorphism in coriander.

Keywords: *Coriandrum sativum* L., molecular characterization, microsatellites, condiments.

RESUMO

Similaridade genética entre genótipos de coentro por marcadores ISSR

Com o surgimento de novas cultivares, uma caracterização genética precisa é essencial, visando à utilização em programas de melhoramento ou para fins de registros e ou proteção de cultivares. Marcadores moleculares vêm complementando a caracterização morfológica e agronômica tradicional, uma vez que são virtualmente ilimitados, cobrem todo o genoma e não são influenciados pelo ambiente. A caracterização genética constitui a base para trabalhos de estimativas de similaridade genética. Portanto, este trabalho teve como objetivo avaliar a similaridade genética entre dez genótipos de coentro (nove cultivares e uma linhagem) por meio de marcadores ISSR. As cultivares utilizadas foram Americano, Asteca, Palmeira, Português, Santo, Supéria, Tabocas, Tapacurá, Verdão e a linhagem experimental HTV-9299. A similaridade genética entre as cultivares foi estimada com base nos marcadores moleculares de ISSR, utilizando-se 227 regiões de bandas de ISSR. O oligonucleotídeo UBC 897 gerou o maior número de fragmentos (16), resultando em um maior polimorfismo. Os resultados indicam que os vinte e nove oligonucleotídeos escolhidos foram satisfatórios para detecção de polimorfismo. Como resultado da análise de agrupamento a partir dos dados de similaridade, verificou-se a presença de dois grupos e dois subgrupos. A similaridade calculada para os genótipos variou de 52 a 75%. A menor similaridade ficou entre Português e Verdão, com 52%. Já a maior similaridade foi obtida entre Português e Palmeira com 75%. O ISSR é eficiente na identificação de polimorfismo de DNA em coentro.

Palavras-chave: *Coriandrum sativum* L., caracterização molecular, microssatélites, condimentares.

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Coriander, *Coriandrum sativum* L., was originated from Europe and the Orient where it has been cultivated for more than 3,000 years (Nascimento & Pereira, 2005). It is a vegetable, whose leaves and fruits are ground up and consumed, and is used as a condiment. In Brazil, it is consumed more in the North and Northeast, but consumption has been expanding in the Centre-south region (Wanderley Júnior & Melo, 2003).

There are a large number of growers and, consequently, it has a high socio-economic importance (Pereira & Nascimento, 2003). The plants can be harvested whole or by making cuts in the petioles and obtaining various harvests (Filgueira, 2008). However, there are few varieties available to growers in Brazil and, in some regions, local materials of unknown origin are cultivated, whose seeds are produced by the growers themselves using a low level

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of technology (Pereira *et al*., 2005).

One of the most important steps in genetic improvement programs is the selection of those genotypes having the desired characteristics using knowledge of the available germplasm (Blank *et al*., 2004). With the development of new cultivars, a precise genetic characterization is essential for genetic improvement programs or for registration or cultivar protection purposes, thereby allowing multiplication and commercialization to be controlled (Priolli *et al*., 2002). It should be emphasized that the information obtained from a study of genetic similarity between different genotypes is an indispensable tool for verifying the duplicity of accessions in germplasm collections or banks, as well as helping in backcross programs.

Molecular markers have been complementing morphological and agronomic characterizations since they are virtually unlimited, cover the whole genome and are not environmentally influenced (Goulão & Oliveira, 2001; Wunsch & Hormaza, 2002; Borba *et al*., 2005). They have been shown to be a powerful tool in genetic analysis due to the simplicity of the technique, easy handling and, in particular, are independent of environmental influences or the plant growth stage. However, some markers are more appropriate than others when information on the genetic diversity of different genotypes needs to be obtained (Milach, 1998).

The ISSR markers (Inter-Simple Sequence Repeat) are considered useful for cultivar identification, the evaluation of phylogenetic relationships, genome mapping and population studies, among other things. Techniques such as ISSR (Zietkiewicz *et al*., 1994), are based on the amplification of regions between 100 and 300 pb, between two opposing microsatellites of the same type. They show a high degree of polymorphism, a low cost (Salimath *et al*., 1995; Borba *et al*., 2005), simplicity and they do not require previous knowledge of the genome sequence which is to be cloned (Reddy *et al*., 2002). This technique is being used in crops, such as tea (Lai *et al*., 2001), cotton (Liu & Wendel, 2001), apples (Goulão & Oliveira, 2001), rice (Sarla *et al*., 2003), coffee (Ruas *et al*., 2003), lettuce (Magalhães, 2006) and sugarcane (Almeida *et al*., 2009).

Among the tools used to estimate genetic similarity between a group of genotypes are included the use of morphological, phenological or molecular markers. However, morphological markers used in the characterization and estimation of genetic divergence are significantly affected by the environment and by the plant growth stage (Tatieni *et al*., 1996; Jesus, 2006). Genetic characterization constitutes a basis for studies to estimate genetic similarity (Conti *et al*., 2002).

The objective of this study was to evaluate the genetic similarity between ten genotypes of coriander (nine cultivars and one line) using ISSR markers.

MATERIAL AND METHODS

Nine coriander cultivars were used for molecular characterization, including: Americano, Asteca, Palmeira, Português, Santo, Supéria, Tabocas, Tapacurá, Verdão and also the experimental HTV-9299 line. The Verdão, Tabocas and Tapacurá cultivars are grown mostly in the North and Northeast regions and the Português in the Southeast, where it is used to produce green matter. The remaining cultivars have been grown on a smaller scale and have been tested in cultivar competition experiments.

The genetic similarity between cultivars was estimated using molecular ISSR markers as described by Zietkiewicz *et al.* (1994).

Six seedlings of each genotype were collected for genomic DNA extraction, using the cotyledon leaves and the first definitive leaf, according to the protocol of Ferreira & Grattapaglia (1998). The DNA was quantified in 0.9% agarose gel, in the presence of a marker with a known molecular weight, lambda 50 ng (Invitrogen). The concentration of each sample was standardized to 20 ng/µL. Thirty-seven ISSR oligonucleotides were selected from a group produced by the University of British Columbia, Vancouver, Canada for *Sphagnum angermanicum* and *Pogonatum dentatum* (Table 1). The amplification reactions were made for a final volume of 25 µL, containing 20 ng of DNA, a unit of Taq DNA polymerase (Invitrogen), 10 mM of Tris-HCL (pH 8.0), 2 mM of $MgCl_2$, 0.25 µM of each desoxyribonucleotide triphosphate (DNTPs) and 0.2 µM of oligonucleotide. The DNA amplifications were done using a MJ Research, Inc. PTC100 Programmable Thermal Controller (Watetown, USA) thermocycler, under the following conditions: 15 minutes at 95°C (initial denaturation), followed by 30 or 35 cycles of 30 seconds at 94°C (denatured), 45 seconds at 50 or 55°C (ringing) and 2 minutes at 72°C (extension), with final extension for 7 minutes at 72°C. The products from the amplifications were separated in 2% agarose gel (Figure 1), crowned with Syber gold (Invitrogen), using the 100 pb Ladder 50 µg (1.0 µg/µL) (Invitrogen) marker and observed under an ultra-violet light and registered in a digital Vilber Lourmat photo-documentor.

The standards of the amplified ISSR products behave like dominant markers (Goulão & Oliveira, 2001) and were tabled as present (1) or absent (0) for the ten coriander genotypes evaluated. The similarity between all the genotypes was calculated using a simple matching coefficient. The computational program NTSYSpc ver. 2.01 (Rohlf, 2000) generated a genetic similarity matrix between the genotypes. In order to construct the dendrogram from the matrix, groups were generated using the unweighted pair group method with arithmetic average (UPMGA). To verify the adjustment between the similarity matrix and the dendrogram obtained, the cophenetic correlation coefficient was calculated (r) (Sokal & Rohlf, 1962).

RESULTS AND DISCUSSION

Of the thirty-seven oligonucleotides tested for ISSR, two did not amplify and six did not show reproducible standards. The remaining twenty-nine oligonucleotides were selected since they exhibited defined amplification standards, which were highly

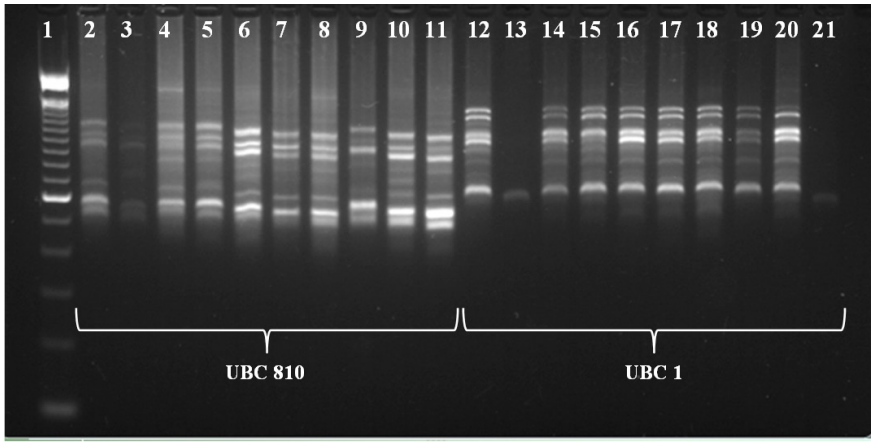

Figure 1. ISSR profile of Americano (2 and 12), Asteca (3 and 13), Palmeira (5 and 15), Português (6 and 16), Santo (7 and 17), Supéria (8 and 18), Tabocas (9 and 19), Tapacurá (10 and 20) and Verdão (11 and 21) coriander cultivars and the HTV-9299 (4 and 14) experimental line, using the UBC 810 and UBC 1 oligonucleotides; Ladder Invitrogen (1) marker with a molecular weight of 100 pb (perfil de ISSR das cultivares de coentro Americano (2 e 12), Asteca (3 e 13), Palmeira (5 e 15), Português (6 e 16), Santo (7 e 17), Supéria (8 e 18), Tabocas (9 e 19), Tapacurá (10 e 20) e Verdão (11 e 21) e da linhagem experimental HTV-9299 (4 e 14), usando os oligonucleotídeos UBC 810 e UBC 1; marcador de peso molecular 100 pb Ladder Invitrogen (1)). Recife, UFRPE, 2006.

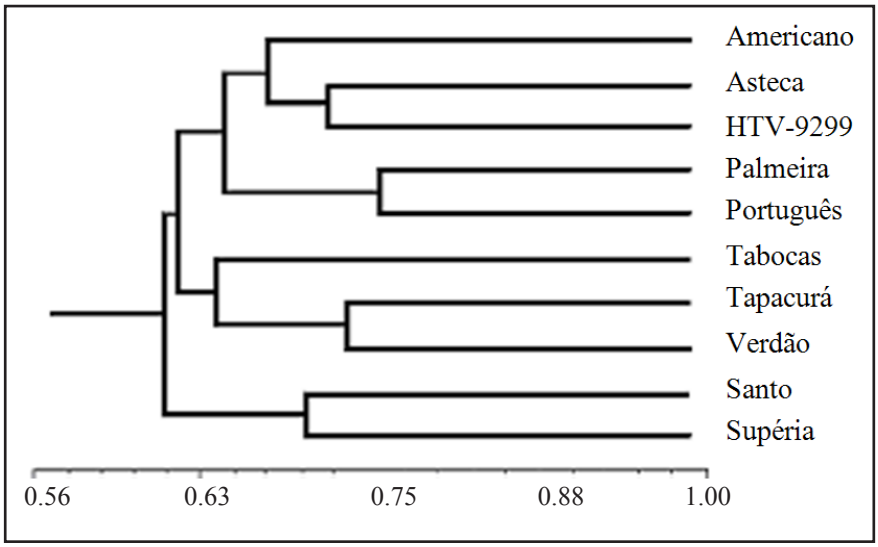

Figure 2. Dendrogram resulting from UPGMA analysis based on the simple matching coefficient estimated from ISSR markers, with a mean similarity of 61% (dendrograma resultante da análise por UPGMA com base no coeficiente coincidência simples estimado a partir de marcadores ISSR, considerando 61% a similaridade média). Recife, UFRPE, 2006.

reproducible and all of which had the dinucleotide 3' repetitions anchored (Table 1).

The 29 oligonucleotides selected for evaluating the 10 genotypes, amplified 227 DNA fragments. The UBC 848, UBC 849, UBC 858 and UBC 868 oligonucleotides resulted in the least number of amplified fragments (3), while the UBC 897 oligonucleotide generated the highest number of fragments (16), resulting in a higher polymorphism (Table 1). According to Liu & Wendel (2001), the method gives highly reproducible results and generates abundant polymorphism in many systems. The mean number of fragments amplified per oligonucleotide was 7.8 and fragment size varied between 300 pb (UBC 2) and 2072 pb (UBC 897). These results show that the 29 oligonucleotides chosen satisfactorily detected polymorphism. ISSR markers have also been used to identify polymorphism in chrysanthemums (Wolff *et al*., 1995), sorghum (Yang *et al*., 1996) and *Rhodiola crenulata* (Lei *et al*., 2006).

The result of the grouping analysis calculated from the similarity data indicated the presence of two groups and two sub-groups, with a mean similarity of 61% (Figure 2). The similarity calculated for the 10 genotypes ranged from 52 to 75%, showing considerable divergence between the 9 cultivars and the line. Those plant species, which have been subjected to genetic improvement programs for longer periods, such as soybeans, dry beans, rice and cotton, show much higher similarity values than those observed in this study (Blair *et al*., 1999; Li *et al*., 2001; Bertini *et al*., 2006; Bonato *et al*., 2006).

The first large group included the Americano, Asteca, HTV-9299, Palmeira, Português, Tabocas, Tapacurá and Verdão cultivars, while the second group consisted of Santo and Supéria. However, the first group is further divided into two smaller groups, the first formed by Americano, Asteca, HTV-9299, Palmeira and Português and the second by Tabocas, Tapacurá and Verdão.

The value for the cophenetic correlation coefficient was 76.67%, indicating that the groups formed were very reliable. The lowest similarity was registered between the Português and Verdão cultivars, at 52%, with the former being the commonest cultivar in São Paulo (Southeast) and the latter in Pernambuco (Northeast). Both cultivars are genetically the most distant. The Português cultivar has a growth cycle of around 53 days for producing green matter, has a lower anthocyanin concentration and a shorter plant, whereas the Verdão cultivar has a shorter cycle for producing green matter (30-35 days), a higher anthocyanin concentration and dark green leaflets, among other differences (Melo *et al*., 2009). A greater similarity was seen between the Português and Palmeira cultivars, at 75%, with these genotypes being genetically closer compared to the others. It is believed that the Português cultivar originated from Europe, more precisely, Portugal, but there is no

Table 1. Selected ISSR oligonucleotides, sequence, regions of the bands developed for the coriander genotypes (oligonucleotídeos de ISSR selecionados, sequência, regiões de bandas reveladas dos genótipos de coentro). Recife, UFRPE, 2006.

Oligonuceotides	Sequence*	Regions of the bands
UBC 1	ACACACACACACACACT	5
UBC 2	GAGAGAGAGAGAGAGAT	13
UBC 3	CTCTCTCTCTCTCTCTG	9
UBC 5	CTCTCTCTCTCTGC	9
UBC 808	AGAGAGAGAGAGAGAGC	6
UBC 810	GAGAGAGAGAGAGAGAT	10
UBC 813	CTCTCTCTCTCTCTCTT	9
UBC 817	CACACACACACACACAA	10
UBC 820	GTGTGTGTGTGTGTGTC	5
UBC 827	ACACACACACACACACG	10
UBC 830	TGTGTGTGTGTGTGTGG	4
UBC 834	AGAGAGAGAGAGAGAGYT	9
UBC 845	CTCTCTCTCTCTCTCTRG	5
UBC 848	CACACACACACACACARG	3
UBC 849	GTGTGTGTGTGTGTGTYA	3
UBC 855	ACACACACACACACACYT	11
UBC 857	ACACACACACACACACTG	7
UBC 858	TGTGTGTGTGTGTGTGRT	3
UBC 864	ATGATGATGATGATGATG	8
UBC 866	CTCCTCCTCCTCCTCCTC	10
UBC 868	GAAGAAGAAGAAGAAGAA	3
UBC 878	GGATGGATGGATGGA	4
UBC 879	CTTCATTTCACTTCA	10
UBC 881	GGGTGGGGTGGGGTG	7
UBC 884	HBHAGAGAGAGAGAGAG	12
UBC 886	VDVCTCTCTCTCTCTCT	8
UBC 887	DVDTCTCTCTCTCTCTC	7
UBC 891	HVHTGTGTGTGTGTGTG	11
UBC 897	CCGACTCGAGNNNNNNATGTGG	16

*Degeneration according to IUPAC (Degeneração de acordo com a IUPAC).

information on the origin of Palmeira. They have some distinct phenotypic characteristics but the formation of this group may be due to the sharing of genes derived from more distant common ancestors, thus explaining the existing genetic similarity.

The second highest similarity registered 72% for Verdão and Tapacurá, which have common morphological characteristics, such as leaflet color, anthocyanin concentration and seed weight (Melo *et al*., 2009). Asteca and HTV-9299 showed 70% similarity, with special characteristics, such as the presence of anthocyanin, mean length of the fifth leaf, a length-width relationship for cotyledons. This cultivar and the line may have common ancestors (Melo *et al*., 2009). Santo and Supéria showed 69% similarity, with very similar morphological characteristics and initiated bolting after an average 55 days. Americano and Supéria, in spite of belonging to different groups, showed a 56% similarity, with some shared characteristics, such as the length of the fifth leaf, plant weight and the number of days to initiate bolting (Melo *et al*., 2009).

Allogamous species, which have been intensely improved, may show a mean similarity of 100% (Vieira & Nodari, 2007). The mean similarity found in coriander was 61%, indicating that these materials have common characteristics.

The ISSR technique was efficient for identifying DNA polymorphism in coriander and permitted a more rapid identification. The data generated can provide a guide for future genotype crossing studies with the aim of improving the genetics of this species.

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REFERENCES

ALMEIDA CMA; LIMA SEN; LIMA GSA; BRITO JZ; DONATO VMTS; SILVA MV. 2009. Caracterização molecular de cultivares de cana-de-açúcar utilizando marcadores ISSR. *Ciência e Agrotecnologia* 33: 1771-1776.

BERTINI CHCM; SCHUSTER I; SEDIYAMA T; BARROS EG; MOREIRA MA. 2006. Characterization and genetic diversity analysis of cotton cultivars using microsatellites. *Genetics and Molecular Biology* 29: 321-329.

BLANK AF; CARVALHO FILHO JLS; SANTOS NETO AL; ALVES PB; ARRIGONI-BLANK MF; SILVA-MANN R; MENDONÇA MC. 2004. Caracterização morfológica e agronômica de acessos de manjericão e alfavaca. *Horticultura Brasileira* 22: 113-116.

BLAIR MW; PANAUD O; McCOUCH SR. 1999. Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 98: 780-792.

BONATO ALV; CALVO ES; GERALDI IO; ARIAS CAA. 2006. Genetic similarity among soybean (*Glycine max* (L) Merrill) cultivars released in Brazil using AFLP markers. *Genetics and Molecular Biology* 29: 692-704.

BORBA RS; GARCIA MS; KOVALLESKI A; OLIVEIRA AC; ZIMMER PD; BRANCO JSC; MALONE G. 2005. Dissimilaridade genética de linhagens de *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) através de marcadores moleculares ISSR. *Neotropical Entomology* 34: 565-569.

CONTI JH; MINAMI K; TAVARES

FCA. 2002. Comparação de caracteres morfológicos e agronômicos com moleculares em morangueiros cultivados no Brasil. *Horticultura Brasileira* 20: 419-423.

FERREIRA ME; GRATTAPAGLIA D. 1998. *Introdução ao uso de marcadores moleculares em análise genética*. Brasília: EMBRAPA-CENARGEM. 220p.

FILGUEIRA FAR. 2008. *Novo manual de olericultura*: agrotecnologia moderna na produção e comercialização de hortaliças. Viçosa: UFV. 421p.

GOULÃO L; OLIVEIRA CM. 2001. Molecular characterization of cultivars of apple (*Malus* x d*omestica* Borkh.) using microsatellite (SSR and ISSR) markers. *Euphytica* 122: 81-89.

JESUS ON. 2006. *Caracterização morfológica e molecular de cultivares de bananeira*. Recife: UFRPE. 83p (Tese mestrado).

LAI JA; YANG WC; HSIAO JY. 2001. An assessment of genetic relationship in cultivated tea clones and native wild tea in Taiwan using RAPD and ISSR markers. *Botanical Bulletin of Academia Sinica* 42: 93-100.

LEI Y; GAO H; TSERING T; SHI S; ZHONG Y. 2006. Determination of genetic variation in *Rhodiola crenulata* from the Hengduan Mountains Region, China using inter-simple sequence repeats. *Genetics and Molecular Biology* 29: 339-344.

LI CD; FATOKUN CA; UBI B; SINGH BB; SCOLES GJ. 2001. Determining genetic similarities and relationships among cowpea breeding lines and cultivars by microsatellite markers. *Crop Science* 41: 189-197.

LIU B; WENDEL JF. 2001. Intersimple sequence repeat (ISSR) polymorphisms as a genetic marker system in cotton. *Molecular Ecology Notes* 1: 205-208.

MAGALHÃES AG. 2006. *Caracterização de genótipos de alface (*Lactuca sativa *L.) em cultivo hidropônico sob diferentes valores de condutividade elétrica da solução nutritiva*. Recife: UFRPE. 83p (Tese mestrado).

MELO RA; MENEZES D; RESENDE LV; WANDERLEY JÚNOR LJG; MELO PCT; SANTOS VF. 2009. Caracterização morfológica de genótipos de coentro. *Horticultura Brasileira* 27: 371-376.

MILACH SCH. 1998. *Marcadores moleculares em plantas*. Porto Alegre: UFRGS. 141p.

NASCIMENTO WM; PEREIRA RS. 2005. Coentro: a hortaliça de mil e uma utilidades. *Horticultura Brasileira* 23, n. 3. Artigo de capa.

PEREIRA RS; MUNIZ MFB; NASCIMENTO WM. 2005. Aspectos relacionados à qualidade de sementes de coentro. *Horticultura Brasileira* 23: 703-706.

PEREIRA RS; NASCIMENTO WM. 2003. Avaliação da qualidade física e fisiológica de sementes de coentro. *Horticultura Brasileira* 21: Suplemento (CD-ROM).

PRIOLLI RHG; MENDES-UUNIOR CT; ARANTES NE; CONTEL EPB. 2002. Characterization of Brazilian soybean cultivars using microsatellite markers. *Genetics and Molecular Biology* 25: 185-193.

REDDY MP; SARLA N; SIDDIQ EA. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* 128: 9-17.

ROHLF FJ. 2000. *NTSYSpc*: numerical taxonomy and multivariate data analysis system, ver. 2.01. Exeter Software: Setauket, New York.

RUAS PM; RUAS CF; RAMPIM L; CARVALHO VP; RUAS EA; SERA T. 2003. Genetic relationship in coffea species and parentage determination of interspecific hybrids using ISSR (Inter-Simple Sequence Repeat) markers. *Genetics and Molecular Biology* 26: 319-327.

SALIMATH SS; OLIVEIRA AC; GODWIN ID; BENNETZEN JL. 1995. Assessment of genome origins and genetic diversity in the genus *Eleusine* with DNA markers. *Genome* 38: 757-763.

SARLA N; BOBBA S; SIDDIQ EA. 2003. ISSR and SSR markers base on AG and GA repeats delineate geographically diverse *Oryza nivara* accessions and reveal rare alleles. *Current Science* 84: 683-690.

SOKAL RR; ROHLF FJ. 1962. The comparison of dendrograms by objective methods. *Taxon* 11: 30-40.

TATIENI V; CANTRELL RG; DAVIS DD. 1996. Genetic diversity in elite cotton germplasms determined by morphological characteristics and RAPD. *Crop Science* 36: 186-192.

VIEIRA RL; NODARI RO. 2007. Diversidade genética de cultivares de alho avaliada por marcadores RAPD. *Ciência Rural* 37: 51-57.

WANDERLEY JÚNIOR LJG; MELO PCT. 2003. Tapacurá: nova cultivar de coentro adaptada às condições subtropicais do Brasil. *Horticultura Brasileira* 21: Suplemento (CD-ROM).

WUNSCH A; HORMAZA JI. 2002. Cultivar identification and genetic fingerprinting of temperate fruit tree species using DNA markers. *Euphytica* 125: 59-67.

WOLFF K; ZIETKIEWICZ E; HOFSTRA H. 1995. Identification of chrysanthemum cultivars and stability of DNA fingerprint patterns. *Theoretical Applied Genetics* 91: 439-447.

YANG W; OLIVEIRA AC; GODWIN I; SCHERTZ K; BENNETZEN JL. 1996. Comparison of DNA marker technologies in characterizing plant genome diversity: variability in chinese sorghums. *Crop Science* 36: 1669-1676.

ZIETKIEWICZ E; RAFALKI A; LABUDA D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176-183.