Genetics and Molecular Biology, 29, 3, 516-524 (2006)

www.sbg.org.br

Research Article

Transferability and use of microsatellite markers for the genetic analysis of the germplasm of some *Arachis* section species of the genus *Arachis*

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Abstract

The *Arachis* section is the most important of the nine sections of the genus *Arachis* because it includes the cultivated peanut, *Arachis hypogaea*. The genetic improvement of *A. hypogaea* using wild relatives is at an early stage of development in spite of their potential as sources of genes, including those for disease and pests resistance, that are not found in the *A. hypogaea* primary gene pool. Section *Arachis* species germplasm has been collected and maintained in gene banks and its use and effective conservation depends on our knowledge of the genetic variability contained in this material. Microsatellites are routinely used for the analysis of genetic variability because they are highly polymorphic and codominant. The objective of this study was to evaluate the transferability of microsatellite primers and the assay of genetic variability between and within the germplasm of some species of the *Arachis* section. Fourteen microsatellite loci developed for three different species of *Arachis* were analyzed and 11 (78%) were found to be polymorphic. All loci had transferability to all the species analyzed. The polymorphic loci were very informative, with expected heterozygosity per locus ranging from 0.70 to 0.94. In general, the germplasm analyzed showed wide genetic variation.

Key words: genetic diversity, germplasm, peanut, transferability, wild species.

Received: November 23, 2004; Accepted: November 11, 2005.

Introduction

The genus *Arachis* L. is composed of 69 described species distributed into nine taxonomic sections (Krapovickas and Gregory, 1994). The most studied section of the genus is the *Arachis* section containing 27 species (Krapovickas and Gregory, 1994) including the cultivated peanut (*Arachis hypogaea* L.), a South America native species which the evidence suggests has its center of origin in Bolivia, where the wild diploid species which are its putative progenitors are found (Gregory *et al*., 1980; Kochert *et al*., 1996). Three *Arachis* species genomes (A, B and D) are known based on chromosomal morphology and crossing relationships, the cultivated peanut being an allotetraploid (AABB) that arose through a single event involving hybridization between two wild diploid section *Arachis* species, *A. duranensis* (the A genome) and *A. ipaënsis* (the B genome) (Kochert *et al*., 1996).

Although the use of wild relatives for the genetic improvement of *A. hypogaea* has been investigated for a large number of agronomic traits and wild species have occasionally been used as a source of genes for the genetic improvement of cultivated peanut (Singh *et al.,* 1996; Simpson and Starr, 2001; Burow *et al*., 1996) breeding programs have typically been undertaken using standard techniques and have only explored the primary gene pool (Garcia *et al*., 1995).

Microsatellite markers are made up of tandemly repeated sequences of one to six nucleotides (Weber and May 1989) and have been widely used to evaluate genetic variability in plant species (Ghebru *et al*., 2002; Hormaza, 2002). Gupta and Varshney (2000) have pointed out that microsatellites have many advantages over other types of markers, including high polymorphism, random distribution in eukaryotic genomes and high frequency, while Plieske and Struss (2001) have shown that microsatellite markers developed for one species can also be used in related species with no additional cost for primer development, a serious restriction to the wider use of microsatellites. Microsatellites also allow easier data integration because they are amplified by using very stringent

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polymerase chain reaction (PCR) conditions and, generally speaking, only single loci are amplified.

Germplasm of most wild *Arachis* species has been collected and maintained in various germplasm banks. An assay of the genetic variability of this germplasm is very important for its most effective use in breeding programs and for its conservation, since knowledge of genetic variation can help in regeneration, the identification of duplicates and the detection of collection sites with high local variability.

Börner *et al.* (2000) used nine microsatellites to evaluate the genetic integrity of *Triticum aestivum* L. germplasm that had been regenerated up to 24 times, demonstrating that microsatellites can be used to analyze seed bulks that had been stored at room temperature in a seed reference collection for more than 50 years. No contamination due to foreign pollen or incorrect handling during the multiplication cycles was discovered and genetic drift was observed for only one accession, showing that microsatellites can be used as a simple and reliable marker system for the verification of the integrity and genetic stability of germplasm bank accessions.

The aim of this study was to evaluate the transferability of microsatellite markers and the genetic variability in the germplasm of wild species of the *Arachis* section of the genus *Arachis* in order to contribute the conservation and better use of this germplasm.

Material and Methods

Biological material

Table 1 lists the 60 *Arachis* section accessions analyzed, all obtained from Dr. José F. M. Valls of the Brazilian agricultural company Empresa Brasileira de Agropecuria (Embrapa), Centro Nacional de Recursos Genéticos (CENARGEN), Brasília, DF, Brazil.

Table 1 - The *Arachis* section accessions analyzed.

Arachis species and describing author(s) (subspecies/variety)	Genome type	Accession number[a]	Brazilian (BRA) accession number	Site[b] (map reference)	Number of specimens
A. aff. Cardenasii		V13721	033723	MT (1609 S; 05827 W)	02
A. aff. Diogoi		V13774	033863	MT (1554 S; 05931 W)	01
A. aff. Simpsonii		V13745	033782	MT (1616 S; 05922 W)	01
		V13746	033791	MT (1616 S; 05922 W)	01
A. batizocoi[1] Krapov. & Gregory	B	K9484	011335	BOL (2005 S; 06314 W)	01
A. cardenasii[1] Krapov & Gregory	A	G10017	013404	BOL (1820 S; 05946 W)	04
A. cruziana Krapov, Gregory & Simpson	B	Wi1302	036919	BOL (n.a.[c])	01
A. decora Krapov, Gregory & Valls	2n = 18	V9953	022985	GO (1301 S; 04642 W)	01
		V13290	030902	GO (1318 S; 04642 W)	01
A. diogoi Hoehne	A	Vp5000	039144	MS (n.a.)	01
A. duranensis Krapov & Gregory	A	V14167	022608	ARG (2445 S; 06526 W)	01
A. glandulifera Stalker	D	V13738	033774	MT (1613 S; 05903 W)	01
		V14730	038687	MT (n.a.)	01
A. gregoryi[d]	n.a.	V14728	038679	MT (n.a.)	01
		V14739	038717	MT (n.a.)	01
		V14760	038792	MT (n.a.)	01
		V6389	012696	MT (1519 S; 06006 W)	01
A. helodes Mart. ex Krapov & Rigoni	A	Co6862	018619	MT (n.a.)	04
		V6325	012505	MT (1552 S; 05604 W)	07
		V10470	024937	n.a.	03
A. hoehnei Krapov & W.C. Gregory	A	V9140	022641	MS (1917 S; 05722 W)	01
		V9146	022659	MS (1914 S; 05716 W)	01
		V13985	034606	MS (1931 S; 05725 W)	01
A. hypogaea Linnaeus (fastigiata/fastigiata)	AB	URY85209	026999	URY (n.a.)	01
(fastigiata/fastigiata)		V10522	025097	SC (n.a.)	01
(fastigiata/aequatoriana)		Mde1640	037427	EQU (n.a.)	01
(fastigiata/peruviana)		Mde1560	037401	EQU (n.a.)	01
(fastigiata/vulgaris)		URY85183	026794	URY (n.a.)	01

Table 1 (cont.)

Arachis species and describing author(s) (subspecies/variety)	Genome type	Accession number[a]	Brazilian (BRA) accession number	Site[b] (map reference)	Number of specimens
(fastigiata/vulgaris)		URY85062	026239	URY (n.a.)	01
(fastigiata/vulgaris)		URY85273	027251	URY (n.a.)	01
(hypogaea/hypogaea)		Pd3147	039229	RS (n.a.)	01
(hypogaea/hypogaea)		V12577	030775	MS (n.a.)	01
(hypogaea/hypogaea)		V12548	030708	MT (n.a.)	01
(hypogaea/hypogaea)		Pd3324	029807	PA (n.a.)	01
(n.a.)		As433	n.a.	n.a.	01
(n.a.)		Mde1600	n.a.	n.a.	01
(n.a.)		Mde1670	n.a.	n.a.	01
(n.a.)		W725	n.a.	n.a.	01
A. ipaënsis Krapov & Gregory	B	K30076	036234	BOL (2100 S; 06325 W)	01
A. kempff-mercadoi: Krapov, Gregory & Simpson	A	V13250	030643	BOL (1745 S; 06310 W)	01
A. kuhlmannii Krapov & Gregory	A	V9394	022629	n.a.	01
		V6404	012653	MT (1537 S; 05848 W)	01
		V8887	020206	MT (1537 S; 05848 W)	01
		V6352	012611	MT (1556 S; 05748 W)	01
		V9479	22586	MS (1955 S; 05530 W)	01
A. magna Krapov, Gregory & Simpson	B	K30097	036871	BOL (1622 S; 06058 W)	01
		V13751	033812	MT (1616 S; 05927 W)	01
		V13748	033804	MT (1616 S; 05924 W)	01
		V13761	033847	MT (1521 S; 06004 W)	01
		V14744	038741	MT (n.a.)	01
A. microsperma Krapov, Gregory & Valls	A	V8711	020397	MS (2205 S; 05631 W)	01
		V7681	017655	MS (2206 S; 05631 W)	01
A. monticola Krapov & Rigoni	AB	V14165	036188	ARG (2407 S; 06573 W)	01
A. palustris Krapov, Gregory & Valls	2n = 18	V13023	030058	TO (0725 S; 04737 W)	01
A. praecox Krapov, Gregory & Valls	2n = 18	V13777	n.a.	n.a.	01
A. schininii[d]	A	V9923	022926	PRY (2223 S; 05620 W	02
A. simpsonii Krapov & Gregory	A	V13728	033740	BOL (1619 S; 05826 W)	01
		V13732	034037	MT (1557 S; 05845 W)	01
A. stenosperma: Krapov & Gregory	A	V10309	024830	MT (1628 S; 05439 W)	01
A. valida Krapov & Gregory	B	V13516	032646	MS (1904 S; 05729 W)	01
A. villosa Benth.	A	V12812	030813	URY (3016 S; 05737 W)	07
Total 27		60			83

[a]*As* - A. O. Scariot; *Co* - L. Coradin; *G* - W.C. Gregory; *K* - A. Krapovickas; *Pd* - G. Pedralli; *Sv* - G.P. Silva; *V* - J.F.M. Valls; *Vp* - V. Pott; *W* - W.L. Werneck; *Wi* - D.E. Williams; [b]Key: ARG = Argentina; BOL = Bolivia; ECU = Ecuador. Brazilian states: GO = Goiás; MS = Mato Grosso do Sul; MT = Mato Grosso; PA = Pará; PRY = Paraguay; RS = Rio Grande do Sul; SC = Santa Catarina; TO =Tocantins; URY = Uruguay. [c]n.a. = not available. [d]undescribed species.

DNA extraction, microsatellite loci and amplification

The DNA of each plant was extracted from leaves by the procedure of Doyle and Doyle (1987) as modified by Grattapaglia and Sederoff (1994), diluted in Tris EDTA buffer (10 mM Tris-HCl mM and 1 mM EDTA), quantified in 0.8% (w/v) agarose gels based on comparison to known DNA dilutions and diluted to 5 ng/μL.

Fourteen microsatellite prime pairs (Table 2) from genomic libraries were used, three (Ag117, Ag140 and Ag171) from *Arachis glabrata* (*Rhizomatosae* section), eight (Ah3, Ah7, Ah11, Ah30, Ah6-125, Ah 126, Ah282 and Ah283) from *A. hypogaea* and three (Ap32, Ap38 and Ap40) from *Arachis pintoi* (*Caulorrhizae* section).

Table 2 - Microsatellite loci analyzed, motifs, primer sequences, expected length in the focal species, $MgCl_2$ concentration per reaction, annealing temperatures, number of alleles per locus and observed (Ho) and expected (He) heterozygosity values.

Locus	Motif	Primer sequences	Length (bp)	Range	$MgCl_2$ (mM)	AT (°C)	Number of alleles	H_0	H_e
Ah3	$(GA)_{15}(AG)_7$ $(GT)_7(GA)_7$	5'TCGGAGAACCAAGCACACATC3' 5'TTGCGCTCTTTCTCACACTC3'	202	176-255	1.5	50	28	0.2692	0.9313
Ah7	$(TG)_8$	5'CAGAGTCGTGATTTGTGCACTG3' 5'ACAGAGTCGGCCGTCAAGTA3'	102	102-110	1.5	50	9	0.3580	0.7983
Ah11	$(TTA)_{15}$	5'AAATAATGGCATACTTGTGAACAATC3' 5'TTCCACCCAAGGCAAGACTATG3'	176	140-204	2.5	55	29	0.0909	0.9219
Ah30	$(GA)_9$	5'TGCTCTTCTTTTCCTTTTCAC3' 5'AACGGCCAAAACTGAAATTA3'	123	121-138	2.0	50	13	0.2388	0.8946
Ah6-125[a]	$(TTC)_{13}$	5'TCGTGTTCCCGATTGCC3' 5'GCTTTGAACATGAACATGCC3'	180	170-210	2.0	60	20	0.1013	0.8972
Ah126	$(GA)_8..(GA)_9$	5'CCCTGCCACTCTCACTCACT3 5'CGTACAAGTCAGGGGGTGAC3'	187	180-216	1.5	60	23	0.3250	0.9139
Ah282	$(TA)_4..(TG)_{45}$	5'GCAAACACACCACATTTCA3' 5'GGCTCCAATCCCAAACACTA3'	213	182-210	1.5	45	21	0.2840	0.9352
Ah283	$(AT)_8(GT)_{13}$	5'GGGGTTCGAAGCTTAATTCC3' 5'CAAGAGCAACTCAATCTTCCTAGA3'	198	160-168	2.5	55	6	0.2125	0.6965
Ap32	$(TC)_{19}$	5'GATCATGCTCATCATCAACACC3' 5'ATAGGGAGAAGGCAGGGAGA3'	170	150	2.5	50	1	0.0000	0.0000
Ap38	$(CT)_{25}$	5'GCGAACAAAGGAGGAAGAGA3' 5'GCGAACAAAGGAGGAAGAGA3'	154	154	2.0	55	1	0.0000	0.0000
Ap40[b]	$(TC)_{17}$	5'CTGTTTGATCGCCGCTATG3' 5'GTCAAGTGCTTCCTCCGATG3'	178	164-220	2.0	50	34	0.2152	0.9410
Ag117	$(GA)_{19}..(AG)_{18}$	5'GAATGACAGAGTGAGAGTCCA3' 5'TCAACAAGTTAGTTACCATTAGTTT3'	241	250	2.5	50	1	0.0000	0.0000
Ag140	$(GA)_{28}$	5'CAGCATTCAATTCAGTTTCG3' 5'TCAACCTCGAACACACAAAA3'	157	124-144	2.5	50	11	0.2099	0.7873
Ag171	$(GA)_{22}$	5'TGACCGTTGGGGTTTTTG3' 5'CAAACCCAAACACACGTCAC3'	197	166-186	2.0	50	15	0.2267	0.8374

[a]Described by Hopkins *et al*. (1999); [b]Described by Palmieri *et al*. (2002). AT: Annealing temperature.

Amplifications were performed using a PTC100 thermal cycler (MJ Research, San Francisco). Reactions were carried out in a total volume of 10 μL and contained 15 ng of template DNA, 0.17 μM of each primer, 0.22 mM of each dNTP, 1X of reaction buffer, 1 U of *Taq* DNA polymerase (Amersham Biosciences, Piscataway) and from 1.5 to 2.5 mM $MgCl_2$ (Table 2). The amplification cycle consisted of an initial denaturation step at 94 °C, followed by 32 cycles of 94 °C for 25 s; (45 °C to 60 °C) for 25 s (Table 2) and 72 °C for 25 s and a final 10 min extension step at 72 °C. The annealing temperatures ranged according to the sequence of the primers (Table 2). The fragments were separated on 4% (w/v) acrylamide gels (19 acrylamide: 1 bis acrylamide) and the gels were silver stained (Promega, 1995) and photographed under white light.

Data analysis

The size of each allele was estimated using the 'EagleSight' software version 3.22 (Stratagene, Austin). Expected (He) and observed (Ho) heterozygosities, genetic distances and number of alleles/locus were calculated with the Population Genetic Analysis program (PopGene, version 1.21 - Yeh *et al*., 1997). A dendrogram was obtained by using the TreeCon for Windows program (version 1.3b; Van de Peer and De Wachter, 1994) that uses the Nei and Li's coefficient. The mean estimated number of repeats in each locus was calculated based on the expected length of fragment and the motif of each locus in focal species.

Results

All 14 loci allowed the amplification of microsatellite loci in all the *Arachis* section species analyzed, eleven loci being polymorphic (78%) and three monomorphic (22%). Two of the monomorphic loci (Ap32 and Ap38) were isolated from the *Caulorrhizae* section species *A. pintoi* and one monomorphic loci (Ag117) from the *Rhizomatosae* section species *A. glabrata*.

We detected 201 putative alleles at the polymorphic loci Ah3, Ah7, Ah11, Ah30, Ah6-125, Ah126, Ah282, Ah283, Ag140, Ag171 and Ap40, with a mean of 18.3 alleles per polymorphic locus which ranged from six for locus Ah283 to 34 for the Ap40 locus (Table 2). The number

of alleles per locus varied according to the origin of the primer, with the eight *A. hypogaea* primers detecting 144 alleles (18 per locus), the three *A. pintoi* primers 35 alleles (11.7 per locus) and the three *A. glabrata* primers 25 alleles (8.3 per locus).

The mean observed heterozygosity for the 11 polymorphic loci was 0.2301, the highest value (0.3580) being that for Ah7 locus and the lowest (0.0909) for the Ah11 locus (Table 2).

The mean number of repeats at the polymorphic loci in the non-focal species was estimated on the basis of observed fragment sizes (Table 3). Variation in the repeat number was very wide, ranging from two repeats at the Ah283 locus to 53 at the Ah3 locus.

We also calculated the variability indices for species with multiple accessions or multiple samples of one accession (Table 4). The average number of different alleles per locus ranged from 1.14 in *Arachis schininii* (undescribed species) to 4.14 in *Arachis helodes* and the mean expected heterozygosity ($\overline{H}_e$) ranged from 0.0446 in *A. schininii* to 0.4706 in *A. helodes.*

Some individual plants had banding patterns composed of two fragments (Figure 1), such plants being analyzed as heterozygous since microsatellites are co-dominant and allow the detection of both alleles in a locus and because most species analyzed were diploid and hence the maximum number of different alleles in a locus was two. The mean observed heterozygosity values ($\overline{H}_0$) ranged from 0 for the *Arachis* species *cardenasii*, *gregoryi*, *magna* and *simpsonii* to 0.1179 for *Arachis kuhlmannii.* (Table 4).

Relationships between accessions based on the data for the 14 loci analyzed are presented in Figure 2. As can be

Table 3 - Number of alleles in the polymorphic loci (A) and mean estimated number of repeats (R) in each locus evaluated in the *Arachis* species.

Species	Loci																					
	Ah3		Ah7		Ah11		Ah282		Ah126		Ah6-125		Ah283		Ag140		Ag171		Ap40		Ah30	
	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
A. aff. cardenasii	1	30	1	11	2	14	2	43	2	28	2	23	2	3	3	14	1	8	1	20	1	15
A. aff. diogoi	1	33	1	7	2	6	1	39	2	27	2	15	1	4	1	19	1	7	1	16	1	15
A. aff. simpsonii	2	36	2	8	2	9	2	41	2	30	2	14	2	3	2	21	2	8	2	18	2	13
A. batizocoi	1	47	1	9	1	9	1	37	1	28	1	20	1	4	1	17	1	11	1	15	1	-
A. cardenasii	1	27	1	8	1	11	2	38	1	18	3	22	1	2	2	13	1	7	1	27	1	15
A. cruziana	1	47	1	3	1	12	1	39	1	26	1	24	1	5	1	17	1	8	1	16	1	8
A. decora	2	53	2	9	2	8	2	37	2	25	2	26	1	4	1	21	1	7	3	22	1	8
A. diogoi	1	32	1	7	1	11	1	33	1	19	1	15	1	4	1	17	1	6	1	10	1	8
A. duranensis	1	36	1	8	-	-	1	44	1	25	1	24	1	6	1	15	-	-	1	20	1	15
A. glandulifera	2	44	3	9	2	15	2	36	3	25	2	12	2	5	1	17	2	9	2	32	1	13
A. gregoryi	3	48	3	9	4	13	3	37	3	25	2	18	2	4	2	16	4	7	3	13	2	11
A. helodes	4	35	4	9	5	9	10	39	5	26	8	23	2	2	3	12	3	12	8	23	4	16
A. hoehnei	2	32	2	8	2	3	2	42	2	33	2	23	1	2	2	14	2	15	2	11	1	13
A. hypogaea	10	37	2	8	6	20	2	41	2	23	1	23	2	4	2	16	2	7	2	17	2	10
A. ipaënsis	1	37	1	8	1	21	1	38	1	29	1	20	1	4	1	17	1	8	1	13	1	15
A. kempff-mercadoi	1	38	1	10	1	19	1	42	1	29	1	22	1	2	1	15	1	9	1	30	-	-
A. kuhlmannii	5	38	4	8	6	9	5	39	4	21	2	18	2	2	1	13	4	9	3	21	3	13
A. magna	3	40	1	8	5	17	4	38	6	25	4	23	2	4	2	16	4	9	3	15	3	12
A. microsperma	2	37	1	8	1	17	3	42	1	17	1	15	1	4	1	17	1	7	2	36	2	14
A. monticola	2	38	2	8	2	24	2	40	2	23	1	18	2	4	2	16	2	7	1	24	2	10
A. palustris	1	37	1	8	1	20	1	40	1	23	1	17	1	4	1	14	1	7	1	16	1	8
A. praecox	1	48	1	11	1	36	1	37	1	25	1	20	-	-	1	21	1	8	1	15	1	8
A. schininii	1	38	3	9	1	6	1	41	1	17	1	22	1	2	1	14	1	14	1	30	1	16
A. simpsonii	2	33	2	8	2	14	2	41	1	31	2	22	2	2	2	19	2	10	3	26	2	14
A. stenosperma	1	37	1	9	1	16	1	40	1	28	1	27	1	6	1	17	1	9	1	27	1	13
A. valida	1	47	1	7	1	26	1	29	1	27	1	17	1	5	1	20	1	9	1	27	1	15
A. villosa	3	28	3	9	2	14	1	45	1	17	4	23	2	5	3	16	2	8	2	24	1	19
Average		38		8		15		39		25		21		4		16		9		21		13

Table 4 - Number of samples analyzed, average number of different alleles per locus, mean observed heterozygosity ($\overline{H}_0$) and mean expected heterozygosity ($\overline{H}_e$).

Species	Number of samples	Average number of alleles	$\overline{H}_e$	$\overline{H}_0$
A. aff cardenasii	2	1.38	0.1346	0.0769
A. aff. simpsonii	2	1.78	0.3929	0.0000
A. cardenasii	4	1.28	0.0982	0.0000
A. decora	2	1.57	0.2589	0.0357
A. glandulifera	2	1.78	0.3393	0.1071
A. gregoryi	4	2.43	0.4514	0.0000
A. helodes	14	4.14	0.4706	0.0997
A. hoehnei	3	1.57	0.2738	0.0714
A. hypogaea	15	2.78	0.4700	-
A. kuhlmannii	5	2.93	0.4615	0.1179
A. magna	5	2.36	0.3829	0.0000
A. microsperma	2	1.64	0.1875	0.0357
A. schinini	2	1.14	0.0446	0.0357
A. simpsonii	2	1.21	0.1071	0.0000
A. villosa	7	1.93	0.2592	0.0663
Average		1.99	0.2759	0.0462

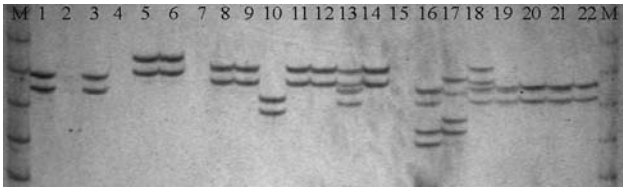

Figure 1 - Silver stained 4% (w/v) acrylamide gel showing polymorphism at locus Ah282 in plants of *Arachis schinini* (plants 1 to 3), *Arachis hoehnei* (plants 4 to 8) and *Arachis helodes* (plants 9 to 22). The number of fragments in the plants ranged from two (plants 1, 3, 5, 6, 8, 9, 11, 12, 14, 19, 20, 21, 22) to four (plants 13, 16, 17, 18). It can also be seen that some plants (17 and 19) from the same species did not share any fragment with each other. M = molecular weight.

seen, all accessions were characterized and the diversity between accessions of a particular species was very high. Many accessions were grouped according to the species to which they belonged with, for instance, A. *helodes* and *A. hypogaea*) accessions being very related to each other, while some accessions were grouped away from the other accessions of the same species, an example being *A. hoehnei* and *A. simpsonii* accessions which were placed in different clusters.

Discussion

Independent of the *Arachis* species (*A. hypogaea, A. glabrata* or *A. pintoi*) from which they were originally developed, all the primers tested allowed the amplification of loci in all the *Arachis* species analyzed. Primer transferability between the *Arachis* section species demonstrated that

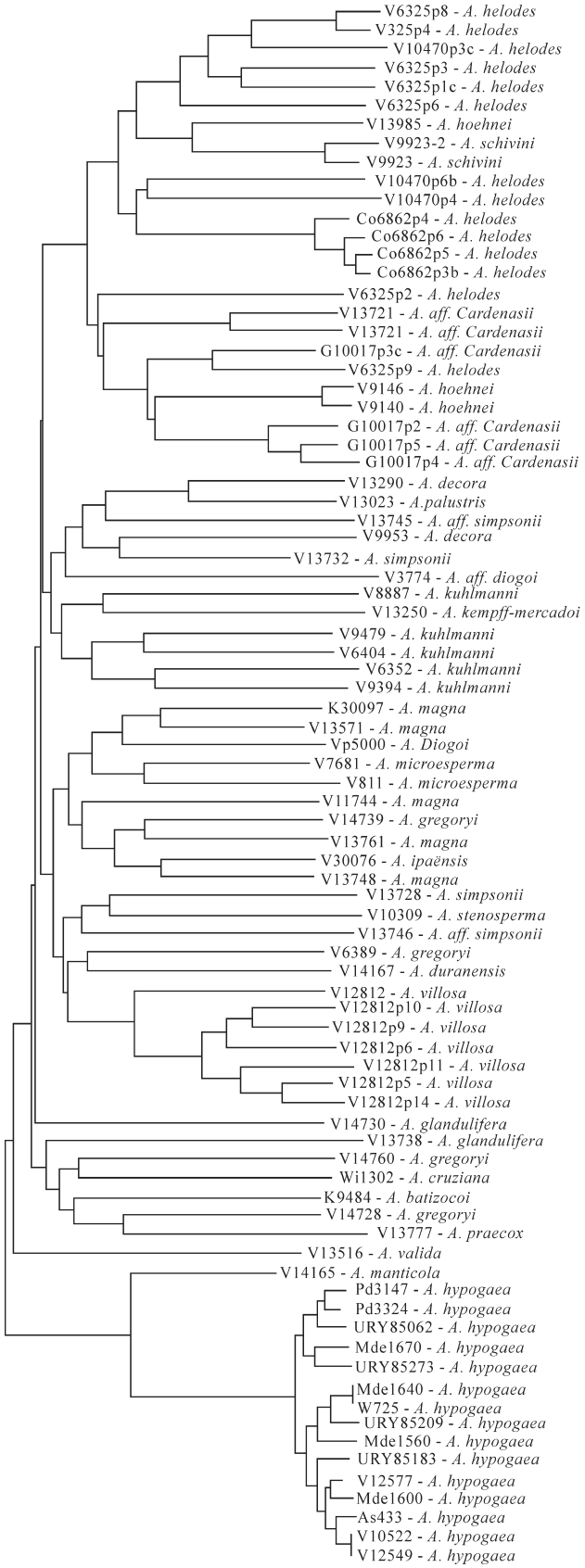

Figure 2 - Dendrogram showing the relationship between 83 plants of 60 genus *Arachis* accessions. The dendrogram was based on the analysis of 14 microsatellite loci.

the regions flanking these microsatellites are conserved enough to allow locus amplification, agreeing with previous work by other authors who have shown that microsatellite flanking regions are conserved, being even used to phylogenetic inferences in a number of species (Matsuoka *et al.,* 2002; Rosseto *et al.,* 2002). We found that the percentage of cross-species transferability was very high considering the number of species analyzed and genetic distance among them. Hopkins *et al*. (1999) who analyzed polymorphism in *A*. *hypogaea* using six *A. hypogaea* microsatellite loci also observed transferability to other *Arachis* species (*A*. *monticola*, *A*. *ipaënsis* and *A*. *duranensis*), but all of them were closely related to *A. hypogaea*. Likewise, Moretzsohn *et al.* (2004) investigated 36 *Arachis* species and observed up to 76% microsatellite primer transferability to *Arachis* section species and up to 45% to species of the other eight sections of the genus *Arachis*. The different transferability levels between our study and that of Moretzsohn *et al.* (2004) was probably because we optimized the annealing temperature and $MgCl_2$ concentration while Moretzsohn *et al.* (2004) optimized only the annealing temperature. Transferability has been observed in many other genera and species, Collevatti *et al.* (1999) having obtained total transferability of 10 *Caryocar brasiliensis* microsatellite loci to five other *Caryocar* species, while Gaitán-Solís *et al*. (2002) developed 68 *Phaseolus vulgaris* microsatellite loci and found that the transferability rate to other four other *Phaseolus* species was almost 50%.

In general the estimated number of repeats in the polymorphic loci in non-focal species were different from the number of repeats found in the same loci in the focal species. For example, the expected allele size of the *A. hypogaea* Ah282 locus was 213 base pairs (bp) with 45 TG repeats (Table 2) while in the species to which it was transferred the estimated repeat number ranged from 29 for *Arachis valida* to 45 for *Arachis villosa* (Table 3), showing that this locus was highly polymorphic with 22 alleles. However, on the other hand, locus Ah283 presented 21 repeats in *A. hypogaea*, two to six repeats in the other species and low polymorphism with only six alleles detected in the whole sample. Our data indicate that the number of repeats as well as the level of polymorphism can vary extensively between focal and non-focal species and that allele length is general longer in focal species than in non-focal species.

Overall, variability levels in the species were moderately high ($\overline{H}_e = 0.2759$). The $\overline{H}_e$ value was high in species for which only a few accessions were analyzed, *e.g. Arachis decora* ($\overline{H}_e = 0.2589$ for two accessions) and *A. kuhlmannii* ($\overline{H}_e = 0.4615$ for five accessions). Our results thus indicate that the germplasm of the species analyzed was generally composed of highly contrasting individuals. The genetic variability of diploid *Arachis* species has been reported in a number of studies using different molecular markers (Galgaro *et al*., 1998; Gimenes *et al.,* 2002). However, these studies used markers, such as amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) which score as dominant markers and lead to an underestimation of genetic variability. Furthermore, in the case of RAPD, data integration and comparison are difficult due to the detection of multiple loci and low repetitiveness.

In general, the mean observed heterozygosity found in the *Arachis* species analyzed was low for all loci, as expected for a self-fertilizing species, indicating that the breeding system of the analyzed species is preferentially autogamous. However, we observed that certain *Arachis* species (*e.g. A*. *glandulifera, A. helodes* and A. *kuhlmannii*) presented moderate levels of observed heterozygosity, *A. helodes* for example presenting $\overline{H}_0 = 0.0997$ indicating that, on average, 9.97% of individual plants analyzed were heterozygous for any the loci analyzed. The frequencies observed in some species such as *A. kuhlmannii* (11.79%) clearly show that allogamy or other mechanisms to retain heterozygosity occur in *Arachis*. Allogamy can have consequences for the conservation of the species germplasm bank since a loss of genetic identity of the species can occur due to pollen contamination.

The genetic relationships between species established based on our microsatellite data (Figure 2) partially agreed with relationships established based on other types of data. For instance, our data placed *A. hypogaea* and *Arachis monticola* together, agreeing with previous work showing that they are highly related to each other (Pattee *et al.,* 1998; He and Prakash, 2001). Also, *A. magna* and *A. ipaënsis* were located close to each other in our tree (Figure 2) and are known to be morphologically very similar to each other (Krapovickas and Gregory, 1994). Furthermore, the *A. decora* V13290 accession and *A. palustris* V13023 were placed very close to each other in our tree (Figure 2), supporting our unpublished data which shows that these two species have 18 chromosomes and show no polymorphism on their rDNA transcribed spacers, indicating that they are closely related to each other phylogenetically.

The *Arachis* species analyzed by us had A, B and D genomes. The A genome species were divided into four groups and the B genome into two groups, with *Arachis glandulifera*, the only D genome species in the genus (Stalker, 1991), being placed close to the B genome species *Arachis batizocoi* and *Arachis cruziana*. Some A genome groups were closer to B genome groups than to other A genome groups. This, and the placement of some accessions away from the other accessions of their species, may have been due to two main factors: the high polymorphism detected in the analyzed loci and/or the occurrence of homoplastic alleles, *i.e.* alleles that present the same size in a gel, are not identical by descent and are found in relatively distantly related species. In some cases, polymorphism within species was so high that the genetic distance was higher between accessions than between some species.

This was the case for *A. simpsonii* accessions, which presented a genetic distance (data not shown) of 0.9359 whereas unrelated species such as *A. magna* and *A. villosa* presented a genetic distance of 0.7506. This means that these species could not be characterized with the 14 microsatellite markers used in our study and that larger samples, which allow better sampling of intraspecific variation, need to be analyzed.

The microsatellite loci used in this study were quite efficient for the analysis of genetic variability in *Arachis* section species because they presented high transferability to species from the same genus and they were polymorphic in the species to which they were transferred, thus permitting the analysis of related species without the development of specific primer pairs. The characterization of the genetic variability of the germplasm analyzed could be used to plan sampling programs, and assist in germplasm maintenance and the formation of species 'core collections'. Moreover, codominance and high polymorphism can determine variation in breeding systems. On the other hand, the markers were not efficient in the establishment of genetic relationships between the species investigated in this study, probably due to the very small number of accessions analyzed for some species, high variability among accessions in the loci analyzed and homoplastic fragments.

Acknowledgments

The authors would like to thank Dr. José F.M. Valls and CENARGEM/EMBRAPA for the plant material, Dr. Edson S. Mori for help with the statistical analysis and Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) for the funding.

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Associate Editor: Márcio de Castro Silva Filho