Notas Científicas

Rescue of a non-viable accession and RAPD analysis of recovered plants of *Arachis retusa*

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Abstract – In vitro regeneration of *Arachis retusa* was examined for the purpose of germplasm renewal and conservation. Random amplified polymorphic DNA (RAPD) fingerprinting was used to evaluate the genetic stability of plants derived from embryo axes and apical segments. Ten arbitrary decamer primers were screened and five of them were selected. Ninety genomic regions were evaluated, with an average of 18 loci per clone. All amplified segments were monomorphic. The results indicate that recovered plants are genetically stable at the assessed genomic regions and that both regeneration processes are suitable for in vitro germplasm preservation of *Arachis* species.

Index terms: germplasm conservation, micropropagation, molecular characterization, genetic stability.

Resgate de um acesso inviável de *Arachis retusa* e análise das plantas recuperadas por RAPD

Resumo – A regeneração in vitro de *Arachis retusa* foi avaliada visando à renovação e conservação de germoplasma. A estabilidade genética de plantas derivadas de eixos embrionários e segmentos apicais foi avaliada por RAPD. Foram analisados dez oligonucleotídeos decâmeros arbitrários, dos quais cinco foram selecionados. Noventa regiões genômicas foram avaliadas, com uma média de 18 loci por clone. Todos os segmentos amplificados foram monomórficos. Estes resultados indicam que as plantas são geneticamente estáveis nas regiões genômicas examinadas e que ambos os processos são adequados para a conservação in vitro do germoplasma de *Arachis*.

Termos para indexação: conservação de germoplasma, micropropagação, caracterização molecular, estabilidade genética.

Some wild species of *Arachis* are endemic to Brazil and occur in areas degraded by human actions. This situation could lead to the loss of germplasm potencially useful for breeding programs and also to the reduction of genetic variability within populations. Therefore, it is highly desirable to develop appropriate in vitro conservation techniques, which may offer an alternative to seed banks for germplasm multiplication, conservation and distribution.

In vitro germplasm conservation relies on micropropagation methods. However, phenotypic and genetic variation are reported to occur during in vitro regeneration processes, originating somaclonal variants (Kaeppler et al., 2000). Thus, the risks of genetic changes induced by tissue culture and the importance of assessing the genetic stability of the biological material along all phases of storage must be considered in the context of conservation.

Random amplified polymorphic DNA (RAPD) fingerprinting has been considered a very suitable method for detecting molecular alterations in plants regenerated in vitro by different processes (Goto et al., 1998; Plader et al., 1998; Rout et al., 1998). In *Arachis*, this technique has also been used to evaluate genetic diversity (He &

Prakash, 1997; Subramanian et al., 2000; Dwivedi et al., 2001), phylogenetic relationships within wild species, interspecific polymorphisms (Galgaro et al., 1998) and introgression analysis of interspecific hybrids (Garcia et al., 1995).

In this work, non-viable seeds of *A. retusa* Krapov., W.C. Gregory & Valls were rescued and multiplied by tissue culture and the genetic stability of plants originated was evaluated by RAPD analysis.

Seeds of *A. retusa* (accession V9950, BRA-022969, originally collected in Goiás State, Central Brazil) were provided by the seed bank of Embrapa Recursos Genéticos e Biotecnologia. Primary cultures were obtained from embryo axes as previously reported (Gagliardi et al., 2000). Primary plants derived from those explants were multiplied by culturing apical segments on MS medium with 2.7 μM NAA (Gagliardi et al., 2002).

Total DNA was extracted from sets of 4-7 plants derived from both explant types, using the CTAB procedure (Doyle & Doyle, 1987). Ten primers were assayed and five of them were selected (Table 1). RAPD reactions were performed in a PTC-100 MJ thermocycler at a final volume of 25 μL containing 10–20 ng of genomic DNA; 10 mM Tris-HCl pH 8.8; 50 mM KCl; 200 μM dNTP; 50 μM each primer; 4 mM $MgCl_2$ and 2U Taq polymerase. After denaturing the DNA at 94°C for 5 min, 45 cycles of 94°C for 1 min, 40°C for 1 min, and 72°C for 2 min were performed, followed by a final extension step at 72°C for 6 min (Vieira et al., 1997). RAPD products were analyzed by electrophoresis (3V/cm) on 1.4% agarose gels and visualized after ethidium bromide staining. Gels were photographed under UV light and data were scored for the presence or absence of amplification products.

The culture of embryo axes in presence of 4.4 μM BAP resulted in multishoot formation and indirect organogenesis after the development of preexisting meristems, confirming previous results (Gagliardi et al., 2000). Shoots were rooted on MS devoid of growth regulators and used for RAPD analysis. The ten primers used in this work were previously employed in order to establish phylogenetic relationships in *Arachis* and *Stylosanthes* (Vieira et al., 1997; Subramanian et al., 2000; Dwivedi et al., 2001). Five of those primers were selected on the basis of the number of RAPD loci and good reproducibility.

Ninety genomic regions (loci) were evaluated, with an average of 18 loci per *A. retusa* clone (Table 1). All RAPD loci were monomorphic in the plantlets obtained both from embryo axes and apical segments (Figure 1). Among the several investigators that have addressed the issue of genetic stability of micropropagated plants using RAPD, many have also found monomorphic loci, showing no intraclonal variation (Isabel et al., 1993; Valles et al., 1993; Rout et al., 1998). RAPD analysis is able to detect variations in the whole genome as the loci are scattered in all chromosomes and both repetitive and non-repetitive sequences are amplified. From this perspective, the recovered shoots evaluated in this work seem to be genetically stable at the genomic regions tested, indicating that these regeneration processes can be used for in vitro conservation of *Arachis* germplasm. Nevertheless, considering that the absence of in vitro-induced polymorphism does not exclude the possibility of chromosomal alterations, additional analysis as cytogenetics and AFLP (Amplified Fragments Length Polymorphism) must be considered to further evaluation of the genetic fidelity of these in vitro clones.

Table 1. Nucleotide sequences and number of amplification products (loci) detected by five arbitrary primers in *A. retusa* clones.

Primer	Sequence	Number of loci
OPA 02	TGCCGAGCTG	15
OPA 07	GAAACGGGTG	18
OPG 02	GGCACTGAGG	17
OPG 03	GAGCCCTCCA	20
OPJ 17	ACGCCAGTTC	20
Total		90

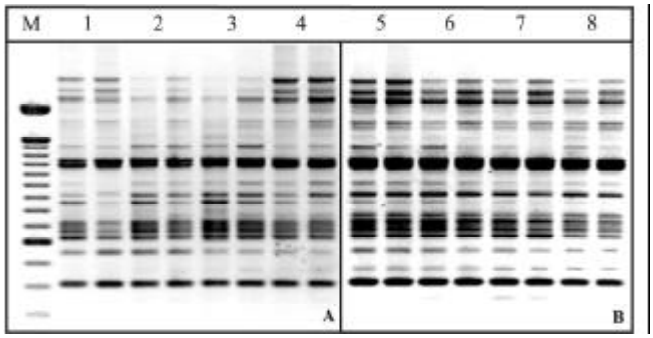

Figure 1. Amplification pattern of 8 DNA samples derived from in vitro *A. retusa* plants using primer OPG02. Each sample was evaluated in two DNA concentrations (10 and 20 ng); A) samples derived from embryonic axes; B) samples derived from apical segments.

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