Molecular characterization and tissue culture regeneration ability of the USA Arachis pintoi (Krap. and Greg.) germplasm collection

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Introduction *Arachis pintoi* Krap. and Greg. is a herbaceous, perennial legume, exclusively native to Brazil. It is considered a multiple use legume, being grown for forage; ground cover in fruits orchards, forest, and low tillage systems; erosion control; and ornamental purposes. Although several cultivars have been released in different countries, little is known about the genetic diversity of the germplasm stored in world genebanks. Our objective was to characterize and evaluate the genetic diversity of the germplasm of 35 accessions of *Arachis pintoi* at molecular level using RAPD markers. Concurrently, two tissue culture protocols were evaluated for their organogenesis ability. Further, variation in band profile was analyzed by comparing "Parent Plants" and tissue culture regenerated plants.

Materials and methods DNA was extracted from leaves of single Parent Plants using a modified CTAB protocol. Eighteen primers of ten nucleotides length from the Operon Technologies kit were used to amplify genomic DNA. Amplification products were separated by electrophoresis on 1.5% agarose gels and banding patterns were visualized by staining the gels in ethidium bromide solutions and viewing under UV radiation. Allele frequency, number of polymorphic loci, Nei's genetic distance, Nei's genetic diversity index, and Shannon-Weaver's genetic diversity index were the parameters calculated. Genetic distance (D= -ln I) was later used as a criterion for differentiation among accessions to prepare a cluster analysis. Two protocols were used to access the organogenic potential among germplasm accessions. Protocol 1 was proposed by Rey *et al*. (2000) and Protocol 2 proposed by Ngo and Quesenberry (2000). To compare these two protocols, callus rating and weight and number of regenerated plants were used. The RAPD band profiles of regenerated plants were compared to the Parent Plants to evaluate induction of somaclonal variability during the tissue culture process.

Results From the 18 primers tested, amplifications were obtained with only eight. Primers A4, B4, B5, C2, D4, D13, E4, and G5 amplified 100 different bands. The average number of amplified bands per primer was 12.5. The size of these 100 fragments ranged from 250 bp to 3500 bp. From the 100 bands amplified, 98 presented polymorphism. The average presence of bands per accession was 32, ranging from 20 to 44 bands. Ten bands were able to discriminate individual germplasm accessions. The proportion of polymorphic RAPD loci was 89%. Genetic diversity of the whole set of germplasm was estimated by Nei's gene diversity (h) and by Shannon-Weaver's diversity index (H). The average h was 0.29 ± 0.16, and average H was 0.45 ± 0.20. Average genetic distance was estimated as 0.36, and indicated that a great genetic diversity exits among the germplasm evaluated in this research. Genetic distances were used to prepare a dendogram for the 35 *A. pintoi* accessions, which separated them in four distinct groups. Callus induction was achieved on two different M.S. basal media protocols after 28 d of incubation. Analysis of variance demonstrated that Protocol 1 was superior to Protocol 2 for both variables related with callus growth. Great variability for these two variables was observed among the accessions. Shoot regeneration was achieved for several accessions on both media with no structures indicative of somatic embryogenesis being detected. Callus growth was not correlated with shoot regeneration. In Protocol 1 shoot regeneration was obtained from 15 accessions, whereas in Protocol 2, shoot regeneration was attained from 18 accessions. Root induction was very difficult to obtain, and invariably many shoots died during this process. At the end, 16 regenerated plants were recovered between the two protocols.

Conclusions Molecular characterization was achieved with RAPD molecular markers, which proved to be very informative and efficient to characterize the genetic diversity and relationships among germplasm accessions of this species. The variables used to assess the genetic diversity of the germplasm indicated that a large amount of genetic diversity exists among the germplasm evaluated in this research. Although differences in callus ratings and weight among protocols were observed we conclude that based on shoot development and plant regeneration both protocols were similar. RAPD band profiles of regenerated tissue culture plants were similar to their parent plants.

References

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