

06.02 - GENÉTICA MOLECULAR VEGETAL

06.02-021 CARACTERIZAÇÃO DE ESPÉCIES DA SECÇÃO *EXTRANERVOSAE* DO GÊNERO *ARACHIS*. Vicente Eugenio de Rosa Junior¹, José Francisco Montenegro Valls², Catalina Romero Lopes³, Claudio Costa³. ¹Dep. Defesa Fitossanitária, FCA/UNESP, C.P.237, Botucatu-SP, CEP 18603-970, ²CENARGEN/EMBRAPA, Brasília-DF, ³Dep.Genética, IB/UNESP, Botucatu, SP. vicente@surfnet.com.br.

O gênero *Arachis* L. (Leguminosae) é tipicamente sul-americano, com espécies distribuídas por vários estados brasileiros, além de Bolívia, Paraguai, Argentina e Uruguai, e têm como principal representante *A. hypogaea*. O gênero é dividido em 9 seções taxonômicas baseadas em morfologia, distribuição geográfica e compatibilidade. A seção *Extranervosae* é de ocorrência exclusiva do território brasileiro e é composta até o momento, de nove espécies, além de uma nova espécie anteriormente classificada como *A. aff pietrarellii*, porém com diversas características morfológicas contrastantes. Para se estabelecer as relações de afinidade nesta seção, foram analisados acessos de *A. villosulcarpa*, *A. burchelli*, *A. lutescens*, *A. macedoi*, *A. pietrarellii*, *A. prostrata*, *A. retusa*, *A. setinervosa*, e da espécie nova. O germoplasma foi proveniente do Banco de Germoplasma de *Arachis* do Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnologia/Empresa Brasileira de Pesquisa Agropecuária (CENARGEN/EMBRAPA, Brasília, DF). Folhas jovens foram utilizadas para a extração de DNA e posterior amplificação por RAPD. Dos 80 "primers" testados, 13 apresentaram polimorfismo evidente, gerando 66 bandas polimórficas, as quais foram avaliadas como caracteres binários por presença e ausência. O grau de similaridade genética utilizou coeficientes de similaridade e de distância taxonômica em programa NTSYS utilizando-se algoritmo UPGMA. Os fenogramas obtidos pelas análises de agrupamento revelaram alta similaridade entre os acessos de cada espécie, sendo facilitada a classificação de alguns acessos. Entre os acessos de *A. villosulcarpa* não houve detecção de variabilidade genética e a espécie nova pôde ser comprovada pela diferenciação dos outros agrupamentos formados. Órgão Financiador: CNPq

06.02-022 SINGLE STRAND BINDING PROTEIN ENHANCES THE PROCESSIVITY OF THE DEOXYNUCLEOTIDYL TRANSFERASE ACTIVITY PROMOTED BY THE THII1 PROTEIN FROM *Arabidopsis thaliana*. Luche D.D.¹, Godoi P.H.C.², Oliva G.², Menck C.F.M.¹. 1- Instituto de Ciências Biomédicas II, USP; 2- Instituto de Física, USP-SC. e-mail: ddluche@usp.br

The *Thi1* gene product from *Arabidopsis thaliana* participates in the biosynthesis of thiamin in organelles and also presents characteristics related to repair/tolerance of damaged DNA, as it complements bacteria (*Escherichia coli*) deficiency in either base or nucleotide excision repair pathways. The amino acid sequence of this protein shows homology to certain domains of prokaryotic DNA polymerases and, in fact, previous results showed that it complements bacteria deficient in DNA Polymerase I. *In vitro* experiments of DNA polymerization, using purified preparations of this protein, indicate that THII1 has the ability to incorporate deoxynucleotides into acid insoluble products, both in the presence and absence of a DNA template, that are sensitive to DNase I. Therefore, the THII1 protein has an activity of deoxynucleotidyl transferase. To confirm that this activity is directly promoted by THII1 protein, and not by a contaminating enzyme, we performed the immunoprecipitation of the protein preparation using anti-THII1 antibody. This complex showed a strong reduction in the deoxynucleotidyl transferase activity, pointing to the fact that it is really a THII1 property. Single strand binding protein (SSB), a known important component for DNA polymerization, was tested on THII1 activity. The data indicate that SSB enhances its processivity, resulting in the polymerization of DNA products of higher molecular weight, either for the presence or absence of template. This might be an important feature for the THII1 repair/tolerance of DNA damaged related activity and it is a strong evidence that THII1, and related homologous proteins, perform a role in the maintenance of organellar genomes. Financial support: FAPESP, PADCT-CNPq

06.02-023 SSR MARKERS AS POWERFUL TOOLS FOR MAIZE ELITE GERMOPLASM FINGERPRINTING. Guimarães, C.T.; Souza, I.R.P.; Parentoni, S.N.; Cameiro, N.P.; Santos, M.X.; Gama, E.E.G.; Pacheco, C.A.; Meirelles, W.F.; Vasconcelos, M.J.V.; Lopes, M.A.; Paiva, E. Embrapa Milho e Sorgo, C.P. 151 Sete Lagoas, MG 35701-970 claudia@cnpmc.embrapa.br

Simple sequence repeats (SSRs) or microsatellites is a class of genetic markers consisting of short nucleotide sequence motifs tandemly repeated and evenly distributed throughout eukaryotic genomes. SSRs show high levels of polymorphisms and provide a codominant marker system based on PCR technique. Due to the availability of high amount of SSR loci well mapped on maize genome (www.agron.missouri.edu/ssr.htm/), this marker can be widely used in genetic fingerprinting and crop protection. In addition, SSR technology presents the potential advantage of reliability, reproducibility and discrimination. The goal of this work was to characterize and to follow the genetic identity of maize elite inbred lines used in

the current Embrapa Maize and Sorghum breeding program and to develop a genetic profile for the commercial hybrids. For this purpose, we screened 230 SSR primer pairs (Research Genetics, Inc.) in order to choose those that generate good amplification patterns. Selected primers were used to amplify genomic DNA of 22 maize inbred lines and 24 hybrids derived from those inbred lines, including commercial ones. PCR products were resolved in 8% non-denaturing polyacrylamide gels stained with ethidium bromide. Gel images were captured by the Eagle-Eye System and the size of each allele was estimated using the RFLPscan Gel Analysis Software 2.1 with 100 bp ladder as size standard. Polymorphic index contents (PIC) were estimated for each SSR locus. PIC value measures the discriminatory power of a SSR locus, considering the number and the relative frequency of the expressed alleles. PCR patterns were also used to evaluate genetic distances among maize elite inbred lines. Genetic distance matrix was used to construct dendrograms using the UPGMA method. Maize elite genotypes were characterized by 74 easily scorable SSRs loci, generating a genetic profile of Embrapa's maize elite germplasm. This study indicates that SSRs may be the optimum approach for the identification and characterization of maize genotypes, compared to other methods currently available. The tropical maize SSR profiles can be readily applied for measuring and monitoring genetic diversity, for supporting Intellectual Property Protection and for future strategies in molecular breeding. This work was supported by grants from PRONEX, CNPq, and SEP/EMBRAPA.

06.02-024 NON MENDELIAN SEGRAGATION IN TRANSGENIC PLANTS. Romano E., Rodrigues K., Proite K., Monte D., Aragão F. EMBRAPA-CENARGEN, romano@cenargen.embrapa.br.

Genes transformed into plants are usually inherited in a regular Mendelian fashion. However, several reports have described transformants in which the transgenes fails to segregate in a simple Mendelian pattern. We have generated hundreds of transgenics beans and soybeans by the biolistic technique. About 10% of the transgenic plants do not transmitted the exogenous genes to the progeny or transmitted in a ratio not expected by Mendelian manner. In this study, we analyzed two non mendelian transgenic lines: I) The transgenic bean line 158, obtained by transformation with pMD4 vector containing the gene block *Rep-TrAP-REn*, from the bean golden mosaic virus (BGMV) genome in antisense orientation and the *gus* gene. This plant is immune to BGMV and the progeny showed complete absence of the introduced DNA. II) The transgenic soybean line 33-3 obtained with pAG1 vector, containing the *ahas* genes that confers resistance against the herbicide imazapyr. Self-pollination of this plant showed a deficiency of transgenic plants (10 AHAS^S: 1 AHAS^R). In subsequent generations, the segregation ratio stabilized at 1 AHAS^S: 1 AHAS^R. The number of seeds per silique was reduced by 50% in this line. Such exceptional segregations ratios could result from a variety of mechanisms. The complete absence of the introduced DNA in 158 line progeny could be result of normal segregation from a chimeric plant or by disruption of genes during the plasmid integration in the transformation process. To address this question, microgametophyte *gus* analysis was performed. This assay showed the normal ratio expected for one transgenic locus, (1 *gus*⁺: 1 *gus*⁻). Thus, we concluded that the distorted segregation observed is not a consequence from a chimeric transgenic plant and probably caused by disruption of genes related with gametogenesis and/or embryogenesis. In order to isolate these genes, we have been using the plasmid rescue approach. Three clones were obtained from each plant. Sequencing analysis of these clones revealed new open reading frames probably related with the non mendelian phenotype. Our results suggests that 158 and 33-3 lines contain plasmid-insertions that delete or disrupt genes related with gametophytic growth and/or development. The next step in this study will be the transformation of bean and soybean with antisense versions of the isolated ORFs obtained in this work.

06.02-025 USO DE MARCADORES MOLECULARES RFLP E AFLP NO ESTUDO DA DIVERSIDADE GENÉTICA DO DENDEZEIRO (*Elaeis guineensis* Jacq. e *E. oleifera* (Kunth) Cortés). E. Barcelos; R. N. V. da Cunha; B. Nouy e N. R. Sousa. Embrapa Amazônia Ocidental/Manaus - barcelos@cmaa.embrapa.br

A Embrapa Amazônia Ocidental dispõe de uma ampla coleção de germoplasma de dendezeiro, compreendendo 296 acessos da espécie africana *E. guineensis* e 182 acessos da espécie americana *E. oleifera*, ocupando um total de 48 hectares de área experimental. A correta caracterização e avaliação da coleção é de grande importância para gestão e uso eficiente do recurso genético conservado, entretanto caracterizar coleções de espécies perenes com base em descritores morfológicos e agronômicos representa um trabalho longo e oneroso, além de problemas como baixa capacidade de discriminação interpopulacional para o caso do dendezeiro. Com o objetivo de estudar a estrutura e o nível de variabilidade genética presente na coleção, empregaram-se marcadores moleculares RFLP e AFLP como ferramentas. RFLP foi determinado pela utilização de 37 sondas cDNA em amostras representando uma ampla cobertura da área de ocorrência