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DEVELOPMENT AND GENETIC ANALYSIS OF A DATABASE OF MULTILOCUS MICROSATELLITE DNA FINGERPRINTS OF THE BRAZILIAN PROTECTED SOYBEAN VARIETIES

Document prepared by experts from Brazil

The analysis of genetic variation at the DNA sequence level is a powerful tool to resolve questions of varietal identification and discrimination in crop plants. It is expected that genetic profiles of highly polymorphic molecular markers will most likely become an important complementary descriptor when requiring the protection of a new variety, particularly in autogamous species of narrow genetic base. With this perspective in mind we developed a database of molecular profiles of 125 soybean varieties currently registered and protected at the National Service of Varietal Protection of the Brazilian Ministry of Agriculture (LADIC/SNPC/MAPA). The main objective of this work was to optimize and evaluate the performance of highly polymorphic microsatellite markers - international standard used in human forensic genetics - for tests of DUS (distinctness, uniformity and stability) in an extensive but closed set of protected soybean varieties characterized by a very narrow genetic base. Official seed samples from each variety were supplied in sealed numbered bags by LADIC/SNPC/MAPA. To avoid experimental bias, the genetic analysis was carried out in double blind experiments where the commercial identification of the samples as well as possible genetic relationships among them was not informed. DNA extraction from seeds and genetic analyses were carried out in duplicate samples from a bulk of 100 seeds ground to a fine powder. Additional analyses of individual seeds was performed in those cases where evidences of seed contamination and /or mixture of lines were observed. A selected battery of 21 microsatellite loci from the SOYBASE were screened and 15 were selected based on a combination of factors including in order: map position, robustness of allelic interpretation and genetic information content in the target gene pool. The 15 loci, twelve of them trinucleotide and three dinucleotide repeats, were typed by multicolor fluorescence detection in four PCR multiplex systems in an ABI 377XL automatic DNA sequencer and the alleles declared in basepairs based on an internal size standard. The genetic differences among the varieties were clearly revealed by the size differences in the alleles at each locus. Of the 125 samples analyzed, 120 could be unequivocally distinguished based on at least two but typically more than four allelic differences at the 15 loci typed. Two samples were indistinguishable, i.e. displayed the same exact multilocus genotype, and three pairs of samples were different by only one marker locus. For these three pairs of samples the analysis of additional markers is necessary to test the hypothesis of the occurrence of a relatively rare but possible mutation or to confirm the distinctness by the observation of further genetic differences. Three samples showed clear evidences of mixture of lines and one sample had evidences of residual heterozigosity. A significant allelic diversity was observed among the 125 samples, although at some loci one or more particular alleles displayed a much higher frequency. On average, the 15 loci had 5.9 alleles with a range from three to nine. Only three out of 15 loci had a genetic diversity (GD) of less than 0.5 while the more informative loci had GD above 0.7. Both the allelic size range and the power of discrimination of these 15 loci are in close agreement with previous estimates from studies on US germplasm. Not surprisingly, however, a number of alleles previously not reported in the US germplasm were detected in the set of 125 samples studied, suggesting the existence of novel germplasm not previously sampled in DNA studies. In conclusion, this work pioneers the establishment of a rapid and economical system for the genetic identification of Brazilian soybean varieties based on a very robust and precise DNA marker technology. This system could certainly add a significant power of resolution for DUS tests especially when closely related varieties are under scrutiny. The database of genetic profiles and allele frequencies could be immediately used to implement genetic identity tests by electronic comparison of multilocus profiles between questioned and reference samples in QA/QC procedures along the seed commercialization chain.

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