WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES,
AND DNA-PROFILING IN PARTICULAR

Thirteenth Session
Brasilia, November 22 to 24, 2011

ADDENDUM

ORGANIZATION OF SOYBEAN OFFICIAL DUS TRIALS IN BRAZIL BASED ON THE
USE OF MOLECULAR MARKERS

Document prepared by experts from Brazil
SECRETARIAT OF AGRICULTURAL DEVELOPMENT AND COOPERATIVISM
DEPARTMENT OF INTELLECTUAL PROPERTY AND AGRICULTURAL TECHNOLOGY
NATIONAL PLANT VARIETY PROTECTION SERVICE

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ORGANIZATION OF SOYBEAN
OFFICIAL DUS TRIALS IN BRAZIL
BASED ON THE USE OF MOLECULAR MARKERS

LUIS GUSTAVO ASP PACHECO
NATIONAL PLANT VARIETY PROTECTION SERVICE
Examiner
PVP in Brazil

✓ Breeder Testing System
  Candidate variety descriptions are provided by the breeders with the application. The information is included on the database of GAIA software and compared with other varieties in order to identify the most similar ones

✓ PVP Office – technical staff:
  1 Coordinator
  6 Examiners
  Examination of applications and Granting of Plant Breeder’s Rights

✓ 1 Laboratory – Live Samples Storage (Seed or DNA)
  2 Experts

✓ Independent Test Lab
  DNA fingerprints of PBR and NLi varieties
  Support for identification of varieties

Soybean - Importance in Brazil

❖ Soybean - most important agricultural commodity
  Yield – 74 million tons
  863 varieties in NLi
  612 protected by PBR:
    - 50 new varieties protected/year

“Narrow” genetic base of Brazilian soybean varieties
Relatively large number of protected varieties
Level of variation on PQ characteristics (environment)
  Increasingly arise the difficulties to establish distinctness with reliability
Identification of Genetic Profiles of SSR markers in DNA

- **Objectives** - Identification of varieties
  - Enforcement of Seed Law (cooperative work within Departments of MAPA)
  - Seed Certification
  - PBR and NLi Varieties
  - Comparison of "new" and "old" varieties
  - Post control

- **Control samples**
  - SNPC provided coded samples
  - Doubled samples from SNPC
  - Negative Control – water / Positive Control – laboratory control sample
  - 2 DNA extractions (bulks of 50 young leaves)
  - Genetic Analysis by 2 different staff in different days – minimize human error

- **SSR Markers**
  - Highly informative
  - Extensively validated in scientific literature

Statistical Analysis

- **Loci polymorphism analysed through PCR**
  - Primers marked with fluorescence blue (FAM), green (HEX) and yellow (NED)

- **Alleles Detection**
  - High resolution Capillary Electrophoresis
  - DNA automated sequencing – ABI Prism 3100

- **Size of Alleles**
  - Estimated by algorithm “Local Southern” – Software Genotyper
  - Discrete allele sizes – Least Squares Minimization Algorithm – Allelobin
  - Di, tri and tetranucleotides – variations of 1.5 pb between different runs and 0.5 pb in the same run – positive control in all tests allows to identify deviations

- **Genetic Similarity**
  - Genetic distance between pairs of entries – NTSYSpc 2.10z
  - Diagonal Matrix of Genetic Distances – UPGMA
  - Dendogram of Genetic Distances - NTSYSpc 2.10z
Organization of official trials

SNPC regularly performs trials for Post Control and to check candidate varieties' characteristics

**2009**
Construction of a Database with soybean varieties DNA profiles
- 556 Samples \(\times\) 15 SSR loci

**2010**
Post Control Trials of varieties with identical genetic profiles
- 690 Samples \(\times\) 15 SSR loci
- 4 protected varieties with identical DNA fingerprints
  Included in a side by side trial on the field
Organization of official trials

For the majority of the cases, 15 SSR markers allow the differentiation of samples profiled.

When genetic distance between the varieties is small and phenotypic differences are weak, included in side by side trials followed by SNPC examiners.
Field Trials

Distinctness test (siblings) – time to maturity

Field Trials

Identical DNA profiles – growth habit, time to maturity
Article 6(1)(c) of the 1961/1972 and 1978 UPOV Acts:
a variety is deemed uniform if it is “sufficiently
homogeneous, having regard to the particular features of
its sexual reproduction or vegetative propagation.”

Article 8 of the 1991 Act
a variety is uniform if, “subject to the variation that
may be expected from the particular features of its
propagation, it is sufficiently uniform in its relevant
characteristics”.

Soybean – self pollinated diploid specie, it is expected
that the tested genotypes should be typically homozygote
in each loci, e.g. one allele duplicated per loci.

When two distinct alleles are observed in one loci,
may be an evidence of residual heterozygosis or mixed
lines, and in this cases, additional field tests are needed.