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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

**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
DEPARTAMENT OF INTELLECTUALPROPERTY AND AGRICULTURAL TECHNOLOGY
NATIONAL PLANT VARIETY PROTECTION SERVICE**

**WORKING GROUP ON BIOCHEMICAL AND MOLECULAR
TECHNIQUES AND DNA PROFILING IN PARTICULAR**

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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 ExaminersExamination 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



Sat_038	Dinucl.	O	ctccaattgagactcta	gttctttaacaacactcactt
Satt586	Trinucl.	-	gcggcctccaactccaaglat	gcgcccaaatgattaactca
Satt045	Trinucl.	E	tggttctacttctataattatt	atgcctctccctcct
Satt042	Trinucl.	A1	gacttaattgcttgctatga	gtgggacacactcactt
Satt070	Trinucl.	B2	taaaaataaaataactagaagacaac	tggcattagaaaatgatatg
Satt038	Trinucl.	G	gggaatcttttttcttataagtt	gggcattgaaatggtttagtca
Satt030	Trinucl.	F	aaaagtgaaccaagcc	tctaaatctatgtgatgc
Satt005	Trinucl.	D1	tatcctagagagaactaaaaaa	gtcgattaggcttgaaata
GMABAB	Dinucl.	N1	caaacataaaaaaggtagaga	aagaacgcacactaatattatt
Satt002	Trinucl.	D2	tgtagggtaaaatagataaaaat	tcatittgaatcgttgaa
Satt009	Trinucl.	N1	ccaactgaaatactagagaaa	cttactagcgtattaaccctt
S45035	Dinucl.	D1	tttgaacgatagaaaatttat	aggggaaaattttaaaga
Satt100	Trinucl.	C2	acctcattttggcataaa	ttggaaaacaagtaataataaca
Satt114	Trinucl.	F	gggttatctccccaata	atatgggatgataagggtgaaa
Satt046	Trinucl.	K	aaaataactaaaatgtctctca	ttggcagattattataagattg



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

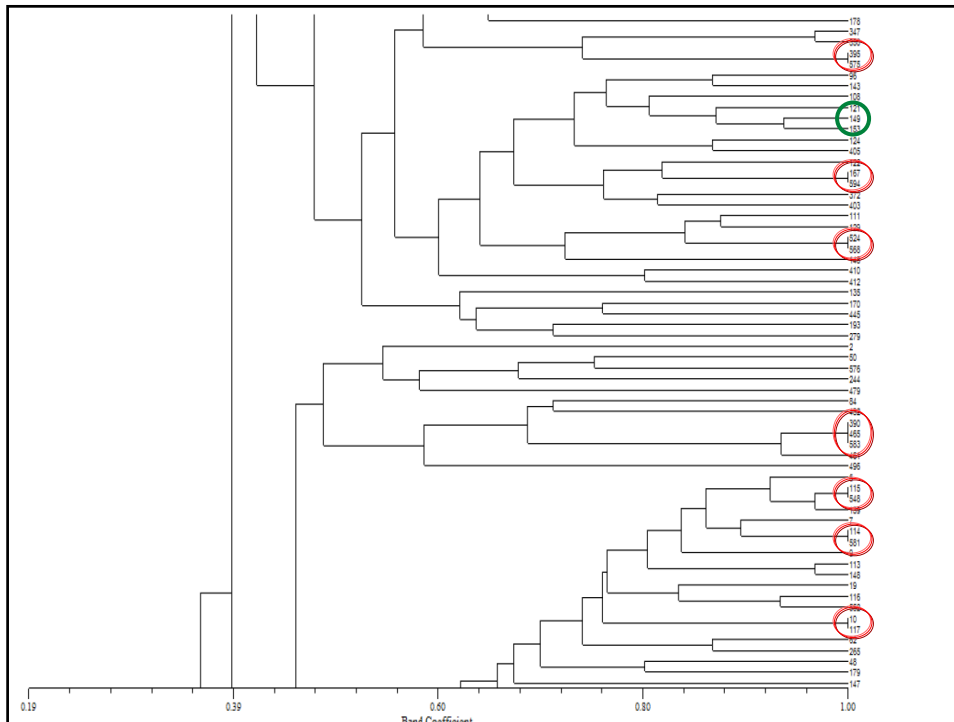
➤ 556 Samples x 15 SSR loci

2010 Post Control Trials of varieties with identical genetic profiles

➤ 690 Samples x 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.



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