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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR SOYBEAN

First Session
Rio de Janeiro, Brazil, September 27, 2002

REPORT

adopted by the Ad hoc Crop Subgroup on Molecular Techniques for Soybean

Opening of the Session

1. The *Ad hoc* Crop Subgroup on Molecular Techniques for Soybean (hereinafter referred to as "the Subgroup") held its first session in Rio de Janeiro, Brazil, on September 27, 2002. The list of participants is reproduced in the Annex to this report.

2. The session was opened by Mr. Marcelo Labarta (Argentina), Interim Chairman of the Subgroup, who welcomed the participants.

Adoption of the Agenda

3. The Subgroup adopted the agenda as reproduced in document BMT-TWA/Soybean/1/1.

Report of Discussions and Developments in UPOV Regarding Possible Use of Molecular Techniques in DUS Testing

4. The Technical Director of UPOV introduced documents TC/38/14-CAJ/45/4, TC/38/14 Add. – CAJ/45/4 Add., BMT/7/2, BMT/7/3.

Report of Work on Molecular Techniques on Soybean

Development and Genetic Analysis of a Database of Multilocus Microsatellite DNA Fingerprints of the Brazilian Protected Soybean Varieties

5. Mr. Grattapaglia introduced document BMT-TWA/1/3, explaining that 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, and that he expected that genetic profiles of highly polymorphic molecular markers would 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, he had 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 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. He added that microsatellite markers are the international standard used in human forensic genetics.

6. 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 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 was necessary to test the hypothesis of the occurrence of a relatively rare, but possible, mutation or to confirm distinctness by the observation of further genetic differences. Three samples showed clear evidence of a mixture of lines and one sample had evidence of residual heterozygosity. 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 a 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.

7. It was concluded that this work pioneered 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. It was thought that 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 used immediately 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.

8. An expert from France reminded the participants that plant varieties were groups of plants, even from different generations (seed lots), and, unlike humans and animals, were not unique individuals. She considered that the same approach for the use of molecular markers for human

and plant varieties would not always be valid. The expert from Brazil clarified that different sources of seed had been used in the study, but he considered that the problems arose when differences in molecular marker profiles were found between two varieties which could not be confirmed by morphological characteristics used in traditional DUS testing. Another expert from France pointed out that the aim of the UPOV system was to promote the creation of variability by encouraging plant breeding. He added that the aim was not to develop techniques which sought to identify very small differences to allow protection of very similar varieties. The expert from Denmark considered that it would be useful to have parallel information on morphological data from the varieties studied. The experts from France considered that it would also be interesting to have information about the origin and pedigree of the varieties included in the study. An expert from Hungary wondered what the situation would be in relation to the enforcement of the plant breeders' rights in the case of two different varieties having the same profile for molecular markers.

Summary of the SSR Soybean Research for DUS Testing Developed by the Molecular Markers Laboratory at the Former *Instituto Nacional de Semillas* (INASE), Argentina:

9. Mr. Marcelo Labarta introduced document BMT-TWA/Soybean/1/2. This work summarized the application of microsatellite markers to characterize and differentiate 271 soybean varieties and landraces of commercial use in Argentina, Bolivia and China. Those countries provided examples of large and small soybean producers with limited genetic variability (Argentina and Bolivia) and a large producer, which is a center of origin for soybean (China). Distinctness was assessed by obtaining a unique genotypic profile of varieties using 20-33 microsatellite markers. Uniformity was assessed by analyzing the relative degree of heterogeneity for microsatellite alleles. By more detailed analysis of seven selected varieties, it was shown that tolerance values used for morphological characteristics would need to be modified to allow molecular markers to be used for protection purposes. Stability was checked over a four-year period by comparing microsatellite patterns of a group of seven Argentine varieties of prolonged commercial use, provided by 32 microsatellite markers. Detailed analysis suggests that the observed "instability" could be attributed to: high mutation rate of the microsatellite loci; a mixture of seeds; cross-pollination, or; alleles that have not been detected before.

10. The main conclusions were that :

- the analysis allowed a fingerprint (unique genotypic identity profile) to be obtained for almost all analyzed varieties.
- In theory, 4-6 SSR would be sufficient for the characterization of soybean genotypes. However, for closely related genotypes, more than 20 SSRs would be needed to distinguish all genotypes efficiently.
- This analysis shows that the number of SSR to be used for distinctness should be carefully chosen to assure a good genotype differentiation, and to avoid identical or similar fingerprints of closely related genotypes.
- The similarity values found using SSR might allow consideration of a possible threshold (of 0.8 or a value close to it), above which a variety would be considered to be uniform.
- If SSR or other DNA markers are to be used for DUS testing, the current number of off-type plants allowed would need to be revised.

- Selection of markers for uniformity and stability testing should take into account two issues. Firstly, use of "neutral" markers may not be as appropriate, in practice, as the use of "trait" characteristics, which assure the farmer of homogeneous agronomic characteristics of the seeds they purchase. Secondly, the frequency of pattern changing of certain microsatellite loci may be higher than for morphological descriptors, suggesting differences in genomic backgrounds or instabilities that are not real.
- Markers might be applicable for germplasm classifications for plant breeders' rights. The analysis of a larger number of SSR and representative varieties would be necessary to establish which and how many SSRs would be adequate for variety registration.

11. An expert from France noted that the work had been undertaken in a set of varieties which had been *a priori* declared as distinct in a DUS examination. He considered it would be interesting to see results from "non distinct" varieties, but recognized that it would be difficult to find such pairs of "non distinct" varieties. An expert from Brazil explained that the technique used (silverstaining) might not be precise enough to obtain different profiles for pairs of closely related varieties or mutations.

Future Work

12. The Subgroup discussed whether the information provided might allow consideration of molecular markers within any of the three options outlined for possible use in DUS testing (see document TC/38/14-CAJ/45/4). The expert from Denmark considered that the information presented at the meeting was enough to consider options 2 or 3. The expert from Germany suggested further research was necessary in order to examine a possible correlation between molecular markers and morphological data as required for option 2.

13. In relation to the three options mentioned above, experts concluded as follows:

Option 1: all the molecular markers used for the two papers presented at the meeting were selected independently of morphological characters, therefore this option was not applicable at the moment.

Option 2: the papers presented gave information which might be developed into a useful approach under this option. However, further research was necessary to develop a harmonized set of molecular markers and provide a correlation between the molecular marker profiles and morphological characteristics.

Option 3: This option was not at a stage of development to be considered further at the moment.

14. The expert from Brazil expressed his willingness to obtain information from other countries in order to develop a common set of molecular markers and study correlation with morphological characteristics. The expert from Denmark considered that this was a necessary step towards the creation of an international DNA database. Experts agreed that several countries could provide information to a database but emphasized that it was necessary to define a harmonized protocol for molecular markers in order to obtain similar DNA profiles between countries and laboratories.

15. An expert from France proposed two concrete actions: a) to define a protocol for molecular markers which could also include the initial development of a database and; b) to use the data (molecular markers and morphological) available at that moment for the purposes of correlation between molecular marker profiles and morphological characteristics in order to progress within option 2. He added that France was developing a tool – the GAÏA software - to calculate phenotypic distance in the examination of distinctness, which would be available in the near future (see documents TWA/30/15 and TGP/9/3.2 Draft 1). The expert from Germany considered that the two countries which had presented papers had large collections of soybean varieties and, if a given set of molecular markers, could provide consistent results with different gene pools, it would be a good step forward. Experts from France offered to contribute data.

16. On the basis of discussions on the documents above, the Crop Subgroup proposed the following future program of work:

(a) the experts from Argentina and Brazil to exchange information on molecular markers used in their respective studies and to seek to develop a common set of molecular markers for use in future studies;

(b) the experts from Argentina and Brazil to study the correlation between morphological data and molecular markers, in the frame of an "Option 2" approach. This study would include morphological data to be provided by the experts from France and would use the GAÏA software for the assessment of phenotypic distance, also to be provided by experts from France;

(c) subject to progress on points (a) and (b) above, the Soybean Crop Subgroup to hold another meeting in association with the thirty-second session of the TWA to be held in Tsukuba, Japan, from September 8 to 12, 2003.

17. This report has been adopted by correspondence.

[Annex follows]

ANNEX

LIST OF PARTICIPANTS

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