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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR SUGARCANE

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

REPORT

adopted by the Ad Hoc Crop Subgroup on Molecular Techniques for Sugarcane

Opening of the Session

1. The *Ad hoc* Crop Subgroup on Molecular Techniques for Sugarcane (hereinafter referred to as "the Subgroup") held its first session in Rio de Janeiro, Brazil, on September 28, 2002. The list of participants is reproduced in the Annex to this report.

2. The session was opened by Mr. Luis Salaices (Spain), 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/Sugarcane/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 Sugarcane

Analysis of Genetic Similarity Detected by AFLP and the Coefficient of Parentage Among Genotypes of Sugarcane (*Saccharum spp.*) (Brazil)

5. Mrs. Milena de Luna Alves Lima introduced document BMT-TWA/Sugarcane/1/2. She explained that the objectives of that study had been to assess the level of genetic similarity (GS) among the sugarcane cultivars and to investigate the correlation between amplified fragment length polymorphism (AFLP)-based GS and coefficient of parentage (*f*), based on pedigree information. The expert had studied 79 cultivars (interspecific hybrids), used as parents in one of the Brazilian breeding programs, and four species of *Saccharum (Saccharum sinens*e, *Saccharum barberi* and two of *Saccharum officinarum*). She explained that twenty-one primer combinations had been used to obtain the AFLP molecular markers, generating a total of 2,331 bands, of which 1,121 had been polymorphic, with an average polymorphism rate of 50% per primer combination. GS's had been determined using Jaccard's similarity coefficient, and a final dendrogram had been constructed using an unweighted pair-group method, using arithmetic average (UPGMA). The analysis of the results showed that AFLP-based GS ranged from 0.28 to 0.89, with a mean of 0.47, whereas *f* ranged from 0 to 0.503, with a mean of 0.057. Cluster analysis, using GS, divided the genotypes into related subgroups suggesting that there was important genetic relationship among the cultivars. Mrs. Lima concluded that AFLP-based GS and *f* were significantly correlated *(r* = 0.42, *P* < 0.001), thus the significance of this *r* value suggested that the AFLP data may help to quantify, more accurately, the degree of relationship between sugarcane cultivars.

6. Experts from France wondered about the reproducibility of AFLP technique among laboratories. It also expressed concern that patent rights on AFLP techniques might limit their availability for DUS testing. The experts also noted that the study might be more useful to the assessment of essential derivation than for DUS testing. The expert from Brazil explained that a ring test had proved the repeatability of the technique used. The Technical Director of UPOV clarified that the assessment of essentially derived varieties was, in principle, a matter to be agreed between breeders rather than to be decided by the national authority.

7. An expert from France highlighted the results showing better precision when the parentage was higher. An expert from Brazil explained that the work had been requested by breeders with the aim of identifying the genetic distance between materials which might be used as parents for the development of new varieties. At the request of the Chairman, Mrs. Lima explained that work to identify microsatellites was underway. The expert from Australia recalled that microsatellites had been developed through an international consortium and that COPERSUCAR from Brazil was involved in this.

DNA Profiling in Sugarcane: Progress Toward Evaluation of SSR as a Tool (Australia)

8. Mr. Piperidis introduced document BMT-TWA/1/3. He explained that his presentation was the continuation of the work presented in document BMT/7/6 "DNA Profiling in Sugarcane: Implications for Varietal Protection". The objective of the work was to develop standard protocols for DNA profiling in sugarcane, which could ensure international reproducibility. He clarified that the work was being carried out by 6 laboratories in different countries and that, at that stage, a list of 20 varieties and 14 primer pairs had been agreed. Among the difficulties for harmonization that he had encountered during the study, Mr. Piperidis noted: the quality of scanned images; different size markers; incorrect scoring and data entry; scoring outside the

expected range and; mislabelling of varieties. As a requirement for standard protocols for sugarcane he suggested the following criteria: primers with simple profiles; limit the range of scoring markers and; the need to specify PCR components and conditions. It was noted that, as well as it being important to have UPOV recognition of the technique once properly developed, the industry placed great importance on the research. He concluded that more results were expected by April 2003, when a sugarcane molecular biology workshop was scheduled in Montpellier, France. During this workshop, it was planned that all interested groups would discuss future directions for continuing this work toward harmonized protocols for DNA profiling of sugarcane.

9. The Technical Director of UPOV noted that the general requirements of a standard protocol for DNA profiling could be an issue for discussion at the BMT itself and the expert from Denmark pointed out the usefulness of data validation for these purposes. Experts from France supported the idea of harmonization and requested information about the assessment of stability with these techniques. The expert from Australia replied that stability had not been specifically tested, but he reported that samples which had been taken from 10 different regions had always shown the same DNA profile. He added that, in the international experiment, each country had used its own samples where possible, but in future experiments it might be more useful to distribute common DNA extractions. He explained that the database which had been developed at that moment included data from 110 cultivars. Experts from Denmark, France and Germany expressed their interest in studying the correlation between molecular markers and morphological characteristics, and it was agreed that the GAÏA program could be used, when available, to calculate morphological distance in this analysis.

Future Work

10. Several experts considered that it was important to have an updated document explaining in simple terms the methods for DNA profiling, and pointing out possible advantages and disadvantages of each one for DUS purposes. An expert from Brazil explained that Brazilian experts were in contact with Mr. Mike Cox (Australia) to set up a common set of varieties as example varieties. The expert from Australia later explained that it was he, not Mike Cox, who should be contacted to set up a common set of varieties as example varieties. It was also proposed that Mrs. Lima and Mr. Piperidis make contact with the aim of testing both (AFLP and SSR) techniques with the same set of varieties, once they had been established. Nevertheless he considered that it was a lot of work and would not be ready before year 2004. Mr. Piperidis supported the proposal and considered that both techniques could add something to the research.

Matters Arising from the 2002 Sessions of the Technical Working Parties, Document TC/39/3

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

(a) UPOV to produce an updated explanation and analysis of available molecular methods, summarizing their advantages and disadvantages for use in DUS testing.

(b) the expert from Australia to prepare a draft standard protocol for the use of molecular markers, to be submitted to the BMT. The requirements for a standard protocol were likely to include: primers with simple profiles, limiting the range of scoring markers

and the need to specify PCR components and conditions. The standard protocol would also address issues such as: the scanning of images; different marker sizes; incorrect scoring and data entry; scoring outside the expected range and; mislabeling of varieties.

(c) the experts from Australia and Brazil to study the correlation between morphological data and molecular markers, in the frame of an "Option 2" approach. The initial aim would be to include 20-30 varieties representing the range of variation within UPOV and for the samples to be obtained from a single source to minimize environmental variation. It was hoped that it would be possible to include a pair(s) of varieties which were not distinct on the basis of morphological characteristics. The study would use the GAÏA software, to be provided by experts from France, for the assessment of phenotypic distance;

(d) the experts from Australia and Brazil to collaborate in the development of a common set of example varieties for which morphological descriptions will be developed, to be completed by 2004;

(e) subject to progress in the study of a possible "Option 2" approach, the Sugarcane 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.

12. This report has been adopted by correspondence.

[Annex follows]

ANNEX

LIST OF PARTICIPANTS

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[End of Annex and of document]