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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR MAIZE

Second Session
Chicago, United States of America, December 3, 2007

REPORT

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

Opening of the Session

1. The *Ad hoc* Crop Subgroup on Molecular Techniques for Maize (hereinafter referred to as the "Crop Subgroup for Maize") held its second session in Chicago, United States of America, on December 3, 2007. The list of participants is reproduced in the Annex to this report.

2. The session was opened by Mrs. Beate Rücker (Germany), Chairperson of the Crop Subgroup for Maize.

Adoption of the Agenda

3. The Crop Subgroup for Maize adopted the agenda as reproduced in document BMT-TWA/Maize/2/1.

Developments in UPOV concerning biochemical and molecular techniques

4. The Crop Subgroup for Maize received a report from the Office of the Union on developments in UPOV concerning biochemical and molecular techniques based on document BMT-TWA /Maize/2/2. A copy of the presentation is reproduced as document BMT-TWA/Maize/2/2 Add.

Molecular techniques in the examination of distinctness, uniformity and stability (DUS)

5. The Office of the Union provided an overview on UPOV guidance for DUS examination based on document BMT-TWA /Maize/2/10.

6. The Crop Subgroup for Maize considered document BMT-TWA /Maize/2/8, presented by Mr. Stephen Smith (Pioneer Hi-Bred International Inc.). The representative of ISF wondered if the type of information obtained by such an exercise might be useful for selecting asterisked characteristics in the UPOV Test Guidelines. He also noted that the approach of combining morphological and molecular information might be considered for the management of reference collections under an "Option 2" approach. Mr. Smith clarified that the aim of the paper was to make some research on characteristics in maize, but that the approach presented might be helpful in the selection of asterisked characteristics and for the management of reference collections, should anyone wish to explore that possibility. An expert from the United States of America noted that molecular markers might provide information which was less influenced by variation due to the environment. Mr. Smith, whilst recognizing that environmental variation could be a problem for morphological characteristics, considered that, before using molecular markers for DUS examination, it was necessary to have a clear understanding of the genetics of the molecular markers involved and to examine the impact it might have on the level of protection. He also clarified that any use of molecular markers for DUS examination should not lead to higher demands on uniformity. An expert from France explained that the type of data recorded (notes or measurements) was important for DUS purposes and highlighted that the variability of the data also needed to be taken into account. He requested an explanation for those morphological characteristics which had shown high discrimination power individually, but low discrimination power when considered together by pairs. Mr. Smith explained that that indicated that both characteristics were reflecting the same difference between varieties.

7. Mr. Smith presented document BMT-TWA /Maize/2/9. An expert from France requested clarification in relation to slide 17, concerning the extent to which decisions on plant breeders' rights could be made on the basis of molecular markers. Mrs. Janice Strachan (Senior Examiner, Plant Variety Protection Office, United States of America) reported that, at that time in the United States of America, molecular markers had been used to provide supplementary data but no plant breeder's right had been granted solely on the basis of molecular marker data. However, she explained that there was no legal basis to exclude such a possibility. In relation to slide 3, an expert from France recalled that the assessment of distinctness was based on differences between varieties, whereas variety identification was based on similarities. He welcomed the clear and informative analysis in the presentation, but noted that it was based on hybrids and wondered if the results would be applicable for other types of varieties. Mr. Smith explained that molecular markers had also proved useful in establishing parentage in soybean and non-hybrid maize varieties. The expert from France noted that, whilst there could be problems with variation due to the environment for morphological characteristics, there was also a problem with the robustness of molecular

marker data. It had previously been suggested that SSR data would be robust, however that had not turned out to be completely true: now it was being claimed that SNP data would be robust and he wondered if that assumption might also be questioned. Mr. Smith noted that SSRs relied on migration rates, which could vary according to conditions, whereas SNPs provided a "digital" system which should be more robust than other markers, and also noted that they were cheaper than SSRs. The representative from ISF agreed that molecular markers might be cheaper than field trials, however it was important to take into account the impact of their use on the level of protection. He considered that, if a difference in a single molecular marker was accepted as for distinctness, it would undermine the plant breeder's right, which would not be acceptable for breeders. Nevertheless, he considered that molecular markers could be used for the assessment of essential derivation.

8. The Crop Subgroup for Maize considered document BMT/10/14 presented by Mr. Joël Guiard (France) and document BMT-TWA /Maize/2/6 presented by Mrs. Cécile Collonnier (France). A copy of Mrs. Cécile Collonnier's and Mr. Guiard's presentations are reproduced as documents BMT-TWA /Maize/2/6 Add. and BMT-TWA/Maize/2/11, respectively. With respect to document BMT-TWA /Maize/2/6, an expert from the United States of America noted some cases where the results of the isozyme analysis conformed to the hybrid formula of the varieties, but the results of the analysis by molecular markers did not. He wondered if such cases might be caused by site mutations (see paragraph 16 of document BMT-TWA /Maize/2/6) which could invalidate the use of such markers. Mrs. Collonnier explained that such cases might invalidate a particular marker but did not necessarily invalidate the approach presented in the document. In particular, she observed that breeders checked for stability in isozymes but, for the time being, that was not the case for molecular markers. In response to discussions on the availability of markers, Mr. Dominique Perret (Euralis Semences / SEPROMA) reported that the set of markers used by SEPROMA was publicly available. In reply to a question from an expert from ISF, Mr. Guiard explained that the morphological data provided by the breeder can be used in GAIA but for most cases it was necessary to use data obtained in the first growing cycle of the official DUS trials.

9. An expert from the Netherlands, considered that it might be useful to explore whether the differences between the results in morphological characteristics and molecular markers could be due to over-, or under-, performance of some morphological characteristics, which could have implications for an Option 2 approach. The representative of ISF observed that the two presentations demonstrated the difficulties in moving from the present system based on morphological characteristics to a new system which includes molecular markers. He explained that breeders had concerns about such a change. However, he noted that the work being carried out by GEVES might help to overcome those concerns when more information became available, whilst emphasizing that any development should be done on a crop-by-crop basis. He added that, at present, breeders did not check uniformity and stability for molecular markers, which might limit their use for variety identification. He referred to the developments in relation to SNPs and pointed out that a new system could use this type of markers. Finally, he enquired how GEVES intended to move forward with their proposal in the context of DUS testing. Mr. Guiard agreed that the introduction of molecular markers in DUS examination should be made step by step in a consensus approach, in particular involving breeders and the Community Plant Variety Office (CPVO). He explained that the characterization of the variety collection by molecular markers should be the first step and noted that this would be a large undertaking which might not be justified for other crops. He added that in France, in addition to maize, studies with molecular markers for DUS in barley, peach and vegetables were being considered. The Chairperson recalled that the management

of reference collections was the first step in the process of the assessment of distinctness. Mr. Guiard observed that the risk of moving to an approach using molecular markers in combination with morphological characteristics needed to be balanced against the weaknesses of the existing system. In that respect, he considered that, in future, there would be situations where it would not be possible to compare all candidate and reference varieties in a field trial.

10. The Chairperson observed that, from the technical point of view, there would be some relationship between the genetic distance used for the management of reference collections and the genetic similarity used for judging essentially derived varieties (EDVs) and for variety identification. The representative of ISF emphasized that the matters of EDV and variety identification were only considered after the granting of plant breeders' rights. Mr. Guiard observed that the matter of EDV would not necessarily involve the use of molecular markers, or might involve different molecular markers to those which might be used for the management of reference collections. He noted that the risk of allowing distinctness on the basis of molecular markers alone was that it could lead to many more cases of EDV.

Molecular techniques in variety identification

11. The Crop Subgroup for Maize considered document BMT-TWA /Maize/2/4 on the basis of a presentation by Mrs. Fengge Wang (China), a copy of which is reproduced as document BMT-TWA/Maize/2/4 Add.

12. In response to questions from the representative of ISF, Mrs. Fengge Wang reported that the markers presented in document BMT-TWA /Maize/2/4 would be published in December 2007. She explained that molecular markers were being used for the checking of variety identity, including in relation to value for cultivation and use (VCU) trials, and clarified that molecular markers were not being used for DUS testing. China was studying the possibility of using molecular markers for DUS testing.

13. An expert from ISF sought further information on the nature of the 230 cases of infringements of plant breeders' rights reported in maize (see document BMT-TWA/Maize/2/4 Add., slide 26). Mrs. Fengge Wang explained that those mostly related to cases between companies where it was alleged that one company was producing seed of another company's protected variety.

14. The Crop Subgroup for Maize considered document BMT-TWA /Maize/2/5 presented by Mrs. Elizabeth Jones (Pioneer Hi-Bred International Inc.), a copy of which is reproduced as document BMT-TWA/Maize/2/5 Add.

15. In reply to a question from an expert from the United States of America, Mrs. Jones explained that the algorithm method for selecting molecular markers for variety identification proved to be more powerful than other methods based on polymorphism and distribution of markers. She reported that there was good correlation between the results using the selected 16 markers and the set of 100 markers, but clarified that it would not be appropriate to use only 16 markers for the purposes of assessing EDV, because it would be too easy to "breed away" from such a small number. An expert from France questioned whether a difference in a single marker would be sufficient for a unique variety identification given the possibility of mutation. Mrs. Jones explained that mutation in SNPs was not such a problem as for SSRs because there was a lot of redundancy. In response to a question concerning the availability of the markers, Mrs. Jones reported that the markers were proprietary, but that Pioneer

Hi-Bred International Inc. would consider publishing those markers if UPOV or the American Seed Trade Association (ASTA) expressed an interest.

16. The representative of ISF noted that, in the conclusions in document BMT-TWA/Maize/2/5 Add., slide 13, the words “distinguishing among” should be replaced by “identifying”.

Molecular techniques in the assessment of essential derivation

17. The Crop Subgroup for Maize considered documents BMT-TWA /Maize/2/7-a, presented by Mr. Bernard Le Buanec (ISF), BMT-TWA/Maize/2/7-b, presented by Mr. Dominique Perret (Euralis Semences / SEPROMA), BMT-TWA /Maize/2/7-c and BMT-TWA /Maize/2/7-c Add., presented by Mr. Martin O. Bohn (University of Illinois), BMT-TWA /Maize/2/7-d presented by Mr. Stephen Smith (Pioneer Hi-Bred International Inc.), BMT-TWA /Maize/2/3 presented by Mrs. Elizabeth Jones (Pioneer Hi-Bred International Inc.) and BMT-TWA /Maize/2/7-a Add., presented by Mr. Michael A. Gumina (Pioneer Hi-Bred International Inc.).

18. Mr. Le Buanec explained that the series of documents (BMT-TWA/Maize/2/7-a to -d) presented the historical developments in ISF which led to the establishment of the ISF Guidelines for the Handling of a Dispute on Essential Derivation of Maize Lines. It was explained that the choice between the SEPROMA- or ASTA- marker set to be used for the assessment of EDV would be decided in the arbitration process. The representative of ISF reported that the relationship between the SEPROMA and ASTA marker sets would be discussed at an ISF meeting later that week. He noted that there was a good correlation between the results obtained from AFLP, RFLP, SSR and SNP markers, but emphasized that the thresholds for EDV might be different. For that reason, he noted that there was a need to establish a collection of original material which could be used for the calibration of new techniques. An expert from France noted that the same issue would arise in relation to the use of molecular markers in DUS testing. He also wondered about the free availability of SNP markers if they were to be used in relation to DUS testing. Mr. Smith observed that there were already around 3,000 published SNP markers and confirmed that Pioneer Hi-Bred International Inc. would publish its SNP markers. An expert from the United States of America wondered whether some of the assumptions on which document BMT-TWA /Maize/2/7-c were based, such as stochastic distribution of markers, might not be valid, in which case it might be possible to reduce the required numbers of marker. Mr. Bohn replied that there were many factors which might have an impact on the optimum number of markers, for example, meiosis frequency, length of chromosomes and the breeding method. He considered that the assumptions of the method were valid and clarified that for molecular markers Mendelian genetics were valid on average but might not be applicable for each individual. In reply to a question from the representative of ISF, Mr. Dominique Perret explained that the threshold presented in document BMT-TWA /Maize/2/7-b was based upon previous studies on restriction fragment length polymorphisms (RFLPs).

Proposals to the Technical Working Party for Agricultural Crops and the Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular

Molecular techniques in the examination of distinctness, uniformity and stability

19. The Crop Subgroup for Maize agreed to propose that the Technical Working Party for Agricultural Crops (TWA), the Technical Committee (TC) and the Administrative and Legal Committee (CAJ) discuss the acceptability of the approach presented in documents BMT/10/14 and BMT-TWA/Maize/2/11, and suggested that the approach might be put forward for consideration at the BMT Review Group as a potential option for the use of molecular markers in DUS examination. It noted that a possible timetable for that process could be for the TWA, TC and CAJ to consider the proposal at their respective sessions in 2008, with a view to convening a possible meeting of the BMT Review Group in April 2009.

20. The Crop Subgroup for Maize agreed that its discussions on document BMT-TWA /Maize/2/8, concerning the discriminatory power of morphological characteristics, should be reported to the TWA.

Molecular techniques in variety identification

21. The Chairperson observed that there had been substantial work on variety identification by breeders and research centers and noted that molecular markers were already being used by breeders in relation to possible cases of infringement.

Essentially derived varieties

22. The Chairperson noted the substantial progress which had been made within ISF on the matter of EDV and welcomed the opportunity which the Crop Subgroup for Maize had provided, by means of good breeder participation, to receive a comprehensive explanation of the developments which had taken place.

23. The Crop Subgroup for Maize agreed that its next session could take place in Autumn/Winter 2009, tentatively in conjunction with the maize and sorghum breeders' meeting in the United States of America. It anticipated that such a timetable would allow for the collection of further substantial data in relation to the approach presented in documents BMT/10/14 and BMT-TWA/Maize/2/11 and would also allow a report the views of the TWA, TC, CAJ and BMT Review Group on that approach.

24. The Crop Subgroup for Maize adopted this report by correspondence.

[Annex follows]

ANNEX

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