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**TECHNICAL WORKING PARTY FOR ORNAMENTAL PLANTS AND FOREST  
TREES**

**Forty-Third Session**  
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**DEVELOPMENTS IN UPOV CONCERNING THE USE OF MOLECULAR  
TECHNIQUES**

*Document prepared by the Office of the Union*

1. The purpose of this document is to report on developments concerning the:
  - (a) UPOV Guidelines for DNA-profiling: molecular marker selection and database construction (BMT Guidelines);
  - (b) proposals for the utilization of biochemical and molecular techniques in the examination of DUS considered by the BMT Review Group;
  - (c) revision of documents TC/38/14-CAJ/45/5 “*Ad Hoc* Subgroup of Technical and Legal Experts on Biochemical and Molecular Techniques (‘The BMT Review Group’)” and TC/38/14 Add.-CAJ/45/5 Add “Recommendations of the BMT Review Group and Opinion of the Technical Committee and the Administrative and Legal Committee Concerning Molecular Techniques”;
  - (d) practical exercise in the development of an exchangeable database;
  - (e) international guidelines on molecular methodologies;
  - (f) statistical methods for data produced by biochemical and molecular techniques;
  - (g) *Ad Hoc* Crop Subgroups on Molecular Techniques (Crop Subgroups); and
  - (h) Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular (BMT).
  
2. An overview of the UPOV bodies involved in the consideration of biochemical and molecular techniques is provided on the first restricted area of the UPOV website at [http://www.upov.int/restrict/en/upov\\_structure\\_index.html](http://www.upov.int/restrict/en/upov_structure_index.html). That overview is also attached as Annex I to this document.

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3. The following abbreviations are used in this document:

CAJ:	Administrative and Legal Committee
TC:	Technical Committee
TC-EDC:	Enlarged Editorial Committee
TWA:	Technical Working Party for Agricultural Crops
TWC:	Technical Working Party on Automation and Computer Programs
TWF:	Technical Working Party for Fruit Crops
TWO:	Technical Working Party for Ornamental Plants and Forest Trees
TWV:	Technical Working Party for Vegetables
TWP(s):	Technical Working Party(ies)
BMT:	Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular
BMT Review Group:	<i>Ad Hoc</i> Subgroup of Technical and Legal Experts on Biochemical and Molecular Techniques
Crop Subgroup:	<i>Ad Hoc</i> Crop Subgroup on Molecular Techniques

#### UPOV GUIDELINES FOR DNA-PROFILING: MOLECULAR MARKER SELECTION AND DATABASE CONSTRUCTION (BMT GUIDELINES)

4. At its eighth session held in Tsukuba, Japan, from September 3 to 5, 2003, the BMT concluded that there was an urgent need to harmonize methodologies for the generation of molecular data in order to ensure that the quality of the data produced would be universally acceptable for use in variety characterization. It was also noted that it would be useful to provide guidance on the planning of databases for molecular data based on different types of markers. On that basis, the BMT agreed that the Office of the Union should prepare a guidance document (BMT Guidelines).

5. At its forty-third session, held in Geneva, from March 26 to 28, 2007, the Technical Committee (TC) considered document BMT Guidelines (proj.8) and agreed to submit the BMT Guidelines to the Council for adoption.

6. At its seventy-fourth session, held in Geneva on October 24, 2007, the Consultative Committee made a preliminary examination of document BMT Guidelines (proj.9), proposed for adoption by the Council. The Consultative Committee made the following recommendations:

“[...]”

“(b) consideration be given to the status of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. with regard to their reference in the introduction of document BMT Guidelines (proj.9); and

“(c) in response to the comments made by some delegations concerning the need for editorial improvements, the Consultative Committee agreed that a circular be sent to the Consultative Committee, the Technical Committee (TC) and the CAJ providing an opportunity to comment within four weeks on documents TGP/4/1 Draft 10, TGP/9/1 Draft 10 and BMT Guidelines (proj.9). Based on the comments received, new drafts of those documents would be prepared for consideration by the [Enlarged] Editorial Committee (TC-EDC) at its meeting on January 8, 2008. Relevant drafts

incorporating the comments by the TC-EDC would subsequently be presented to the TC, the CAJ, the Consultative Committee and the Council in April 2008.”

7. In accordance with the recommendations of the Consultative Committee, Circular E-606 was issued, inviting comments on document BMT Guidelines (proj.9) to be sent to the Office of the Union.

8. The Office of the Union received comments from China, Ukraine and the United States of America on document BMT Guidelines (proj.9). Those comments were incorporated in document BMT Guidelines (proj.10), which was considered by the TC-EDC, at its meeting on January 8, 2008. The TC-EDC noted that a number of the comments received concerned technical aspects of the BMT Guidelines and concluded that those were matters which would need to be addressed by the TC in conjunction with the BMT, as considered appropriate.

9. At its forty-fourth session, held in Geneva from April 7 to 9, 2008, the TC noted the comments from China, Ukraine and the United States of America incorporated in document BMT Guidelines (proj.11). It noted that a number of the comments concerned technical aspects of the BMT Guidelines and concluded that, in the first instance, those were matters which would need to be addressed by the BMT. The TC agreed that the BMT should be invited to consider those matters in the form of a new draft of the BMT Guidelines at its eleventh session, to be held in Madrid, from September 16 to 18, 2008.

10. The TC noted the request of the Consultative Committee that consideration be given to the status of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. with regard to their reference in the introduction of document BMT Guidelines (proj.11). The TC noted that documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. would need to be reviewed in conjunction with discussions on the approach presented in documents BMT/10/14 and BMT-TWA/Maize/2/11, as explained in document TC/44/7, paragraph 30. On that basis, it agreed that it would be appropriate to submit a revised version of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. to the Council in conjunction with the BMT Guidelines.

11. At its fifty-seventh session, held in Geneva on April 10, 2008, the CAJ considered document BMT Guidelines (proj.11) and the oral report on the conclusions of the TC at its forty-fourth session. Based on that oral report of the conclusions of the TC at its forty-fourth session, the CAJ agreed that the BMT should be invited to consider a new draft of the BMT Guidelines at its eleventh session, to be held in Madrid from September 16 to 18, 2008. The CAJ noted that the TC had agreed that it would be appropriate to submit a revised version of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. to the Council in conjunction with the BMT Guidelines.

12. At its eleventh session, held in Madrid, from September 16 to 18, 2008, the BMT considered document BMT Guidelines (proj.12), and made its recommendations. Those recommendations were incorporated in document BMT Guidelines (proj.13), which was considered by the TC-EDC at its meeting on January 8, 2009.

13. The TC-EDC made no proposals to amend document BMT Guidelines (proj.13), as agreed by the BMT at its eleventh session.

14. The TC considered document BMT Guidelines (proj.14) at its forty-fifth session, held in Geneva from March 30 to April 1, 2009, and agreed that no changes were required to that document. However, it noted that the French, German and Spanish translations of the original English text would be checked by the relevant members of the Editorial Committee prior to submission of the document for adoption by the Council (see documents TC/45/7 and TC/45/16 “Report”, paragraphs 148 and 149).

15. The CAJ, at its sixtieth session, held in Geneva on October 19, 2009, considered document BMT Guidelines (proj.15) “Guidelines for DNA Profiling: Molecular Marker Selection and Database Construction” in conjunction with document CAJ/60/7. Document BMT Guidelines (proj.15) contained no changes in relation to document BMT Guidelines (proj.14), other than for the purposes of presenting the draft to the CAJ. The CAJ made no comments on document BMT Guidelines (proj.15).

16. The TC (see document TC/45/16 “Report”, paragraph 149) and CAJ (see document CAJ/60/10 “Report on the Conclusions”, paragraph 41) agreed that, on the basis of the conclusions of the TC and CAJ at their sessions in 2009, a draft of the BMT Guidelines should be prepared for approval by the TC and CAJ in March 2010, in anticipation of adoption of the BMT Guidelines by the Council in 2010. The TC noted that that timetable also anticipated the submission of a revised version of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. to the Council for adoption in conjunction with the BMT Guidelines (see below).

17. Document BMT Guidelines (proj.16), which contained no changes in relation to the text of documents BMT Guidelines (proj.14) and BMT Guidelines (proj.15), was considered by the TC at its forty-sixth session, held in Geneva from March 22 to 24, 2010, and by the CAJ at its sixty-first session, held in Geneva on March 25, 2010.

18. The TC agreed that document BMT Guidelines (proj.16) should be amended as indicated in the document, but agreed that no further modifications were required. The TC agreed that, subject to agreement by the CAJ, document BMT Guidelines (proj.16) should be put forward for adoption by the Council at its forty-fourth ordinary session. The TC noted that the French, German and Spanish translations of the original English text would be checked by the relevant members of the Editorial Committee prior to submission of the draft of document BMT Guidelines to the Council (see document TC/46/15 “Report on the Conclusions”, paragraphs 39 and 40).

19. The CAJ noted that no proposals for modification to document BMT Guidelines (proj.16) had been made by the TC at its forty-sixth session. The CAJ agreed that document BMT Guidelines should be put forward for adoption by the Council at its forty-fourth ordinary session, to be held in Geneva on October 21, 2010, on the basis of document BMT Guidelines (proj.16). It noted that the French, German and Spanish translations of the original English text would be checked by the relevant members of the Editorial Committee prior to submission of the draft of document BMT Guidelines to the Council (see document CAJ/61/11 “Report on the Conclusions”, paragraphs 58 and 59).

## PROPOSALS FOR THE UTILIZATION OF BIOCHEMICAL AND MOLECULAR TECHNIQUES IN THE EXAMINATION OF DUS CONSIDERED BY THE BMT REVIEW GROUP

### Background

20. At its forty-fourth session, held in Geneva from April 7 to 9, 2008, the TC agreed to propose to the CAJ that the approach presented in documents BMT/10/14 and BMT-TWA/Maize/2/11 “Possible use of molecular techniques in DUS testing on maize: how to integrate a new tool to serve the effectiveness of protection offered under the UPOV system”, prepared by experts from France, be put forward for consideration at the BMT Review Group as a potential option for the use of molecular markers in DUS examination (see document TC/44/13 “Report”, paragraph 152(c)).

21. At its fifty-seventh session, held in Geneva on April 10, 2008, the CAJ agreed with the proposal of the TC, that the approach presented in documents BMT/10/14 and BMT-TWA/Maize/2/11 should be put forward for consideration at the BMT Review Group as a potential option for the use of molecular markers in DUS examination (see document CAJ/57/7 “Report”, paragraph 28). The composition of the BMT Review Group is provided in Annex II to this document.

### Proposal considered by the BMT Review Group

22. At its meeting on April 1, 2009, the BMT Review Group considered document BMT-RG/Apr09/2 “Proposal for use of molecular techniques in DUS testing of Maize” and a presentation made by Mr. Joël Guiard (France), a copy of which is reproduced as document BMT-RG/Apr09/2 Add. The BMT Review Group concluded that the proposal to be considered was set out in the Annex to document BMT-RG/Apr09/2, subject to the addition of the slide in the presentation (BMT-RG/Apr09/2 Add., slide 11), concerning the visual assessment by maize crop experts of a scale of similarity, and the clarification of certain points (see document BMT-RG/Apr09/3 “Report”, paragraphs 7, 8 and 12).

23. The proposal considered by the BMT Review Group, on the basis set out above, is reproduced as Annex III to this document.

### Recommendations of the BMT Review Group

24. At its meeting on April 1, 2009 the BMT Review Group:

(a) concluded that the proposal in the Annex to document BMT-RG/Apr09/2 “Proposal: System for combining phenotypic and molecular distances in the management of variety collections”, incorporating the clarifications set out in document BMT-RG/Apr09/3, paragraphs 7 and 8 (Annex III to this document), where used for the management of variety collections, was acceptable within the terms of the UPOV Convention and would not undermine the effectiveness of protection offered under the UPOV system;

(b) agreed that the proposal in the Annex to document BMT-RG/Apr09/2 (Annex III to this document) represented a model that might be applicable to other crops provided that the elements of the proposal were equally applicable. In that respect, it noted, for example, that the proposal in the Annex to document BMT-RG/Apr09/2 (Annex III to

this document) applied only to maize parental lines and did not extend to other types of maize. The BMT Review Group concluded that it was important to consider on a case-by-case basis whether the model would be applicable; and

(c) noted that some of the elements of the proposal in the Annex to document BMT-RG/Apr09/2 (Annex III to this document) were similar to the Option 2 approach “Calibration of threshold levels for molecular characteristics against the minimum distance in traditional characteristics”, as set out in documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. However, the BMT Review Group concluded that it would not be appropriate to classify the proposal under Option 2 and agreed that the proposal should be referred to as the “System for combining phenotypic and molecular distances in the management of variety collections”.

### Opinion of the CAJ

25. The CAJ, at its sixtieth session, held in Geneva on October 19, 2009, endorsed the recommendations of the BMT Review Group, as set out above (see document CAJ/60/10 “Report on the Conclusions”, paragraph 43). The CAJ noted that the TC, at its forty-sixth session, would be invited to express its opinion on the recommendations of the BMT Review Group, in conjunction with the opinion of the CAJ.

### Opinion of the TC

26. The TC endorsed the recommendations of the BMT Review Group, as set above (see document TC/46/15 “Report on the Conclusions”, paragraph 42).

## REVISION OF DOCUMENTS TC/38/14-CAJ/45/5 AND TC/38/14 ADD.-CAJ/45/5 ADD.

### *Background*

27. Documents TC/38/14-CAJ/45/5 “*Ad Hoc* Subgroup of Technical and Legal Experts on Biochemical and Molecular Techniques (‘The BMT Review Group’)” and TC/38/14 Add.-CAJ/45/5 Add “Recommendations of the BMT Review Group and Opinion of the Technical Committee and the Administrative and Legal Committee Concerning Molecular Techniques”, summarize the consideration of possible application models proposed by the TC, on the basis of the work of the BMT and crop subgroups, for the utilization of biochemical and molecular techniques in the examination of Distinctness, Uniformity and Stability.

28. At its seventy-fourth session, held in Geneva on October 24, 2007, the Consultative Committee made a preliminary examination of document BMT Guidelines (proj.9), proposed for adoption by the Council. One of the recommendations of the Consultative Committee was that “consideration be given to the status of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. with regard to their reference in the introduction of document BMT Guidelines (proj.9)” .

29. With regard to the status of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add., the Consultative Committee, at its seventy-eighth session, held in Geneva on October 22, 2009, agreed that, unless otherwise agreed by the Council,

documents which set out UPOV policies or guidance, once approved by the relevant UPOV Committees, as appropriate, must be adopted by the Council. In cases where a rapid presentation of a UPOV policy or guidance is required, such that adoption could not be achieved by presentation of a document to the Council, approval would be sought by correspondence from the representatives to the Council of the members of the Union (see document C/43/16 “Report”, paragraph 14(i)).

30. At its forty-fourth session, held in Geneva from April 7 to 9, 2008, the TC noted the request of the Consultative Committee that consideration be given to the status of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. with regard to their reference in the introduction of document BMT Guidelines. The TC noted that documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. would need to be reviewed in conjunction with discussions on the approach presented in documents BMT/10/14 and BMT-TWA/2/11 “Possible use of molecular techniques in DUS testing on maize: how to integrate a new tool to serve the effectiveness of protection offered under the UPOV system” (see document TC/44/13 “Report”, paragraph 150). On that basis, it agreed that it would be appropriate to submit a revised version of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. to the Council in conjunction with the BMT Guidelines.

31. At its forty-fifth session, held in Geneva from March 30 to April 1, 2009, the TC recalled that, at its forty-second session, held in Geneva, from April 3 to 5, 2006, it had “reaffirmed its support for the presentation of the situation, set out in documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add., which presented the proposals developed in the *Ad hoc* Crop Subgroups, the recommendations of the BMT Review Group concerning those proposals and the opinion of the TC and the CAJ regarding the recommendations of the BMT Review Group. [...]”. Therefore, it did not consider that it would be appropriate to make major changes to the structure and form of the information provided in documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. However, to assist the Office of the Union in the preparation of the revision of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add., with the aim of developing a document for adoption by the Council, the TC agreed:

- (a) to consolidate document TC/38/14-CAJ/45/5, paragraphs 9 and 10 and the Annex, and document TC/38/14 Add.-CAJ/45/5 Add., paragraphs 3 to 7, into a single document;
- (b) subject to a positive assessment by the BMT Review Group of the approach presented in documents BMT/10/14 and BMT-TWA/Maize/2/11 and endorsement by the TC and CAJ, to add a section concerning the approach presented in documents BMT/10/14 and BMT-TWA/Maize/2/11; and
- (c) to emphasize the importance of the assumptions to be met in each of the options and proposals and to clarify that it is a matter for the relevant authority to consider if the relevant assumptions set out in documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. are met.

32. Subject to a positive assessment by the BMT Review Group of the approach presented in documents BMT/10/14 and BMT-TWA/Maize/2/11 and an endorsement by the CAJ at its sixtieth session, the TC agreed that a first draft of the revised version of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. should be prepared for consideration by the TC at its forty-sixth session and by the CAJ at its sixty-first session, both in



March 2010 (see document TC/45/16 “Report”, paragraphs 152 and 153). On that basis, the TC noted that a document could be presented for adoption by the Council in 2010, in conjunction with the BMT Guidelines.

33. The CAJ, at its sixtieth session, agreed that a first draft of the revised version of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. should be prepared for consideration by the TC at its forty-sixth session and by the CAJ at its sixty-first session, both in March 2010. On that basis, the CAJ noted that a document could be presented for adoption by the Council in October 2010, in conjunction with the BMT Guidelines (see document CAJ/60/10 “Report on the Conclusions”, paragraph 46).

34. In accordance with the procedure set out above, the Office of the Union prepared a revised version of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add., with the aim of developing a document for adoption by the Council. That document (document BMT/DUS Draft 1 “Possible Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)”) was presented to the TC-EDC, at its meeting on January 7, 2010.

35. The TC-EDC, at its meeting on January 7, 2010, did not comment on document BMT/DUS Draft 1 in detail, considering that it would be more appropriate for the TC to make a first assessment of that document at its forty-sixth session. On that basis, document BMT/DUS Draft 2, considered by the TC at its forty-sixth session, and by the CAJ at its sixty-first session, contained no changes to the text of document BMT/DUS Draft 1. However, the TC-EDC recommended that the TC consider whether the document might take on the reference “document TGP/15” (TGP/15 currently has the title “New Types of Characteristics”), subject to an appropriate change of title of document TGP/15.

36. At its forty-sixth session, held in Geneva from March 22 to 24, 2010 (see document TC/46/15 “Report on the Conclusions”, paragraphs 43 to 45), the TC agreed the following amendments to document BMT/DUS Draft 2:

Title	to read “Possible Use of Molecular Markers in the Examination of Distinctness, Uniformity and Stability (“DUS”)”
Structure	to differentiate the models into those which received a positive endorsement by the BMT Review Group, CAJ and TC and those where there was no consensus on their acceptability. Within the models which received a positive endorsement by the BMT Review Group, CAJ and TC, to consider a further separation of the models for which further work was required.
Title of models	to seek to develop a short title for each model

37. The TC noted that the CAJ, at its sixtieth session, had agreed that a document could be presented for adoption by the Council in October 2010, in conjunction with the document BMT Guidelines. However, the TC agreed that a new draft of the document BMT/DUS should be prepared by the Office of the Union, in conjunction with the TC Chairman and the BMT Chairman, for consideration by the BMT and the TWPs at their sessions in 2010 and a further draft prepared on the basis of the comments of the BMT, TWPs and CAJ for consideration by the TC at its forty-seventh session.

38. The TC agreed that the possibility of document BMT/DUS Draft 2 becoming document TGP/15, with an appropriate change of title for TGP/15, should be considered at a later stage.

39. The CAJ, at its sixty-first session, held in Geneva on March 25, 2010 (see document CAJ/61/11 “Report on the Conclusions”, paragraphs 60 to 62), noted the conclusions of the TC on document BMT/DUS Draft 2. The CAJ noted that the TC had agreed that a new draft of the document BMT/DUS should be prepared by the Office of the Union, in conjunction with the TC Chairman and the BMT Chairman, for consideration by the BMT and the TWPs at their sessions in 2010 and a further draft prepared on the basis of the comments of the BMT and TWPs for consideration by the TC at its forty-seventh session.

*Comments on document BMT/DUS Draft 3 by the BMT and the Technical Working Parties at their sessions in 2010*

40. This section contains the comments made on document BMT/DUS Draft 3 by:

(a) the BMT, at its twelfth session, held in Ottawa from May 11 to 13, 2010, considered document BMT/DUS Draft 3 (see document BMT/12/24 “Report”, paragraphs 7 and 8);

(b) the Technical Working Party for Agricultural Crops (TWA), at its thirty-ninth session, held in Osijek, Croatia, from May 24 to 28, 2010 (see document TWA/39/27 “Report”, paragraphs 29 to 32); and

(c) the Technical Working Party for Vegetables (TWV), at its forty-fourth session, held in Veliko Tarnovo, Bulgaria, from July 5 to 9, 2010 (see document TWV/44/34 “Report”, paragraphs 25 to 27)

41. The Technical Working Party on Automation and Computer Programs (TWC), at its twenty-eighth session, held in Angers, France, from June 29 to July 2, 2010, made no comments on document BMT/DUS Draft 3.

General	(BMT, agreed by TWA and TWV) to delete all references to the terms “Option” and “Proposal” and to replace with the terms “Model” and “Example”
	(BMT, agreed by TWA and TWV) to replace all references to “molecular characteristics” with an appropriate term such as “molecular markers”.  The TWA noted that “molecular markers” were a tool and concluded that the term “molecular markers” might not be an appropriate term to refer to the data or information generated by those markers. It considered that terms such as “molecular data”, “molecular marker data” and “molecular polymorphism”, should be considered.  The TWV agreed that the term “molecular data” would be a suitable broad term.
	(TWA, agreed by TWV) to seek to develop shorter names for the models and to avoid any use of numbering in association with the models, i.e. to remove the indications of 3.1.1, 3.1.2, 3.1.3 and

	3.2.1.
3.1.2	<p>(BMT, agreed by TWA and TWV) to clarify that the phenotypic distance is based on phenotypic characteristics and to indicate that the GAIA threshold would need to be selected on a case-by-case basis.</p> <p>The TWV noted that the model “System for combining phenotypic and molecular distances in the management of variety collections” would not necessarily require the GAIA method to be used to calculate phenotypic distance, but noted that any other method would need to be based on a similar “combination of differences observed on phenotypic characteristics, where each difference contributes to the distance according to the reliability of the characteristics, especially regarding its variability and its susceptibility to environment” (see document BMT/DUS Draft 3, Annex 4, Section 1.4.1) in order to fall within the model.</p>
3.1.3	<p>(BMT, agreed by TWA) to read “Calibration of molecular distances in the management of variety collections (see Annex 2)”</p> <p>The TWV agreed that the title should read “Calibration of molecular and traditional distances in the management of variety collections (see Annex 2)” or “Calibration of distances in the management of variety collections (see Annex 2)”</p>

42. The BMT agreed that document TGP/15 should be developed separately, but in parallel, to document BMT/DUS. The content of document BMT/DUS would be similar to BMT/DUS Draft 3, i.e. it would explain the development and consideration of all models within UPOV. However, document TGP/15 would contain only models that had received a positive assessment and for which accepted examples could be provided, i.e. Models “Characteristic-specific molecular markers” (Section 3.1.1) and “Combining phenotypic [characteristics] and molecular distances in the management of variety collections” (Section 3.1.2) for the time being.

43. The TWA and TWV agreed that document TGP/15 should be developed separately, but in parallel, to document BMT/DUS on the basis that document BMT/DUS would provide a report on the development and consideration of all models within UPOV and that document TGP/15 would provide guidance for the use of those models that had received a positive assessment and for which accepted examples could be provided, i.e. Models “Characteristic-specific molecular markers” (Section 3.1.1) and “Combining phenotypic [characteristics] and molecular distances in the management of variety collections” (Section 3.1.2) for the time being. The TWA and TWV agreed that the purpose of both documents should be clarified within the documents and noted that both documents would need to be adopted by the Council. The TWA and TWV agreed that consideration should be given to how to maintain both documents in an efficient way.

#### PRACTICAL EXERCISE IN THE DEVELOPMENT OF AN EXCHANGEABLE DATABASE

44. At the twenty-third session of the Technical Working Party on Automation and Computer Programs (TWC), held in Ottawa, Canada, from June 13 to 16, 2005, and at the

ninth session of the BMT, held in Washington D.C., United States of America, from June 21 to 23, 2005, Mr. Sylvain Grégoire (France), drafter of Section 6 “Databases” of the BMT Guidelines, suggested that it would be useful to move forward with a practical exercise, involving a small number of crops, in the development of an exchangeable database. He noted that, from an IT perspective, such an exercise would be straightforward, but that it would require all participating partners to identify the markers to be used and to clarify and agree on the status of the information to be included in the database and the accessibility of that data, e.g. to contributing partners or to all interested experts from members of the Union.

45. At its forty-second session, held in Geneva from April 3 to 5, 2006, the TC agreed to investigate the possibility of a practical exercise, involving a small number of crops, in the development of an exchangeable database. It agreed that it would be necessary to set clear terms of reference for that work and agreed that such terms of reference should be considered at its forty-third session. In the meantime, it agreed to invite the BMT, at its tenth session, to suggest suitable crops where such a practical exercise might be appropriate.

46. At its tenth session, held in Seoul, Republic of Korea, from November 21 to 23, 2006, the BMT agreed to suggest oilseed rape, potato and rose as suitable crops where a practical exercise in the development of an exchangeable database might be appropriate. It was agreed that the terms of reference to be established by the TC for that work should clarify what was meant by an exchangeable database and whether it referred to the structure of the database or the quality of the data and whether it would involve a test data set rather than the complete set of data which an authority had for the crop concerned. At its forty-third session, the TC agreed that the *Ad Hoc* Crop Subgroups on Molecular Techniques (Crop Subgroups) for Rose, for Potato and for Oilseed Rape should be invited to consider how to take that matter forward. With respect to the terms of reference for such an exercise, the TC agreed that the exercise should consider both the quality and structure of the data.

47. The Crop Subgroup for Potato held its second session in Quimper, France, on April 17, 2007. It agreed that it would be useful for the experts working on the Community Plant Variety Office of the European Community (CPVO) project and at the French Federation of Potato Seed Growers (FNPPPT) to cooperate in order to investigate the compatibility of data obtained using different technologies (see documents BMT-TWA/potato/2/2, BMT-TWA/potato/2/2 Add., BMT-TWA/potato/2/4 and BMT-TWA/potato/2/4 Add. at [http://www.upov.int/restrict/en/bmt\\_cropsubgroups/potato\\_2.htm](http://www.upov.int/restrict/en/bmt_cropsubgroups/potato_2.htm)).

48. At the thirty-sixth session of the Technical Working Party for Agricultural Crops (TWA), held in Budapest, Hungary, from May 28 to June 1, 2007, an expert from the United Kingdom reported that NIAB was working on the use of molecular techniques for variety identification in potato. The TWA agreed that it would be useful for that expert to contact the coordinator of the CPVO project who was discussing with the *Institut national de la recherche agronomique* (INRA, France) the possibility to cooperate in order to investigate the compatibility of data obtained using different technologies.

49. At its second session, held in Angers, France, on April 18, 2007, the Crop Subgroup for Rose was informed that the TC had invited BMT Crop Subgroup for Rose to consider how to proceed with a practical exercise in the development of an exchangeable database. The Crop Subgroup for Rose did not have any proposals with regard to such an exercise.

50. At its eleventh session, held in Madrid, from September 16 to 18, 2008, the BMT agreed that it would be more appropriate to change the title of the agenda item “Practical

exercise in the development of an exchangeable database of molecular data of plant varieties” to “Development of common database structure for molecular data” (see document BMT/11/29 “Report”, paragraph 112).

51. At its forty-fifth session, held in Geneva from March 30 to April 1, 2009, the TC noted from the developments reported in document TC/46/7 “Molecular Techniques” and those reported in document TC/45/9 “Publication of Variety Descriptions”, that members of the Union were developing databases containing morphological and/or molecular data and, where considered appropriate, were collaborating in the development of databases for the management of variety collections, particularly on a regional basis. The TC agreed that it could be beneficial to offer the possibility for members of the Union to report on that work in a coherent way to the TC, the Technical Working Parties (TWPs) and the BMT. On that basis, the TC agreed to replace the various agenda items concerning such databases with an item for “Variety description databases” on the agendas of the forthcoming sessions of the TC, TWPs and the BMT. In that respect, it recalled the importance of the list of criteria for consideration for the use of descriptions obtained from different locations and sources as set out in document TC/45/9, paragraph 3. The TC also agreed that the information presented would not need to be related to the publication of descriptions (see document TC/45/16 “Report”, paragraph 173).

#### INTERNATIONAL GUIDELINES ON MOLECULAR METHODOLOGIES

52. At its tenth session, held in Seoul, Republic of Korea, from November 21 to 23, 2006, the BMT discussed the BMT Guidelines. In relation to Section B: 5.2 “Quality criteria”, the BMT was informed that the International Organization for Standardization (ISO) and the Codex Alimentarius Commission were developing guidelines. The BMT agreed that it would be useful to invite relevant experts to make a presentation on those guidelines at the eleventh session of the BMT.

53. At the forty-fourth session of the TC, held in Geneva from April 7 to 9, 2008, Mrs. Katalin Ertsey (Hungary), President of the Executive Committee of the International Seed Testing Association (ISTA), reported that ISTA and the Organisation for Economic Co-operation and Development (OECD) were jointly working on harmonization in molecular markers for variety verification and confirmed that ISTA would be glad to make a presentation on that subject at the eleventh session of the BMT. The TC agreed to invite ISTA and OECD to make a presentation on their joint work on harmonization in the development of sets of markers which might be used for variety verification, at the eleventh session of the BMT.

54. At its eleventh session, held in Madrid, from September 16 to 18, 2008, the BMT received a presentation by ISO, based on document BMT/11/25 and a presentation by Ms. Selma Doyran, Senior Food Standard Officer, Food and Agriculture Organization of the United Nations (FAO), based on document BMT/11/26.

55. At its eleventh session, the BMT was informed that ISTA was unable to attend the BMT session but had provided some information: there was no formal collaboration arrangements between ISTA and OECD regarding harmonization in the development of sets of markers to be used for variety verification. In 2007, the ISTA Variety Committee had established a Working Group on DNA-Based Methods, which had finalized its first

comparative test with SSR markers to distinguish varieties of several species. First results had been reported at the ISTA Annual Meeting in June 2008.

56. At its forty-fifth session, the TC noted the developments concerning international guidelines on molecular methodologies, as set out above.

57. The Technical Working Party for Agricultural Crops (TWA), at its thirty-eighth session, held in Seoul, Republic of Korea, from August 31 to September 4, 2009 noted that ISTA had been unable to attend the eleventh session of the BMT, held in Madrid, from September 16 to 18, 2008. It agreed that the Office of the Union should contact ISTA to investigate how the work of UPOV and ISTA might, where appropriate, be coordinated. On that basis, the Vice Secretary-General of UPOV wrote to Mr. Muschick, Secretary General of ISTA on October 29, 2009, to explore where the work of UPOV and ISTA might, where appropriate, be coordinated in that regard. It was suggested that, in the first instance, a report on ISTA's activities in this area could be made at the twelfth session of the BMT, to be held in Ottawa, Canada, from May 11 to 13, 2010.

#### STATISTICAL METHODS FOR DATA PRODUCED BY BIOCHEMICAL AND MOLECULAR TECHNIQUES

58. At the eleventh session of the BMT, held in Madrid, from September 16 to 18, 2008, no papers were presented for the item on statistical methods for data produced by biochemical and molecular techniques.

59. The BMT agreed that it would be more appropriate to replace the item with an item on methods for analysis of molecular data to cover, for example, calculation of distances. It noted that the TWC might wish to consider that matter.

60. The TC, at its forty-fifth session, agreed to request the TWC to consider if the item on the BMT agenda for "statistical methods for data produced by biochemical and molecular techniques" should be replaced by an item "methods for analysis of molecular data" to cover, for example, calculation of distances.

61. At the twenty-seventh session of the TWC, held in Alexandria, Virginia, United States of America, from June 16 to 19, 2009, several participants considered that the TWC could provide assistance to the BMT in the development of statistical tools as well as databases of molecular markers and encouraged cooperation between the TWC and BMT.

62. At its forty-sixth session, held in Geneva from March 22 to 24, 2010, the TC noted that the item on the BMT/11 agenda for "statistical methods for data produced by biochemical and molecular techniques" had been replaced by an item "methods for analysis of molecular data" in the agenda for the twelfth session of the BMT.

#### AD HOC CROP SUBGROUPS ON MOLECULAR TECHNIQUES (CROP SUBGROUPS)

63. There have been no meetings of the Crop Subgroups since 2008.

#### Chairmanship of the Crop Subgroup for Oilseed Rape

64. The TC, at its forty-sixth session, approved Mrs. Laetitia Denecheau (France) as new Chairperson of the Crop Subgroup for Oilseed Rape.

#### Planned Meetings of the Crop Subgroups

65. The TC agreed the following plans for meetings of Crop Subgroups:

Crop Subgroup for Maize: no subgroup meeting planned. The TC noted that the Office of the Union had contacted the American Seed Trade Association (ASTA) to see if it would be interested in receiving a report on the outcome of the consideration within UPOV on the approach presented in documents BMT/10/14 and BMT-TWA/Maize/2/11 “Possible use of molecular techniques in DUS testing on maize: how to integrate a new tool to serve the effectiveness of protection offered under the UPOV system” at the meeting of the maize and sorghum breeders’ meeting in the United States of America in 2010;

Crop Subgroup for Oilseed Rape: no subgroup meeting planned;

Crop Subgroup for Potato: no subgroup meeting planned. To consider a future meeting according to developments in on-going projects reported at the eleventh session of the BMT;

Crop Subgroup for Soybean: to consider a meeting to include a report on the work of Argentina and Brazil in the context of a possible Option 2 approach in due course; and

Crop Subgroup for Wheat and Barley: no subgroup meeting planned. Any on-going work, such as the work in France on barley in the context of a similar approach to that presented for maize in document BMT/10/14, would be reported at the twelfth session of the BMT.

#### WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES, AND DNA-PROFILING IN PARTICULAR (BMT)

66. It is recalled that, in order to encourage the presentation of information in relation to the use of molecular techniques in the consideration of essential derivation and in variety identification, the BMT agreed at its tenth session that it would be appropriate to dedicate a specific day to the agenda items “The use of molecular techniques in the consideration of essential derivation” and “The use of molecular techniques in variety identification”, at the eleventh session of the BMT. In particular, breeders and other experts would be offered the possibility to attend for that specific day (the “Breeders’ Day”). At its eleventh session, the BMT proposed to continue that approach for its twelfth session.

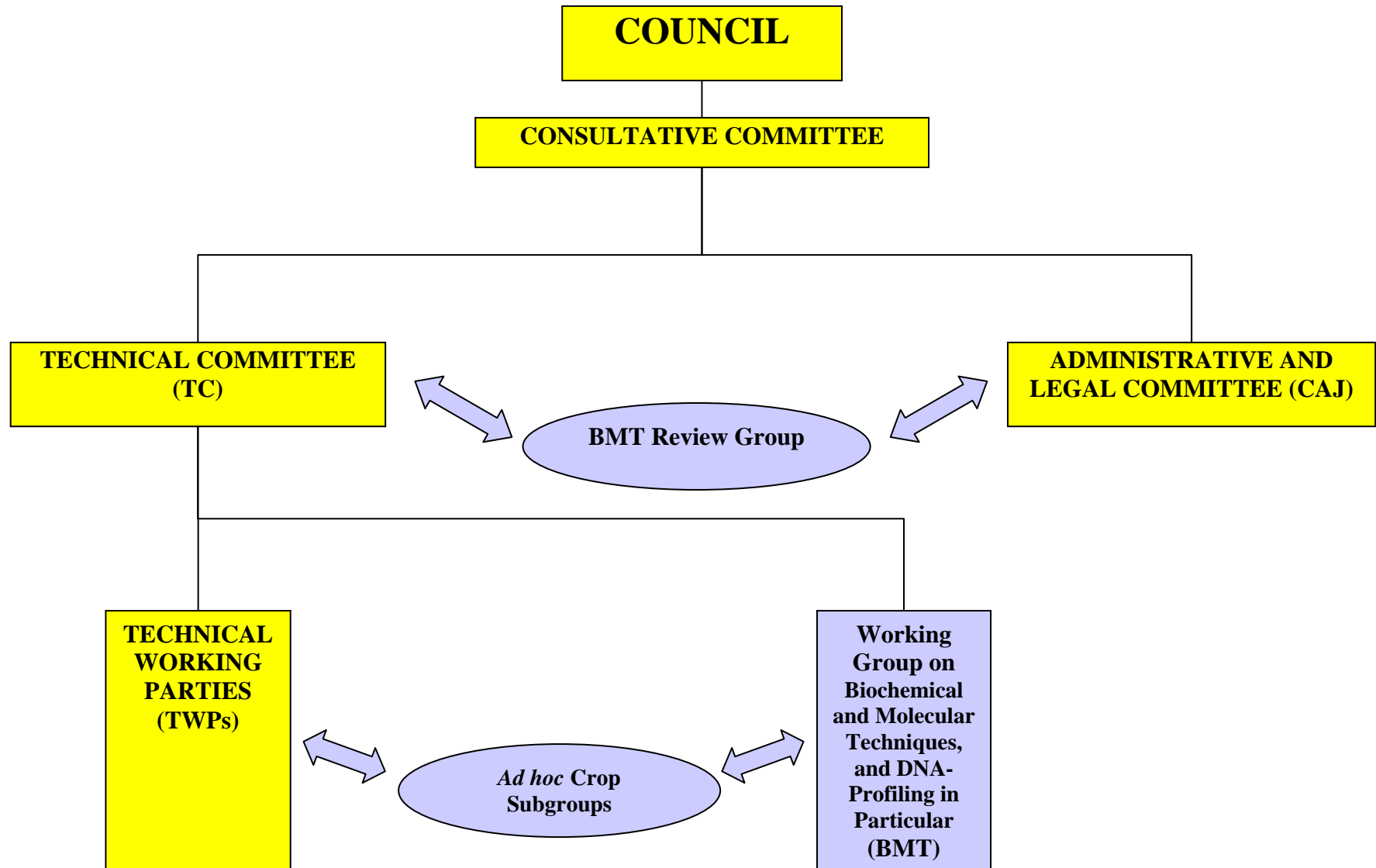
67. The twelfth session of the BMT was held in Ottawa, Canada, from May 11 to 13, 2010, with the preparatory workshop on May 10, 2010. The specific day for the agenda items “The

use of molecular techniques in the consideration of essential derivation” and “The use of molecular techniques in variety identification” (the “Breeders’ Day”) was May 11, 2010.

[Annex I follows]



# UPOV Structure: Biochemical and Molecular Techniques



**ROLE OF THE  
WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES, AND  
DNA-PROFILING IN PARTICULAR (BMT)**

*(as agreed by the Technical Committee at its thirty-eighth session, held in Geneva,  
from April 15 to 17, 2002 (see document TC/38/16, paragraph 204))*

The BMT is a group open to DUS experts, biochemical and molecular specialists and plant breeders, whose role is to:

- (i) Review general developments in biochemical and molecular techniques;
- (ii) Maintain an awareness of relevant applications of biochemical and molecular techniques in plant breeding;
- (iii) Consider the possible application of biochemical and molecular techniques in DUS testing and report its considerations to the TC;
- (iv) If appropriate, establish guidelines for biochemical and molecular methodologies and their harmonization and, in particular, contribute to the preparation of document TGP/15, "New Types of Characteristics." These guidelines to be developed in conjunction with the Technical Working Parties;
- (v) Consider initiatives from TWPs, for the establishment of crop specific subgroups, taking into account available information and the need for biochemical and molecular methods;
- (vi) Develop guidelines regarding the management and harmonization of databases of biochemical and molecular information, in conjunction with the TWC;
- (vii) Receive reports from Crop Subgroups and the BMT Review Group;
- (viii) Provide a forum for discussion on the use of biochemical and molecular techniques in the consideration of essential derivation and variety identification.

**TERMS OF REFERENCE OF *AD HOC* SUBGROUP OF TECHNICAL AND LEGAL  
EXPERTS ON BIOCHEMICAL AND MOLECULAR TECHNIQUES  
("BMT REVIEW GROUP")**

*(as agreed by the Administrative and Legal Committee at its forty-third session,  
held on April 5, 2001 (see document CAJ/43/8, paragraph 58))*

1. The BMT Review Group should assess possible application models proposed by the Technical Committee, on the basis of the work of the BMT and crop subgroups, for the utilization of biochemical and molecular techniques in the examination of Distinctness, Uniformity and Stability in relation to the following:
  - (a) conformity with the UPOV Convention, and
  - (b) potential impact on the strength of protection compared to that provided by current examination methods and advise if this could undermine the effectiveness of protection offered under the UPOV system.
2. In conducting its assessment, the BMT Review Group may refer specific aspects to the Administrative and Legal Committee or the Technical Committee for clarification or further information as considered appropriate.
3. The BMT Review Group will report its assessment, as set out in paragraph 1 above, to the Administrative and Legal Committee, but this assessment will not be binding for the position of the Administrative and Legal Committee.

## **AD HOC CROP SUBGROUPS ON MOLECULAR TECHNIQUES (CROP SUBGROUPS)**

At its thirty-sixth session, held in Geneva, from April 3 to 5, 2000, the Technical Committee agreed to the creation of the *Ad hoc* Crop Subgroups proposed by the BMT at its sixth session, held in Angers, France from March 1 to 3, 2000 (see document TC/36/11, paragraph 123).

*Extract from document TC/36/3 Add.*

“23. [At its sixth session, held in Angers, France from March 1 to 3, 2000] The BMT agreed that real progress could not be expected without intensive discussion in small groups on specific species. It therefore decided to propose establishing *ad hoc* crop subgroups during the eighteen month interval until the next session to make real progress in discussions on possibilities and consequences of the introduction of molecular techniques in DUS testing, the management of reference collection and the judgement of essential derivation.

“24. The BMT discussed the role of *ad hoc* crop subgroups and its relationship with the Technical Working Parties. It agreed that testing experts in the Technical Working Party should be involved with the discussion in the *ad hoc* crop subgroups. It also agreed that the chairmen of the *ad hoc* crop subgroups should be chosen from experts in the Technical Working Party in question. The role of the *ad hoc* crop subgroups would not be to make any decisions, but to prepare documents that could be a basis of further discussions in the BMT, the Technical Working Parties and the Technical Committee. The BMT confirmed that the Technical Working Parties should be the decision-making bodies for the introduction of new characteristics into DUS testing for each species.

[...]

“26. The BMT discussed the selection of species for the subgroups. A majority of experts supported two criteria, (i) the need for the introduction of molecular techniques in DUS testing (species for which a limited number of characteristics are available and species which urgently need effective methods for the management of reference collection) and (ii) the availability of DNA profiling data and on-going studies.”

At its forty-third session, held in Geneva, from March 26 to 28, 2007, the Technical Committee agreed to invite the Crop Subgroups to develop proposals concerning the possible use of molecular tools for variety identification in relation to the enforcement of plant breeders' rights, technical verification and the consideration of essential derivation.

The list of Crop Subgroups established by the Technical Committee (TC) is as follows:

<u>Crop Subgroup for:</u>	<u>TWP</u>	<u>Chairperson</u>	<u>TC Session which established</u>
<b>Maize</b>	TWA	Mrs. Beate Rücker (Germany)	thirty-sixth session (2000)
<b>Oilseed Rape</b>	TWA	Mrs. Laetitia Denecheau (France)	thirty-sixth session (2000)
<b>Potato</b>	TWA	Mrs. Beate Rücker (Germany)	thirty-eighth session (2002)
<b>Rose</b>	TWO	Mr. Joost Barendrecht (Netherlands)	thirty-sixth session (2000)
<b>Ryegrass</b>	TWA	Mr. Michael Camlin (United Kingdom)	forty-second session (2006)
<b>Soybean</b>	TWA	Mr. Marcelo Labarta (Argentina)	thirty-eighth session (2002)
<b>Sugarcane</b>	TWA	Mr. Luis Salaices (Spain)	thirty-eighth session (2002)
<b>Tomato</b>	TWV	Mr. Richard Brand (France)	thirty-sixth session (2000)
<b>Wheat and Barley</b>	TWA	Mr. Michael Camlin (United Kingdom)	thirty-sixth session (2000) / forty-second session (2006)

[Annex II follows]

ANNEX II

BMT REVIEW GROUP

- Chairman: Mr. Rolf Jördens (Office)
- Members: Ms. Carmen Gianni (AR and Chair of the CAJ)  
Mr. Doug Waterhouse (AU and President of the Council)  
Mr. Bart Kiewiet / Mr. Carlos Godinho (European Union)  
Mr. Michael Köller (DE)  
Ms. Nicole Bustin (FR)  
Mr. Joël Guiard (FR)  
Mr. Yasuhiro Kawai (JP)  
Mr. Henk Bonthuis (NL) (ex-Chairman of the BMT)  
Mr. Chris Barnaby (NZ) (Chairman of the TC)  
Mr. Michael Camlin (GB)  
Mr. Andy Mitchell (GB and Chairman of the BMT)  
Mrs. Beate Rücker (DE) (Chairperson of the *Ad Hoc* Crop Subgroup on  
Molecular Techniques for Maize)
- Observers: International Community of Breeders of Asexually Reproduced Ornamental  
and Fruit-Tree Varieties (CIOPORA)  
International Seed Federation (ISF)
- Office: Mr. Peter Button  
Mr. Raimundo Lavignolle  
Mr. Makoto Tabata  
Mrs. Yolanda Huerta

[Annex III follows]

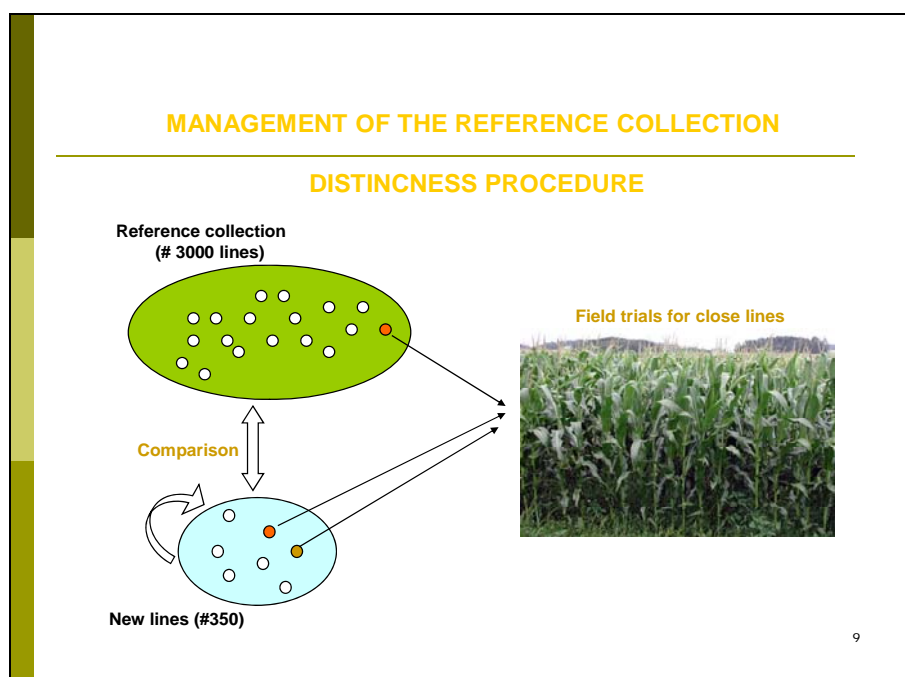
Proposal: “System for combining phenotypic and molecular distances  
in the management of variety collections”

*considered by the Ad hoc Subgroup of Technical and Legal Experts of Biochemical and  
Molecular Techniques (BMT Review Group) at its meeting on April 1, 2009*

1. Description

- 1.1 A key feature of the process of eliminating varieties of common knowledge prior to the DUS growing trial is that the threshold for deciding which varieties can be safely excluded (i.e. are distinct on the basis of descriptions), can be set with a suitable margin of safety, because those varieties which are eliminated, will not be included in the growing trial. This threshold, with a safety margin, is termed the “Distinctness plus” threshold which means that the distances between a candidate variety and “distinct plus” varieties are robust enough to take a decision without direct comparison in the growing trial.
- 1.2 The objective of this proposal is to develop an efficient tool, based on a combination of phenotypic and molecular distances, to identify within the variety collection, those varieties which need to be compared with candidate varieties (see Figure 1) in order to improve the selection of “distinct plus” varieties and so to limit the workload without decreasing the quality of the test. The challenge is to develop a secure system that:
- only selects varieties which are similar to the candidate varieties; and
  - limits the risk of not selecting a variety in the variety collection which needs to be compared in the field, especially when there is a large or expensive variety collection.

*Figure 1*



- 1.3 The new system has been elaborated on the following background:

- (a) Studies done on molecular distances in maize for DUS testing and essential derivation, which showed the link with the parentage between varieties (see documents BMT/3/6 “The Estimation of Molecular Genetic Distances in Maize or DUS and ED Protocols: Optimization of the Information and new Approaches of Kinship” and document BMT/3/6 Add.)
- (b) An experiment done by GEVES on a set of maize parental lines, which showed that there is a link between the evaluation of distinctness by experts (global assessment) and a molecular distance computed on Simple Sequence Repeat (SSR) molecular data (see Figure 2).

#### 1.4 Components of the system

##### 1.4.1 GAIA distance

The GAIA distance component is computed with the GAIA software developed by GEVES. The GAIA distance is a combination of differences observed on phenotypic characteristics, where each difference contributes to the distance according to the reliability of the characteristics, especially regarding its variability and its susceptibility to environment. The larger the size of the difference and the greater the reliability of the characteristic, the more the difference contributes to the GAIA distance. Only differences that are equal or larger than the minimum distance required for each individual characteristic are included.

##### 1.4.2 Molecular distance

The molecular distance component is computed on the differences observed on a set of markers. Different types of molecular markers and distances can be used. In the case of the study done in France on maize, 60 SSR markers and Roger’s distance have been used. It is important that sufficient markers, with a good distribution on the chromosomes, are used. The type of markers, the effect of the number of markers and the distribution of the markers need to be considered according to the species concerned.

1.4.3 Before combining these two components, an evaluation of the link between molecular distance and a global assessment of distinctness by a panel of experts needs to be done on a set of pairs of varieties. In the case of maize, that evaluation was made on the following basis:

Material : 504 pairs of varieties tested in parallel with molecular markers

Field design : pairs of varieties grown side by side  
(1 plot = 2 rows of 15 plants)

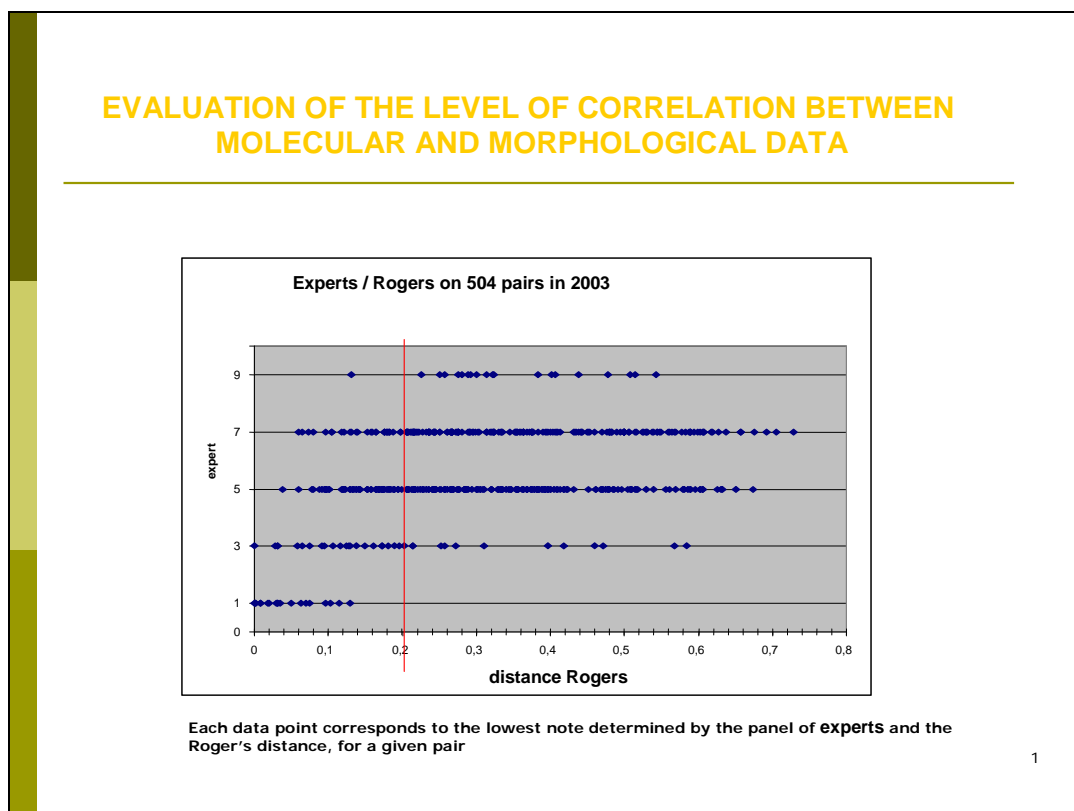
Visual assessment by maize crop experts:

Scale of similarity:

1. the two varieties are similar or very close
  3. the two varieties are distinct but close
  5. the comparison was useful, but the varieties are clearly distinct
  7. the comparison should have been avoided because the varieties are very different
  9. the comparison should have been avoided because the varieties are totally different
- (“even” notes are not used in the scale)

In the case of maize, this evaluation showed that no parental lines with a molecular distance greater than 0.15 were considered as similar or very close by a DUS expert evaluation (see Figure 2).

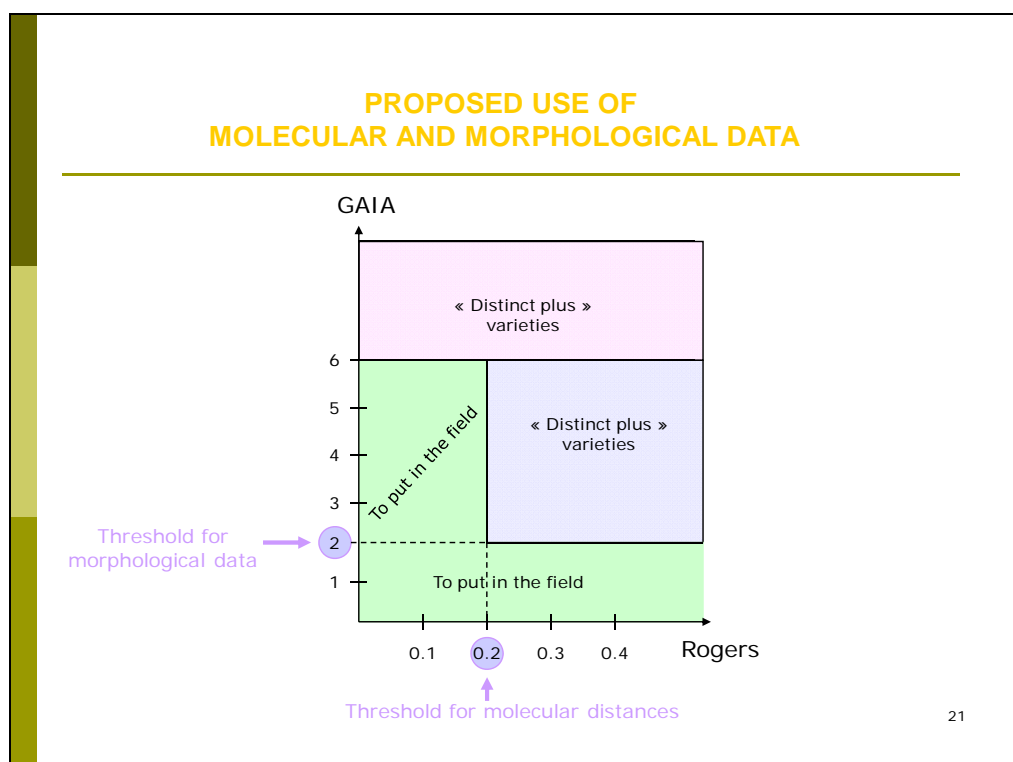
Figure 2





1.4.4 On the basis of that result, the combination of morphological and molecular distances offers the possibility to establish a decision scheme as follows (see Figure 3):

Figure 3



1.4.5 All pairs of varieties with a GAIA distance equal to, or larger than, 6 and all varieties with a GAIA distance between 2 and 6, plus a molecular distance equal to, or larger than, 0.2 are declared “Distinct plus”.

1.4.6 This scheme shows that less parental lines need to be observed in the field compared to the situation where only a GAIA distance of 6 is used on its own.

1.4.7 The robustness of this system has been studied with different GAIA and molecular distances.

## 2. Advantages and constraints

### 2.1 Advantages

- (a) Improvement of the management of variety collections with less varieties needing to be compared in the field;
- (b) Use of morphological and molecular distances with thresholds defined by DUS experts. GAIA was also calibrated against DUS experts' evaluations when developed by GEVES;
- (c) Use of molecular data that are not susceptible to the environment; the set of markers and the laboratory protocol are well defined;
- (d) Use of only phenotypic characteristics with a good robustness and possibility to use descriptions coming from different origins under close cooperation (The maize database that has been developed in cooperation between Germany, France,

Spain and the Community Plant Variety Office of the European Union (CPVO) is a good example to illustrate the value of this approach with a variety collection shared between different offices);

- (e) Electrophoresis characteristics can also be replaced; and
- (f) There is no influence of lack of uniformity in molecular profiles provided enough markers are used and the number of variants is low. In the case of maize parental lines, the level of molecular uniformity is high but could be a problem in some other crops.

## 2.2 Constraints

- (a) Not efficient, or less efficient, for species with synthetic varieties or populations;
- (b) Necessity to have enough good DNA markers and enough phenotypic characteristics with low susceptibility to environment; and
- (c) Preliminary work with calibration in comparison with DUS expert evaluation of distinctness.

[End of Annex III and of document]