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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

ADMINISTRATIVE AND LEGAL COMMITTEE

Sixty-First Session
March 25, 2010

MOLECULAR TECHNIQUES

Document prepared by the Office of the Union

1. The purpose of this document is to report and consider 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 Distinctness, Uniformity and Stability considered by the *Ad hoc* Subgroup of Technical and Legal Experts of Biochemical and Molecular Techniques (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"; and

(d) twelfth session of the Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular (BMT), to be held in Ottawa, Canada, from May 11 to 13, 2010

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*. That overview is also attached as Annex I to this document.

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:	*Ad Hoc* Subgroup of Technical and Legal Experts on Biochemical and Molecular Techniques
Crop Subgroup:	*Ad Hoc* Crop Subgroup on Molecular Techniques

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

4. The Technical Committee (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).

5. 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).

6. 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).

7. Document BMT Guidelines (proj.16) contains no changes in relation to documents BMT Guidelines (proj.14) and BMT Guidelines (proj.15), other than for the purposes of presenting the draft to the TC at its forty-sixth session, to be held in Geneva from March 22 to 24, 2010, and the CAJ at its sixty-first session, to be held in Geneva on March 25, 2010.

8. Any changes to the text of document BMT Guidelines (proj.16) proposed by the TC at its forty-sixth session will be reported to the CAJ at its sixty-first session. Subject to agreement of a common text by the TC and the CAJ, document BMT Guidelines (proj.16) will be put forward for adoption by the Council at its forty-fourth ordinary session, to be held in Geneva on October 21, 2010. The French, German and Spanish translations of the original English text will be checked by the relevant members of the Editorial Committee prior to submission of the draft of document BMT Guidelines to the Council.

9. The CAJ is invited to consider document BMT Guidelines (proj.16) as the basis for adoption of document BMT Guidelines by the Council, as set out in paragraph 8.

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

Background

10. At its forty-fourth session, 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)).

11. 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

12. 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).

13. The proposal considered by the BMT Review Group, on the basis set out in paragraph 12, is reproduced as Annex III to this document.

Recommendations of the BMT Review Group

14. 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

15. The CAJ, at its sixtieth session, held in Geneva on October 19, 2009, endorsed the recommendations of the BMT Review Group, as set out in paragraph 14, 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

16. At its forty-sixth session, to be held in Geneva from March 22 to 24, 2010, the TC will be invited to note the opinion of the CAJ at its sixtieth session, concerning the recommendations of the BMT Review Group, as set out in paragraph 15 and to express its opinion concerning the recommendations of the BMT Review Group, as set out in paragraph 14. The opinion of the TC will be reported to the CAJ at its sixty-first session.

17. The CAJ is invited to note that the opinion of the TC, at its forty-sixth session, concerning the recommendations of the BMT Review Group, as set out in paragraph 16, will be reported to the CAJ at its sixty-first session.

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

18. 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.

19. 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)" .

20. 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)).

21. 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.

22. 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.

23. 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 (see paragraph 8 above).

24. 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).

25. In accordance with the procedure set out in paragraphs 21 to 23, 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.

26. 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, to be considered by the TC at its forty-sixth session, and by the CAJ at its sixty-first session, contains 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. A report of the conclusions of the TC will be reported to the CAJ at its sixty-first session.

27. The CAJ is invited to consider document BMT/DUS Draft 2 in conjunction with the conclusions of the TC.

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

28. 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, held in Seoul, Republic of Korea, from November 21 to 23, 2006, 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

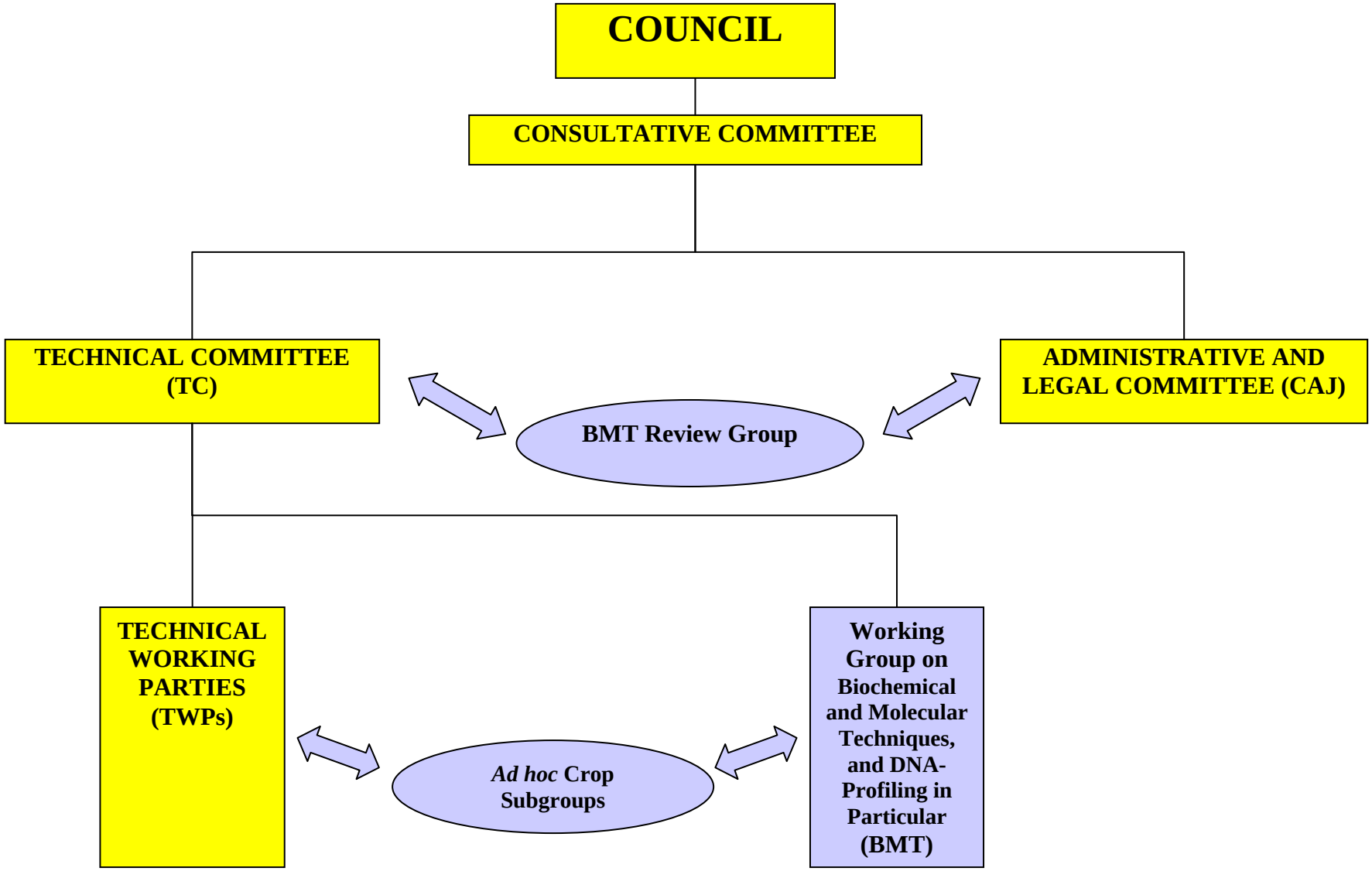
that specific day (the "Breeders' Day"). At its eleventh session, the BMT proposed to continue that approach for its twelfth session.

29. The twelfth session of the BMT will be held in Ottawa, Canada, from May 11 to 13, 2010, with the preparatory workshop to be held 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") will be May 11, 2010.

30. The CAJ is invited to note that, at the twelfth session of the BMT, to be held in Ottawa, Canada, from May 11 to 13, 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") will be 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:

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

ANNEX III

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

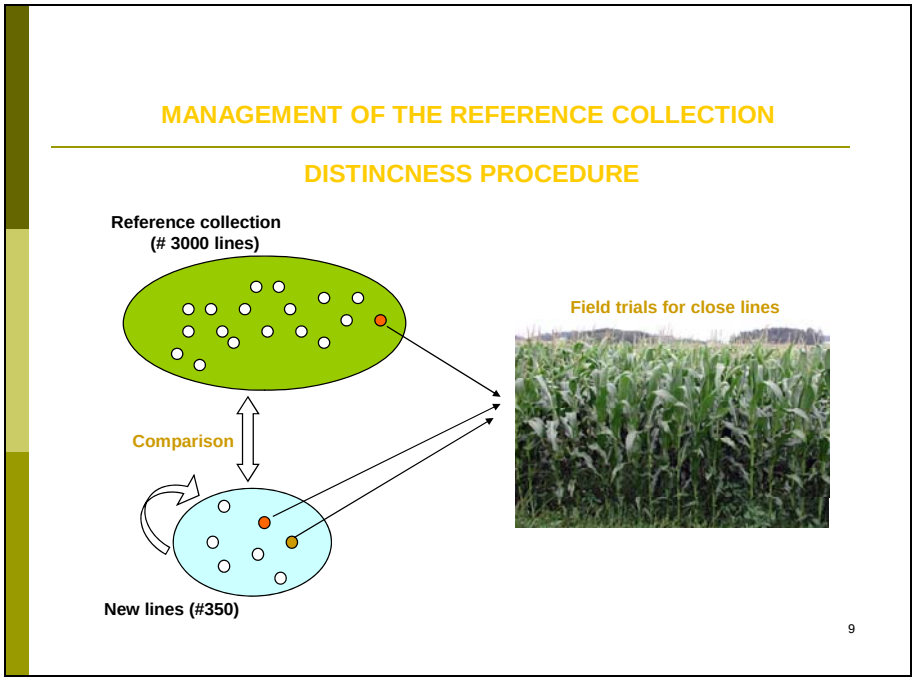
1. Description

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:

(a) only selects varieties which are similar to the candidate varieties; and
(b) 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:

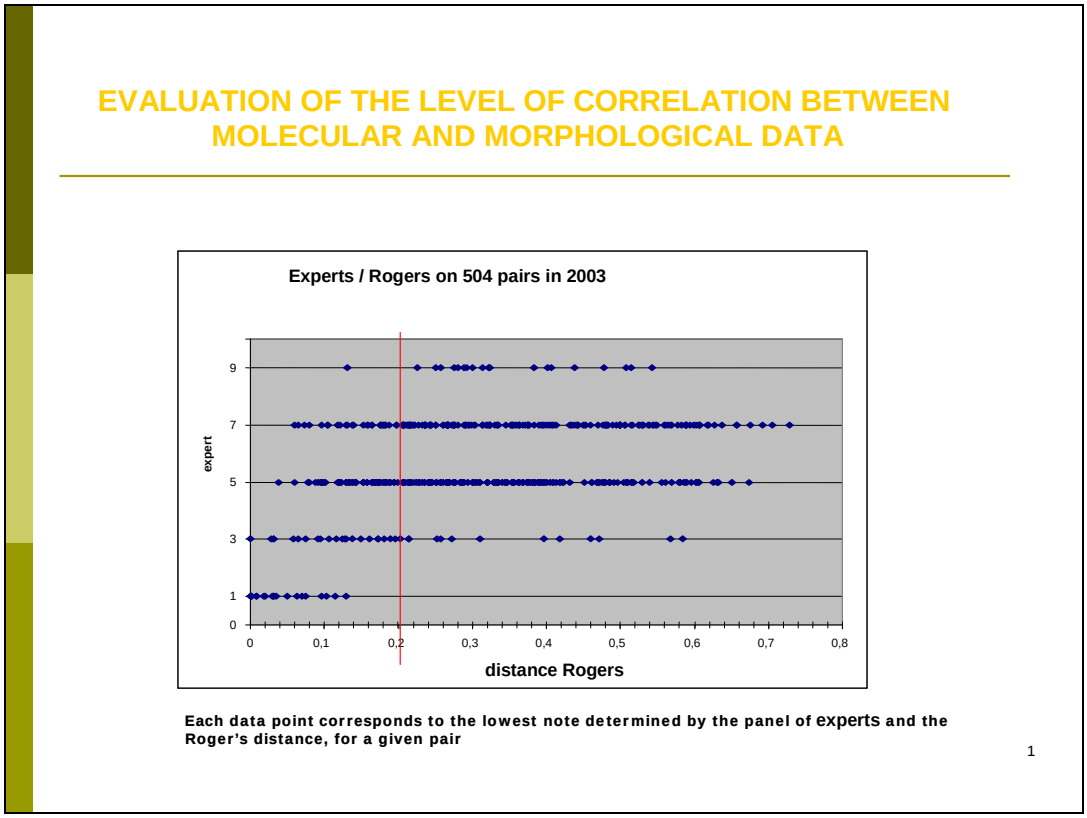
Scale of similarity:

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)

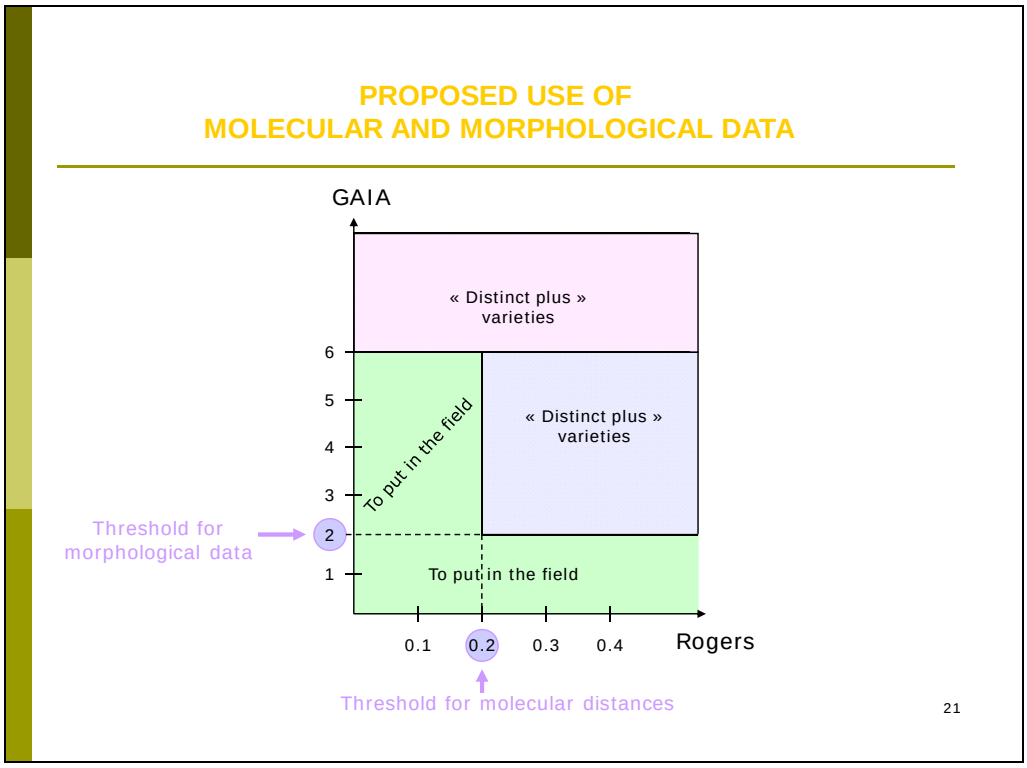
In the case of maize, this evaluation showed that no parental lines with a molecular distance greater than 0.15 were considered as similar or very close by a DUS expert evaluation (see Figure 2).

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]