



**BMT/13/2**

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**INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS**  
GENEVA

**WORKING GROUP ON BIOCHEMICAL AND MOLECULAR  
TECHNIQUES AND DNA PROFILING IN PARTICULAR**

**Thirteenth Session**  
**Brasilia, November 22 to 24, 2011**

**REPORTS ON DEVELOPMENTS IN UPOV CONCERNING BIOCHEMICAL AND  
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) statistical methods for data produced by biochemical and molecular techniques; and
  - (e) *Ad Hoc* Crop Subgroups on Molecular Techniques (Crop Subgroups).
2. Developments concerning international guidelines on molecular methodologies and the development of a common database structure for molecular data (variety description databases) will be considered under agenda items 7 and 8 respectively.
3. 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/export/sites/upov/restrict/en/upov\\_structure/upov\\_structure\\_bmt\\_rev\\_1.pdf](http://www.upov.int/export/sites/upov/restrict/en/upov_structure/upov_structure_bmt_rev_1.pdf). That overview is also attached as Annex I to this document.

4. 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

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

5. 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).

6. The UPOV Guidelines for DNA-profiling: molecular marker selection and database construction (BMT Guidelines) were adopted by the Council, at its forty-fourth ordinary session, held in Geneva on October 21, 2010. The BMT Guidelines are presented as document UPOV/INF/17/1 (see [http://www.upov.int/export/sites/upov/en/publications/pdf/upov\\_inf\\_17\\_1.pdf](http://www.upov.int/export/sites/upov/en/publications/pdf/upov_inf_17_1.pdf)).

7. It is recalled that the purpose of the BMT Guidelines is “to provide guidance for developing harmonized methodologies with the aim of generating high quality molecular data for a range of applications. The BMT Guidelines are also intended to address the construction of databases containing molecular profiles of plant varieties, possibly produced in different laboratories using different technologies. In addition, the aim is to set high demands on the quality of the markers and on the desire for generating reproducible data using these markers in situations where equipment and/or reaction chemicals might change. Specific precautions need to be taken to ensure quality entry into a database” (see document UPOV/INF/17/1, “Introduction”).

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

8. 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.

9. 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)”.

10. 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)).

11. 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.

12. 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.

13. At its sixty-fourth session, held on October 17, 2011, the Council adopted document BMT/DUS/1 “Possible use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)” on the basis of document BMT/DUS/1 Draft 6. In accordance with the sequential numbering of information materials, the reference of the document has been changed from “BMT/DUS/1” to “UPOV/INF/18/1”.

Development of document TGP/15

14. The BMT, at its twelfth session of the BMT, held in Ottawa, Canada, from May 11 to 13, 2010, 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 5, 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.

15. The TWA, TWV, TWO and TWF, at their respective sessions in 2010, 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.

16. At its forty-seventh session, held from April 4 to 6, 2011, the TC 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. It 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 TC also agreed that consideration should be given to how to maintain both documents in an efficient way.

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

17. There have been no meetings of the Crop Subgroups since the twelfth session of the BMT.

18. The TC, at its forty-sixth session, held in Geneva from March 22 to 24, 2010, agreed the following plans for meetings of Crop Subgroups (TC/46/16 “Report”, paragraph 137):

“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;

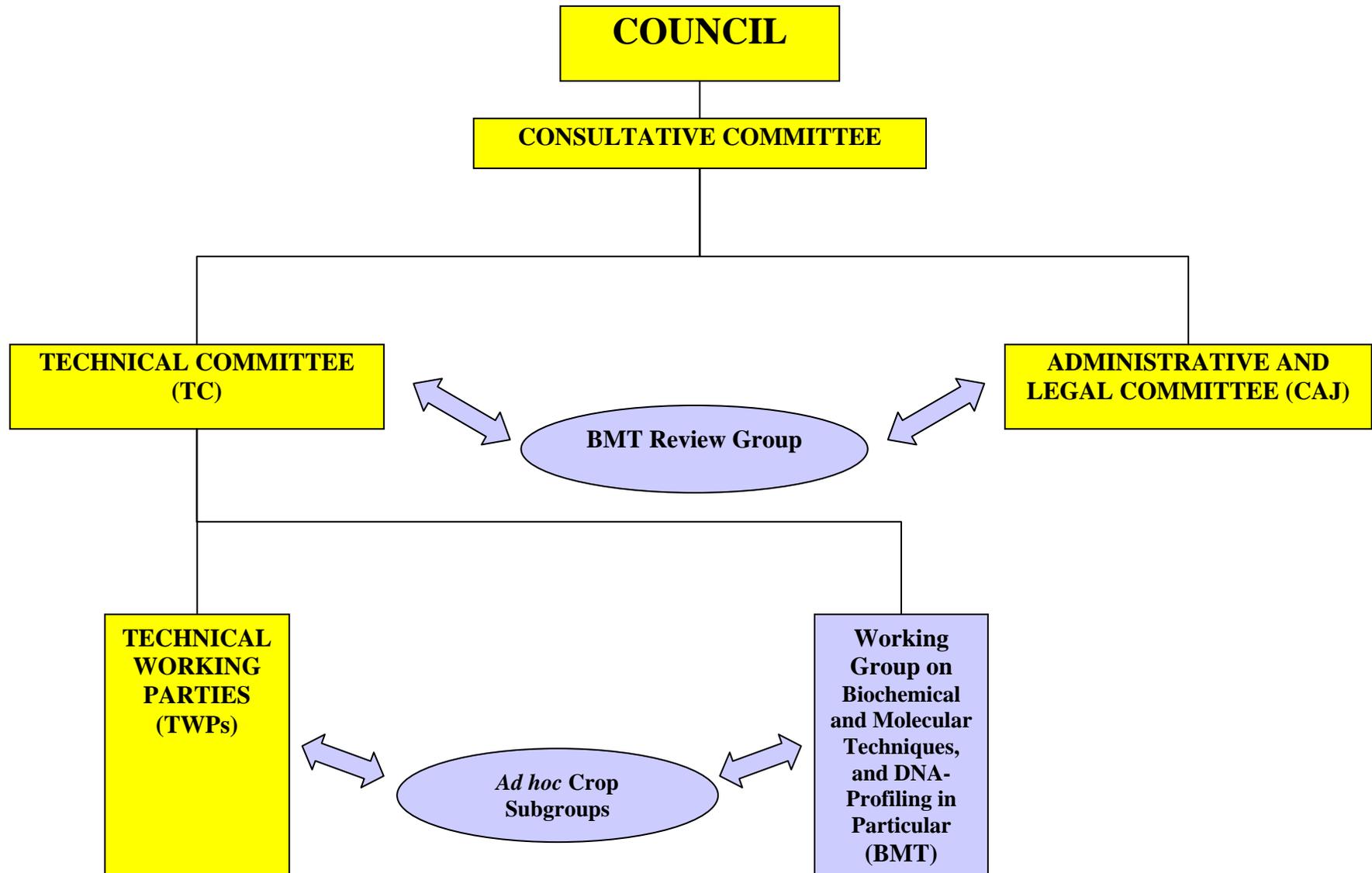
“Crop Subgroup for Potato: no subgroup meeting planned. To consider a future meeting according to developments in on-going projects reported at the eleventh session of the BMT;

“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.”

[Annexes follow]

# 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	(vacant)	n/a
<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]

BMT/13/2

ANNEX II

BMT REVIEW GROUP

- Chairman: Mr. Peter Button (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. Raimundo Lavignolle  
Mrs. Yolanda Huerta  
Ms. Julia Borys  
Mr. Fuminori Aihara

[Annex III follows]

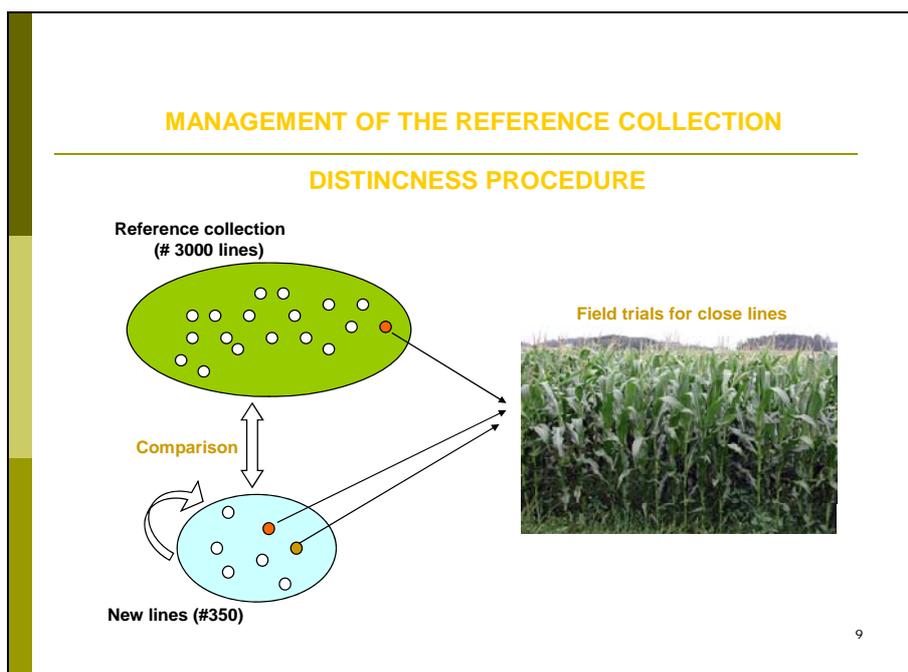
Proposal: “System for combining phenotypic and molecular distances  
in the management of variety collections”

*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)

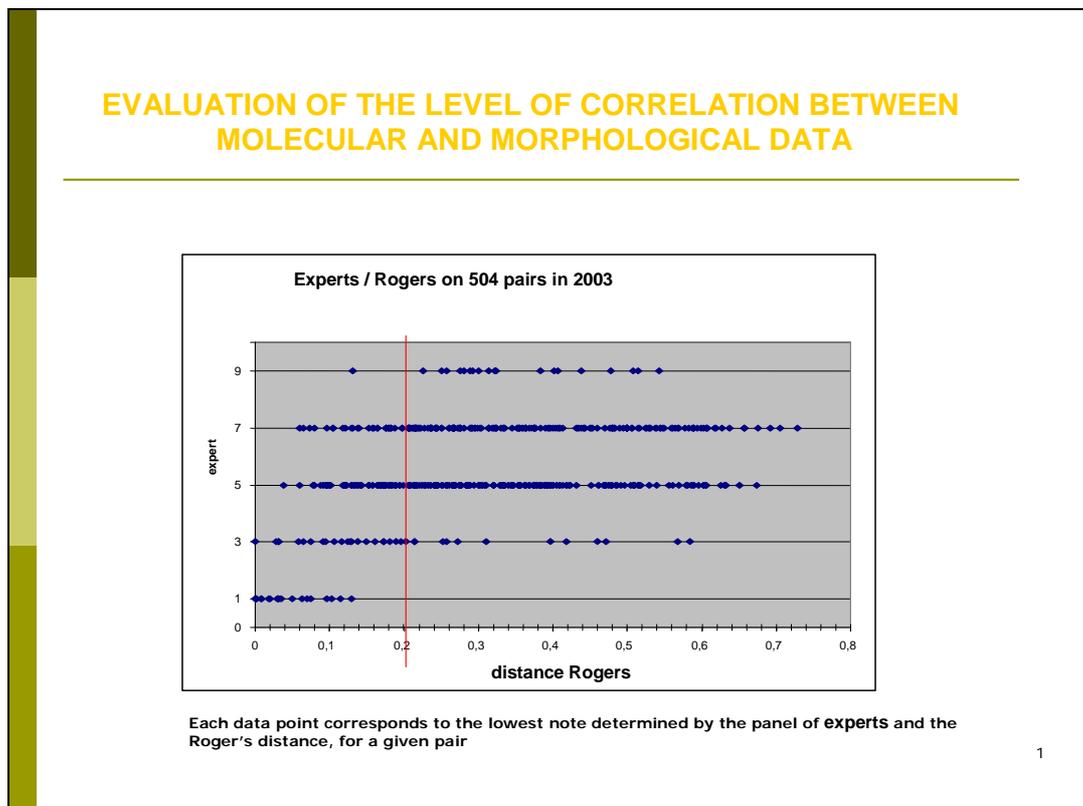
Visual assessment by maize crop experts:

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)

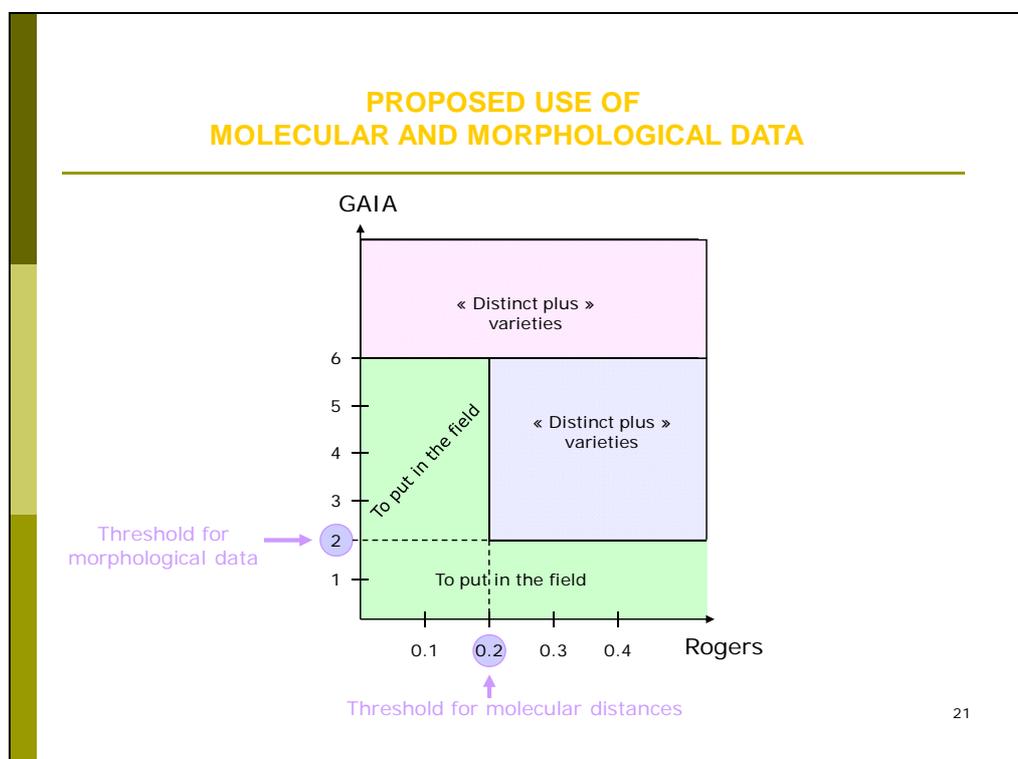
In the case of maize, this evaluation showed that no parental lines with a molecular distance greater than 0.15 were considered as similar or very close by a DUS expert evaluation (see Figure 2).

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]