



BMT/8/28

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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**

Eighth Session

Tsukuba, Japan, September 3 to 5, 2003

REPORT

*adopted by the Working Group on Biochemical and Molecular Techniques
and DNA-Profiling in Particular*

1. The Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular (hereinafter referred to as "BMT") held its eighth session in Tsukuba, Japan, from September 3 to 5, 2003, under the chairmanship of Mr. Gerhard Deneken (Denmark). The list of participants is reproduced in Annex I to this report.
2. Mr. Akira Nagata, Director of the Plant Variety Protection Office of Japan, welcomed the participants and provided an overview of the work of his Office. In particular, he noted the need for harmonization in plant variety protection and effective enforcement of plant breeders' rights and informed the BMT that Japan had recently amended its laws to improve its enforcement measures. He further remarked on the need to rapidly identify varieties and the importance of molecular markers in this respect.
3. Mr. Koutaro Iwashita, Director, Business Management Department, welcomed the participants to the National Center for Seeds and Seedlings, which is an independent administrative institution conducting DUS testing.
4. The BMT welcomed, as observers, ten experts who were participating in a training course on plant variety protection organized by the Japan International Cooperation Agency (JICA) from August 13 to October 25, 2003.

Adoption of the Agenda

5. The BMT unanimously adopted the revised agenda as reproduced in document BMT/8/1 Rev.

Report on Development in UPOV Concerning Biochemical and Molecular Techniques

6. The Office of the Union introduced documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. explaining the recent developments in UPOV concerning the use of biochemical and molecular techniques for DUS testing. Presentations on the models presented to the *Ad hoc* Subgroup of Technical and Legal Experts on Biochemical and Molecular Techniques (hereinafter referred to as “the BMT Review Group”) were made by the experts from France (Option 1(a) – Proposal 1; Option 2 – Proposals 2, 3 and 4)), the Netherlands (Option 3 – Proposal 5) and the United Kingdom (Option 3 – Proposal 6). The BMT noted, in particular, the recommendations made by the BMT Review Group concerning the possible use of molecular techniques in DUS testing and the opinions of the Technical Committee (TC) and the Administrative and Legal Committee.

7. Mr. Elena (Community Plant Variety Office (CPVO)) explained that Regulation 2100/94 of the European Union required that decisions on distinctness be based on phenotypic traits. In this respect he noted that the use of molecular markers in pre-screening involved taking decisions on distinctness. Mr. Le Buanec (International Seed Federation (ISF)) noted that the ISF position was consistent with that of the CPVO and pointed to the need to improve the relationship between morphological and molecular distances. Mr. van Deynze (ISF) proposed that, in document TC/38/14 Add.-CAJ/45/5 Add., paragraph 5, it would be better to refer to the need to have a “good relationship”, rather than to “improve” the relationship. Concerning Proposals 5 and 6 in paragraph 3 of document TC/38/14 Add.-CAJ/45/5 Add., Mr. Cooke (United Kingdom) considered that it was not necessarily correct to assume that it would always be possible to find differences between varieties by using a limitless number of molecular markers.

Report on the Work of the Crop Subgroups

8. In the absence of Mr. Nico van Marrewijk (Netherlands), Chairman of the *Ad hoc* Subgroup on Molecular Techniques for Mushroom, the Office of the Union made an oral report on the first session of the Crop Subgroup for Mushroom which had met in Tsukuba, Japan, on September 13, 2002, on the basis of the report made in document BMT-TWV/Mushroom/1/4. The comments on that document made by the Technical Committee (TC) (document TC/39/15, paragraph 11) and the Technical Working Party for Vegetables (TWV) (document TWV/37/8, paragraph 9) were also reported. With regard to paragraph 19 of document BMT-TWV/Mushroom/1/4, Mr. Guiard (France) emphasized that it was not appropriate to search for differences between varieties if there was no variation and that, furthermore, the aim of breeders should be to breed for variety improvement and not for variety distinctness.

9. Mr. Luis Salaiques (Spain), Chairman of the *Ad hoc* Subgroup on Molecular Techniques for Sugarcane, reported on the outcome of the first session of the Crop Subgroup for Sugarcane which had met in Rio de Janeiro, Brazil, on September 27, 2002, on the basis of

document BMT-TWA/Sugarcane/1/4. The BMT noted, in particular, the importance that that Subgroup had attached to the development of standardized protocols for the use of molecular markers.

10. In the absence of Mr. Marcelo Labarta (Argentina), Chairman of the *Ad hoc* Subgroup on Molecular Techniques for Soybean, the Office of the Union made an oral report on the first meeting of the Crop Subgroup for Soybean which had met in Rio de Janeiro, Brazil, on September 27, 2002, on the basis of document BMT-TWA/Soybean/1/4. An expert from the United Kingdom considered that the apparent “instability” mentioned in paragraph 9 of the report was, in his experience, very unlikely to have been caused by mutation in the microsatellite loci. Mr. van Deynze (ISF) added that another possible cause of the apparent “instability” could be a lack of repeatability in the markers, leading to stutter bands. Mr. Le Buanec (ISF) noted that the document highlighted three main issues for ISF concerning the use of molecular markers, namely that: there would be a need to modify the assessment of distinctness; the number of off-types would need to be changed for the assessment of uniformity; and instability would need to be investigated. He noted that all these issues would need to be addressed before a new system, based on molecular markers, could be established.

Short Presentations on New Developments in Biochemical and Molecular Techniques by DUS Experts, Biochemical and Molecular Specialists, and Plant Breeders

11. Mr. Wray (United Kingdom) reported that, following the outcome of the BMT Review Group, the United Kingdom had reviewed its research project on molecular markers in Oilseed Rape and Wheat with a view to developing an Option 2 type approach, but retained an option to develop an Option 3 approach. On this basis, his country was collaborating with France in the use of GAÏA and PREDIP methodologies. He also reported that the United Kingdom was introducing the use of protein electrophoresis in its procedure for authenticating stocks of seed submitted for trials on value for cultivation and use.

12. Mr. Guiard (France) informed the participants that France was planning to collaborate with the United Kingdom on the utilization of microsatellite markers for Oilseed Rape, in order to obtain more data on which to develop, in particular, the PREDIP method. He explained that, unlike for GAÏA, the PREDIP software package was not yet available for release.

13. Mr. Vosman (Netherlands) reported that he was working on a project with Naktuinbouw (the Netherlands Inspection Service for Horticulture) in the Netherlands to characterize 90 varieties of tomato using AFLP, microsatellites, SNPs and morphological characteristics, with a view to developing an option 2 approach.

Report of Work on Molecular Techniques on a Crop-Specific Basis (Including Methods to Assess the Potential Impact on the Strength of Variety Protection)

BMT/8/27 Genomic characterization of Mexican Nopal accessions

14. The BMT noted the presentation made by Mrs. Valadez Moctezuma (Mexico).

BMT/8/26 Genetic mapping of 66 new microsatellite (SSR) loci in Bread Wheat

15. The BMT noted the presentation made by Mrs. Valadez Moctezuma (Mexico).

BMT/8/22 Development of SSR markers and genetic identification of pear varieties

16. The BMT noted the presentation made by Mr. Kimura (Japan).

BMT/8/21 SSR markers and the application to cultivar profiling in Peach

17. The BMT noted the presentation made by Mr. Hayashi (Japan).

BMT/8/3 The Use of SSRs for DUS Testing of Wheat: 1. Uniformity and Stability of Varieties

18. The BMT discussed the presentation made by Mr. Cooke (United Kingdom). Mr. Guiard (France) suggested that the failure of the molecular markers to identify “tall” off-type plants might be because such off-types are natural hybrids showing the banding pattern of the female parent. Mrs. Rucker (Germany) observed that, at the level of uniformity found for molecular markers, it would be necessary to amend the current uniformity standards to accept all the varieties which had satisfied the uniformity standard for morphological characteristics. She also wondered why it would be necessary to find additional markers to improve the discrimination between varieties when, with morphological characteristics, it was accepted that distinctness would not always be found. Mr. Cooke observed that the varieties in the study had all been found to be distinct using morphological characteristics.

BMT/8/4 The Use of SSRs for DUS Testing of Wheat: 2. Genetic and Phenotypic Distances

19. The BMT discussed the presentation made by Mr. Cooke (United Kingdom). Mr. Guiard (France) reported that studies using PREDIP had indicated that rare alleles had a very strong influence on the genetic distance and that elimination of such alleles might significantly improve the relationship between molecular and phenotypical distances. Mr. Cooke (United Kingdom) observed that initial separation of varieties into groups, such as winter and spring types, might also improve the relationship between molecular and morphological distances.

BMT/8/5 Single nucleotide polymorphisms in Barley using tetra-arms PCR

20. The BMT received a presentation from Mr. Cooke (United Kingdom). Mr. Cooke, noted that the high level of discrimination between varieties using only five markers suggested that these five markers had been pre-selected for their discriminatory power, although he had not checked this with Montana State University. In response to a question from Mr. Wray (United Kingdom), Mr. Cooke suggested that SNPs should become available

for Oilseed Rape and Wheat and that, although it was not possible to know at that stage, he considered that these markers should be able to satisfy the requirements for consistency and repeatability.

BMT/8/6 SNPs for variety identification in Tomato

21. The BMT received a presentation from Mr. Vosman (Netherlands) and heard that a similar project on SNPs was underway for Potato. The Chairman wondered if the lack of SNPs in coding sequences meant that it would be difficult to develop these techniques for an Option 1(a) approach. Mr. Vosman replied that it should be possible, because although SNPs are found very infrequently in exonic DNA (coding sequences), they are frequently observed in the introns and markers in introns are also useful for an option 1 (a) approach.

BMT/8/7 Genetic variability and relationships among Sunflower inbred lines from a French Reference Collection assessed using a set of well-characterized SSR markers

22. The BMT discussed the presentation made by Mr. Zhang (France). Mrs. Blouet (France) noted that the information obtained on SSRs in Sunflower could be used to replace field checks and electrophoresis in the verification of submitted seed samples and could also be used to check the parental formula for hybrid varieties. Mr. Cooke (United Kingdom) noted that, as with Wheat, there appeared to be relatively high levels of uniformity in SSRs despite the fact that selection had only been based on morphological characteristics.

BMT/8/8 Characterization of a set of SSR markers for maize genotyping and estimation of SSR analysis cost for routine use

23. The BMT noted the presentation made by Mr. Zhang (France). Mr. Cooke (United Kingdom) observed that the major cost of SSR analysis was now the extraction step and that there was a need for manufacturers to concentrate on automating sample preparation. Mr. van Deynze (ISF) noted that the use of multiple markers on the same sample would reduce the relative cost of extraction.

BMT/8/9 Intra- and Inter-Varietal variability in Oilseed Rape varieties measured by DNA markers

24. The BMT received a presentation from Mr. Le Buanec (ISF). He remarked that the large intravarietal variability of “wide population” varieties suggested that it may be necessary to work with an average value for the population, rather than the range, in order to be able to develop thresholds for essential derivation. In response to a question from Mr. Wray (United Kingdom), he anticipated that, eventually, it should be possible to conduct assessment of essential derivation using only 20 to 40 markers, subject to further consideration.

BMT/8/19 Proposal of a breeding method prerequisite to cultivar identification in allogamous crops, e.g. bunching onion

25. The BMT noted the presentation made by Mr. Kojima (Japan).

BMT/8/20 Identification of Strawberry varieties using DNA markers

26. The BMT received a presentation from Mr. Matsumoto (Japan). Mr. Elena (CPVO) welcomed the development of such work on the identification of varieties for the purpose of enforcement of plant breeders' rights and reported that the CPVO had, in the Crop Subgroups, made similar proposals for such an approach.

BMT/8/10 Genetic background analysis to help solving some Soybean registration problems

27. In the absence of experts from Argentina, Mrs. Rücker (Germany) introduced document BMT/8/10. The BMT noted the document and agreed that it should be considered further by the Soybean Crop Subgroup.

BMT/8/23 Study on detection of specific color gene introduced into Carnation

28. The BMT noted the presentation made by Mr. Osono (Japan). It was clarified that the use of molecular markers was restricted to the identification of genetically modified varieties, but that the method would require further development before being used in the examination of distinctness, uniformity and stability.

BMT/8/24 Protection of plant breeders' rights and use of advanced technology

29. The BMT noted the presentation made by Mr. Ban (Japan). Mr. Elena (CPVO) welcomed the document and reported that a Council Regulation of the European Union was in the process of being amended to allow the possibility of action against infringements of plant breeders' rights at customs controls.

BMT/8/2 Cultivar identification of Rice by PCR method and its application to processed rice products

30. The BMT noted the presentation made by Mr. Otsubo (Japan).

BMT/8/25 Identification of White Common Bean varieties (Otebo, Phaseolus Vulgaris L.) by DNA polymorphism using PCR amplification

31. The BMT noted the presentation made by Mr. Kamiya (Japan).

Marker Selection and Database Construction for Variety Characterization

32. The BMT discussed document BMT/8/13, presented by Mr. Ben Vosman (Netherlands).

33. Consideration was given to the selection of markers in relation to various possible objectives and, in particular, whether an agreed set of markers could be established for these objectives. With regard to an Option 1(a) approach, it was noted that markers could not be anticipated in advance. For an Option 2 approach, it was noted that the first step was to establish acceptable calibration between phenotypic and molecular distances on a crop-by-crop basis and this would need to be taken forward in the Crop Subgroups before consideration could be given to finalizing a set of markers. With regard to the possible use of markers for variety identification, the BMT noted that, in the light of discussions in the Administrative and Legal Committee (CAJ), it would not be appropriate for the BMT to pursue methods specifically aimed at variety identification at this time, but that the BMT could remain a forum for discussion on matters concerning variety identification.

34. 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. On this basis, the BMT agreed that the Office of the Union should prepare a guidance document ("BMT Guidelines") based on documents BMT/8/13 and BMT/8/14 (see below). A first draft of the BMT Guidelines would be produced by the Office by the end of November 2003 and circulated to Mr. Guiard (France), Mr. Piperidis (Australia), Mr. Vosman (Netherlands), Mr. Wray (United Kingdom) and Mr. Le Buanec (ISF) for comment after consultation with their respective experts. Once agreed, the BMT Guidelines would be circulated to the Technical Committee, the Crop Subgroups and the BMT and would be considered further by the BMT at its ninth session. It was agreed that the introduction to the BMT Guidelines should present these as good practice for molecular techniques, whilst clarifying that UPOV had only approved the use of molecular techniques in the framework of an Option 1(a) or Option 2 approach.

Review of the Costs of Molecular Techniques

35. The BMT noted the costs of molecular techniques, presented by Mr. Le Buanec (ISF) on the basis of document BMT/8/11, and heard that these costs were based on high throughput numbers. It was clarified that the cost of US\$2.5 to 5.0 to fingerprint a variety on the basis of 50 to 100 datapoints, presented in the final paragraph of document BMT/8/11, did not include the cost of extraction, but the other costs in the document did include extraction costs.

Statistical Methods for Data Produced by Biochemical and Molecular Techniques

BMT/8/14 Band scoring, distances, use for distinctness and uniformity, data storage

36. The BMT received a presentation from Mr. John Law (United Kingdom), based on document BMT/8/14, and heard that a copy of the presentation would be prepared as an addendum to the aforementioned document to be circulated with the report of the meeting. It was noted that consultation between crop experts and the Technical Working Party on Automation and Computer Programs (TWC) would be desirable to achieve consensus on the choice of distance measurement method. It was also observed that, at least initially, different distance measurement methods might be required for different situations.

TWC/21/3 PREDIP, a software package to predict phenotypic distances with molecular data

37. The BMT discussed the use of the PREDIP software on the basis of document TWC/21/3, presented by Mr. Guiard (France). It was explained that the PREDIP software was designed to predict phenotypic distances on the basis of molecular data. In contrast, the GAÏA software was used to calculate phenotypic distance from phenotypic values, and electrophoresis data where appropriate, using values which were already available for the object variety. The BMT also noted that the weighting of differences used in the GAÏA software was not employed in PREDIP. It was reported that it was hoped that results from the use of PREDIP on a large set of maize inbred lines would be ready for presentation to the Maize Crop Subgroup in 2004.

38. With regard to the possible impact of mutant varieties on the PREDIP model, for example in ornamental varieties, Mr. Guiard explained that there would probably be an effect because there would be morphological differences but no detectable molecular differences. He also recalled his earlier remarks on the effect of rare alleles on the model and the need to consider removing those from the model. In response to a question on the influence of the environment on the model he explained that it was too early to know whether different models would need to be produced for different locations and/or years.

The Use of Molecular Techniques in Examining Essential Derivation

39. The BMT received presentations from Mr. Cooke (United Kingdom), Mr. Vosman (Netherlands) and Mr. Law (United Kingdom), on the basis of documents BMT/8/15, BMT/8/16 and BMT/8/17 respectively. It was noted that a copy of the presentation, made on the basis of document BMT/8/17, would be attached as an addendum to the latter document and circulated with the report of the meeting. A number of experts expressed surprise at the low level of differences between the second, third and fourth generation backcrosses and the parents; however, it was noted that there was insufficient information on the particular backcross population to know whether this was a typical situation.

40. The BMT received a report from Mr. Le Buanec (ISF), based on document BMT/8/18. Mr. Le Buanec emphasized that demonstration of a high degree of similarity was not sufficient to consider a variety to be essentially derived, since, according the 1991 Act of the UPOV Convention, it also had to be predominantly derived. With regard to the proposed threshold of 0.96, as a trigger point for starting a dispute on essential derivation in Butterhead lettuce, he explained that ISF had three options to be considered by the ISF General Assembly in May 2004, namely:

- (a) allow the result to be used by breeders in whatever way they wished;
- (b) establish a voluntary code of conduct in the same way as for ryegrass; or
- (c) draft an agreement, to be signed by breeders, leading to binding ISF arbitration or settlement in court.

41. Mr. Le Buanec advised that the threshold for Butterhead lettuce would be reviewed after five years to allow new information to be reviewed. In response to a question from Mr. van Deynze (ISF), Mr. Le Buanec explained that, in the future, at least two independent laboratories would be able to conduct the AFLP marker analysis on lettuce. He noted that

other types of markers, such as SSRs, would probably produce different threshold values, but the classification of varieties would very likely be the same. Mrs. Rücker (Germany) wondered if the establishment of a threshold might encourage breeders to target this threshold in their breeding efforts. Mr. Le Buanec suggested that it might, in fact, encourage breeders to increase the genetic base of their breeding programs. He confirmed that the introduction of a *Bremia* resistance into an existing variety would probably result in an essentially derived variety, but noted that such activities were usually undertaken by the owner of the initial variety. In response to a question from Mrs. Weitz (CPVO), Mr. Le Buanec agreed to ask the ISF experts to compare the morphological differences in those varieties which exceeded the threshold value.

Proposals for the Future Work of the Existing Crop Subgroups and for the Establishment of New Crop Subgroups

42. The BMT noted that there were no proposals from the Technical Working Parties for new crop specific subgroups. With regard to the work of the existing Crop Subgroups, the BMT agreed that these Crop Subgroups should be encouraged to use the BMT Guidelines in their work. It noted that the program of work for these Subgroups would be established by the respective Chairpersons of the Crop Subgroups concerned.

Future Program, Date and Place of the Next Session

43. In response to the invitation received from the United States of America, the BMT agreed to hold its ninth session in the United States of America in June 2005. It also noted that the Republic of Korea had offered to host the tenth session of the BMT at the end of 2006.

44. During its ninth session, the BMT planned to discuss the following items:

1. Opening of the session
2. Adoption of the agenda
3. Short presentations on new developments in biochemical and molecular techniques by DUS experts, biochemical and molecular specialists, and plant breeders
4. Reports from the BMT Review Group, Technical Committee and Crop Subgroups
5. Report of work on molecular techniques on a crop-by-crop basis
6. Recommendations on the establishment of new crop specific subgroups
7. BMT Guidelines
8. Construction and standardization of databases of molecular characteristics of plant varieties
9. Statistical methods for data produced by biochemical and molecular techniques
10. The use of molecular techniques in examining essential derivation

11. Date and place of next session
12. Future program
13. Report on the conclusions of the session (if time permits)
14. Closing of the session

45. With regard to item 9 on the program for the ninth session, the BMT agreed that one day of the session should be set aside for that item and the TWC should be informed of this development to encourage participation by the members of the TWC.

46. Mr. John Law was awarded a UPOV Bronze Medal in recognition of his Chairmanship of the Technical Working Party on Automation and Computer Programs (TWC) for the period 1997 to 1999.

47. The BMT adopted this report at the close of the session.

[Annex follows]

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