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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

Eleventh Session Madrid, September 16 to 18, 2008

REPORT

adopted by the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular (BMT)

1. The Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular (BMT) held its eleventh session in Madrid, from September 16 to 18, 2008. The list of participants is reproduced in Annex I to this report.

2. The BMT was welcomed by Mrs. Alicia Villauriz, General Secretary of Rural Affairs, Ministry of Environment and Rural and Marine Affairs. A copy of the welcoming address presented by Mrs. Alicia Villauriz is reproduced in Annex II to this document.

3. The session was opened by Mr. Henk Bonthuis (Netherlands), Chairman of the BMT, who welcomed the participants.

Adoption of the Agenda

4. The BMT adopted the Agenda as reproduced in document BMT/11/1 Rev.2

Reports on developments in UPOV concerning biochemical and molecular techniques

5. The Office of the Union (the Office) provided a report on developments in UPOV concerning Biochemical and Molecular Techniques, on the basis of document BMT/11/2. In

addition, Mr. Joël Guiard (France) made a presentation on the approach presented in documents BMT/10/14 and BMT-TWA/Maize/2/11 to be put forward for consideration at the BMT Review Group as a potential option for the use of molecular markers in DUS examination. A copy of that presentation is provided as document BMT/11/2 Add..

The use of molecular techniques in examining essential derivation:

Concepts of Dependence and Essential Derivation: The possible use of DNA markers

6. The BMT considered document BMT/11/24, as presented by Mr. Marcel Bruins (International Seed Federation (ISF)).

7. Mr. David Calvache (Spain) requested information on varieties which had been officially recognized as essentially derived varieties. Mr. Bruins explained that he was not aware of any "officially recognized" essentially derived varieties, because the existing court cases concerning essentially derived varieties were still under appeal.

Essentially Derived Varieties (EDV) in the Area of Asexually Reproduced Ornamental and Fruit Varieties

8. The BMT considered document BMT/11/22 and a presentation made by Mr. Edgar Krieger (International Community of Breeders of Asexually Reproduced Ornamental and Fruit-Tree Varieties (CIOPORA)).

9. Mr. Huib Ghijsen, (ISF) observed that the "Me-too-varieties" referred to by CIOPORA appeared to have many similarities to varieties obtained by backcrossing, and recalled that backcrossing was an example of one of the means by which an essentially derived variety might be obtained according to the 1991 Act of the UPOV Convention.

10. In relation to "Me-too-varieties", Mr. Cecilio Prieto (Spain) requested clarification on the possibility of considering non-UPOV Test Guidelines characteristics as essential characteristics, particularly where such characteristics were of commercial importance. Mr. Krieger clarified that non-UPOV Test Guidelines characteristics would not be considered as essential characteristics, even if they were important for the purposes of exploitation.

11. Mr. Joël Guiard (France) noted that many of the varieties in fruit crops such as apples were mutant varieties and wondered what the consequences for the plant variety protection system would be if all such varieties were considered to be essentially derived varieties. In particular, he requested information on any studies on the economic impact of such an approach. Mr. Krieger replied that there had been no study on the economic impact, but recalled that an essentially derived variety would only be "dependent" on an initial variety if the initial variety was protected by a plant breeder's right. He also noted that an arrangement could be made between the breeder of a protected initial variety and the breeder of an essentially derived variety, in order to allow the commercialization of an essentially derived variety; however, he noted the importance of providing breeders with an incentive to engage in innovative breeding.

12. Mrs. Marisé Borja (ISF) raised the example of the breeder of the first variety of a "hanging petunia" type and sought clarification on whether all subsequent varieties of "hanging petunia" type would be considered to be essentially derived varieties from that first

variety, according to the "Me-too-varieties" concept set out by CIOPORA. Mr. Krieger clarified that all the essential characteristics would need to be retained for the varieties to be considered to be essentially derived varieties; for example, if the flower color was different a variety would not be an essentially derived variety. He considered that there would be very few "Me-too-varieties".

13. Mr. Pere Arús (ISF) recalled that Mr. Krieger had given the example of a first blue rose variety in his presentation and wondered if all blue rose varieties would be considered to be essentially derived varieties from the first blue rose variety. Mr. Krieger clarified that, as for the example of the "hanging petunia" type, a variety would only be considered to be an essentially derived variety if it retained all the essential characteristics: therefore, a variety would not be considered to be an essentially derived variety if the only characteristic in common was the color of the flower. However, if all the characteristics of the variety were the same except for one or two characteristics which were not of importance for exploitation of the variety, e.g. prickle color and color of the underside of the leaf, that variety should be considered to be a "Me-too-variety" and would be an essentially derived variety.

14. Mrs. Lysbeth Hof (Netherlands) sought clarification of the means by which the threshold of 0.90 Jaccard for the shift of burden of proof for mutant varieties had been developed and wondered if that threshold would be appropriate for all species. Mr. Krieger explained that the threshold had been developed by a group of experts, including scientists and breeders, which had considered genetic studies in several ornamentals such as geranium, carnation etc. That threshold allowed mutant and non-mutant varieties to be separated. Mr. Krieger explained that it would not be feasible to develop crop-specific thresholds because there were over 1,000 species to consider. However, a crop-specific threshold could be proposed in future if the information supported a different threshold. He recalled that the 0.90 threshold was for a shift of the burden of proof and not for a determination of essential derivation.

15. With regard to "Me-too-varieties", Mrs. Beate Rücker (Germany) observed that the consideration of essential characteristics in terms of exploitation seemed to be similar in concept to the value for cultivation and use (VCU) criteria for National Listing purposes. She wondered about the case of a red rose variety for which the characteristics were the same as another variety except for the prickle color and color of the underside of the leaf. Mr. Krieger clarified that National Listing and VCU were not relevant for ornamental varieties. With regard to the red rose example, he considered that if all the characteristics of the variety were the same except for prickle color and color of the underside of the leaf, that variety should be considered to be a "Me-too-variety" and would be an essentially derived variety.

16. With regard to mutation varieties, Mr. Antonio Villarroel (Spanish Plant Breeders' Association (ANOVE)) noted that there were important breeding developments arising from mutation induction; for example seedless varieties of grape and citrus. He wondered if any distinction was made between natural and induced mutation varieties and wondered if there had been cases of recognized mutant essentially derived varieties. Mr. Krieger clarified that all mutation varieties were considered to be essentially derived varieties, irrespective of whether the mutations were natural or induced. He was not aware of any recognized cases of mutant varieties already considered as essentially derived varieties.

The Use of Molecular Techniques When Infringement of PBR or Essential Derivation are Suspected

17. The BMT considered document BMT/11/28, as presented by Mrs. Hedwich Teunissen (Netherlands).

18. In the case of freesias reported in the presentation, Mr. Huib Ghijsen (ISF) sought information on whether there would be a comparison of the morphology of varieties A and B. Mrs. Teunissen explained that, in the first instance, only bulbs had been available and that a comparison of morphology would only be possible next year.

Comparing Wheat Varieties With Their Offspring by Molecular Markers

19. The BMT received a presentation by Mr. Michael Gohn (Austria), based on document BMT/11/7. A copy of the presentation is provided as document BMT/11/7 Add.

20. With regard to the case of the Probstdorfer Saatzucht variety registered in 1997 and the competitors variety registered in 2001, Mr. Joël Guiard (France) enquired whether there were morphological differences between the two varieties. Mr. Gohn confirmed that there were morphological differences. He noted that in a court case to consider essential derivation, a judge would take into account a range of information such as molecular data, morphology and breeding history. It was important for breeders to have a level of agreement in order to minimize the need for court cases, because it was not sure that judges would follow the guidance developed within the industry.

21. In reply to a question by Mr. Daniel Palmero Llamas (Spain), Mr. Gohn explained that 'Capo' had not been eligible for protection by the Community Plant Variety Office of the European Community (CPVO). With regard to the 84 molecular markers used in the study, Mr. Gohn explained that there was no information on their linkage to phenotypic expression.

The Assessment of Essential Derivation in Grapevine

22. The BMT received a presentation by Mr. Javier Ibañez (Spain), based on document BMT/11/16, a copy of which is provided in document BMT/11/16 Add..

23. In response to a request by the Chairman for clarification of the status of the proposals in the document on the assessment of essential derivation in grapevine, Mr. Ibañez clarified that the proposals had been developed by the Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA). He anticipated that breeders would support those proposals.

24. Mrs. Anne Weitz (European Community) requested a definition of the term "clone". Mr. Ibañez explained that only specified varieties could be grown in a particular region and new varieties would not be accepted. Therefore, selections to improve such varieties were not registered as new varieties, but were known as clones. He considered that such clones were probably distinct. Mr. Joël Guiard (France) reported that experience in France had indicated that clones were not distinguishable and wondered if Mr. Ibañez had investigated the matter in Spain. Mr. Ibañez explained that distinctness of clones had not been studied.

25. Mr. Joël Guiard (France) referred to the indication in the presentation that an essentially derived variety was a variety which came from the same original embryo, and questioned

whether this was an appropriate assumption. Mr. Ibañez explained that it was intended to mean that varieties from the same embryo were mutation varieties.

Putting the EDV Concept Into Practice for Maize: SSRs Today and SNPs Tomorrow?

26. The BMT received a presentation by Ms. Elizabeth Jones (ISF), based on document BMT/11/18 Rev.

27. Mr. Huib Ghijsen, (ISF) considered that it was not logical to move from SSRs to SNPs and wondered if that might allow breeders to see how close they could get to the limit of essential derivation on the basis of the SNP markers. Mr. Philippe Moreau (SEPROMA) wondered if the varieties in the study included flint varieties. Ms. Jones explained that the study had not included flint varieties, but reported that the approach had been tested on a collection of European Union protected varieties, which had contained a large number of flint varieties, and had produced good results. She reported that the SNP results were well correlated with the pedigree of the varieties.

28. The Chairman noted that Ms. Jones had referred to the lower error rate for SNPs and requested further information on that aspect. Ms. Jones explained that specification of a protocol was not as critical for SNPs and reported that the error rate in SNPs was considered to be 5 to 10 times lower than for SSRs.

The use of molecular techniques in variety identification

The Spanish Experience (GESLIVE-IRTA) on the Enforcement of Plant Variety Rights: DNA-Fingerprinting: Part 1

29. The BMT received a presentation by Mr. Antonio Villarroel (GESLIVE, Spain), based on document BMT/11/13, a copy of which is provided as document BMT/11/13 Add..

The Spanish Experience (GESLIVE-IRTA) on the Enforcement of Plant Variety Rights: DNA-Fingerprinting: Part 2

30. The BMT received a presentation by Mr. Pere Arús (IRTA, Spain), based on document BMT/11/12, a copy of which is provided as document BMT/11/12 Add..

31. Mr. Huib Ghijsen (ISF) sought clarification of how to address a situation where the DNA-profile or DNA sample in the collection differed from that taken from material collected 20 years later, where mutations might produce some differences at the DNA level, even though the morphology would be the same. Mr. Arús reported that they had 10 years of experience with *Prunus*, but had not experienced such problems.

32. In reply to a question by Mrs. Marisé Borja (ISF), Mr. Villarroel reported that their enforcement work was undertaken both on a routine, systematic basis and in response to specific complaints.

33. In response to a question concerning the remedial actions which GESLIVE might require in cases of infringement, Mr. Antonio Villarroel emphasized that, before starting legal actions, GESLIVE always sought to find amicable solutions with growers. He reported that

several relevant decisions of Spanish courts were available on the CPVO website in the case law section.

Development of SSR Markers and Their Application for Identification in Rose (document BMT/11/14)

34. The BMT received a presentation by Mr. Tetsuya Kimura (Japan), based on document BMT/11/14, a copy of which is provided in document BMT/11/14 Add..

35. In response to a question from Mr. Joël Guiard (France), Mr. Kimura clarified that molecular techniques were not being used in DUS testing in Japan; however, consideration was being given to how such techniques might possibly be used in the future.

Identification System for Soybean Based on the Most Frequent SSR Alleles

36. The BMT received a presentation by Ms. Ana Laura Vicario (Argentina), based on document BMT/11/19, a copy of which is provided in document BMT/11/19 Add..

37. Mr. Huib Ghijsen (ISF) noted that reference was made to the role of the office in controlling seed in commerce. Ms. Vicario explained that the office had a role in checking variety identity.

Comparative Genomic Hybridization for Identifying Mutation Varieties

38. The BMT received a presentation by Mr. Manuel Talón (Spain), based on document BMT/11/23, a copy of which is provided as document BMT/11/23 Add..

39. In his introduction, Mr. Talón emphasized that the inherent barriers to crossing in *Citrus* meant that most new varieties were produced by mutagenesis. In that respect, he noted that the CIOPORA position of considering all mutation varieties to be essentially derived varieties could result in a lack of incentive for breeding new varieties of *Citrus*.

40. In reply to a question from Mrs. Marisé Borja (ISF) concerning the cost of developing a microarray, Mr. Talón estimated that the cost would be around \in 50,000, although he explained that microarrays had already been developed for many crops such as *Citrus* and *Prunus*. The cost of a single variety identification test was estimated at around \in 300.

41. Mr. Ben Vosman (Netherlands) wondered if two varieties had ever been compared using the microarrays. Mr. Talón explained that that had not been done.

Preparation of Guideline for Method Validation of DNA Identification for the Enforcement of Plant Breeder's Rights in Japan

42. The BMT received a presentation by Mr. Takeshi Sugisawa and Mr. Nobuyoshi Takahashi (Japan), based on document BMT/11/15, a copy of which is provided as document BMT/11/15 Add..

A Practical Example of the Possible Use of Molecular Techniques in Variety Identification

43. The BMT received a presentation by Mr. Carlos Godinho (European Community), based on document BMT/11/20, a copy of which is reproduced as document BMT/11/20 Add..

44. Mr. Huib Ghijsen (ISF) noted that the courts did not always accept descriptions provided by the CPVO as evidence and wondered what implications that would have for the proposal. Mr. Godinho agreed that it would be a matter for the courts to decide whether to accept DNA-profiles provided by the CPVO; however, the CPVO had been active in organizing seminars to inform judges on such matters.

45. Mr. Pedro Chomé (Spain) explained that the *Oficina Española de Variedades Vegetales* (OEVV) maintained plant collections and observed that it might be less expensive to collect DNA samples from those plants as and when required, rather than collecting and maintaining DNA samples of all varieties in a systematic way. Mr. Godinho explained that the main cost would be in the cost of DNA extraction and that the proposal would not be prohibitively expensive, particularly taking into account the risks of losing living plant material.

46. Mr. Joël Guiard (France) wondered what the consequences would be if the DNA-sample held in store diverged from the DNA of the most recent living plant material of the variety as a consequence of DNA mutations which did not have an impact on the morphological characteristics of the variety. Mrs. Anne Weitz (European Community) noted that the approach would have implications for breeders in that respect.

47. On an assumption that breeders accepted the proposal of the collection of a DNA-sample and DNA-fingerprint, Ms. Lysbeth Hof (Netherlands) sought clarification on whether the DNA-fingerprint database might be used for DUS purposes. Mr. Philippe Moreau (SEPROMA) requested clarification on whether the proposal was also aimed at seed-propagated crops.

48. Mrs. Anne Weitz (European Community) clarified that, in the first instance, the approach would be considered for roses and breeders would be sent a questionnaire to indicate their preference for an Option A or Option B approach. If there was an interest, the approach could be considered for other crops. Mrs. Weitz explained that a report on developments would be made at the twelfth session of the BMT.

Reports on the work of the Ad Hoc Crop Subgroups on Molecular Techniques ("Crop Subgroups")

49. In the absence of Mr. Joost Barendrecht, Chairman of the Crop Subgroup for Rose, the BMT Chairman made a report on the second session of the Crop Subgroup for Rose, held in Quimper, France, on April 18, 2007, on the basis of document BMT/11/2. He further explained that the third session of the Crop Subgroup for Rose, which had been scheduled to be held on September 15, 2008, had been cancelled because of the lack of participation by rose breeders.

50. Mrs. Beate Rücker (Germany), Chairperson of the Crop Subgroup for Maize and Chairperson of the Crop Subgroup for Potato, made a report on the second session of the Crop Subgroup for Maize, held in Chicago, United States of America, on December 3, 2007 and

the second session of the Crop Subgroup for Potato, held in Quimper, France, on April 17, 2007, on the basis of document BMT/11/2.

51. Mr. Bert Scholte (European Seed Association (ESA)) reported that ESA were planning to hold a first meeting to discuss thresholds for essentially derived varieties in potato.

52. The BMT noted that the Technical Committee had approved Mr. Michael Camlin (United Kingdom) as Chairman of the Crop Subgroup for Wheat and Barley.

53. The BMT heard that no meetings of the Crop Subgroups for Oilseed Rape, Ryegrass, Soybean, Sugarcane, Tomato or Wheat and Barley had taken place since its last session.

Short presentations on new developments in biochemical and molecular techniques by DUS experts, biochemical and molecular specialists, and plant breeders

54. An expert from <u>Brazil</u> reported that the large number of protected soybean varieties in Brazil and the narrow genetic background of that species had made it difficult to distinguish varieties based on morphological characteristics. He reported that a study on the reliability of SSR markers for variety identification of soybean varieties, which might be used as a complement to the variety description, had been initiated. He explained that 20 SSR markers, well distributed throughout the whole genome and located in different linkage groups; and 9 soybean varieties, clearly distinct and very similar varieties, had been selected by Brazilian breeders. He added that the project was coordinated by the Brazilian authority on plant breeder's rights and involved a ring test between six laboratories. He clarified that the objective of the study was the identification of at least 15 SSR markers capable of identifying all the protected soybean varieties in Brazil.

55. An expert from <u>China</u> reported that in 2007 the Ministry of Agriculture had adopted guidelines for the use of DNA for variety identification for the purposes of enforcement of plant breeder's rights for maize and rice. She added that the guidelines had also been used to check material in the official court growing trials as well as in seed market control. She explained that molecular markers had not been used for DUS assessment in any species, but that they had been used since 2002 to identify synonymies in VCU assessment, which was conducted by another part within the Ministry of Agriculture. She also reported that a maize DNA database containing information of 4,000 varieties had been established by the Maize Research Centre of Beijing.

56. An expert from the <u>European Community</u> reported that the Community Plant Variety Office of the European Community (CPVO) had sponsored projects on oilseed rape, potato and tomato, which would be presented at the meeting and that there was a new project starting on peach.

57. An expert from <u>France</u> reported that a project, supported by CPVO, for the development of SSR markers for peach, in cooperation with Spain, Hungary and Italy was under development. She explained that the project was intended to develop a database with morphological characteristics, molecular markers and digital images. She added that France had explored the possibility of applying to oilseed rape and barley the combined approach presented on maize in the introduction. The preliminary results would be presented at the twelfth session of the BMT. She concluded by saying that France was intending to extend the approach to lettuce.

58. An expert from <u>Japan</u> reported that DNA-based techniques for variety identification were regularly used in his country for 10 species, including strawberry, beans, sweet cherry, rush, Japanese pear, and tea, amongst others. Further work would be done to develop similar techniques for other crops.

59. An expert from <u>Mexico</u> reported that a project to develop SSR markers for variety identification on Amaranth had been initiated. He explained that the objective was to combine morphological and molecular data and that a report could be presented at the twelfth BMT session.

60. An expert from the <u>Netherlands</u> reported that, in addition to those projects which would be presented during the BMT session, there was a project for the development of expressed SSR markers on Brazilian rice varieties, which might fall within an Option 1(a) approach.

An expert from the Republic of Korea reported that the Korea Seed and Variety Service 61. (KSVS) had used molecular techniques for seed management, including plant variety protection. He added that KSVS collaborated with national research authorities to deal with cases of infringement of plant breeders' rights and other seed-related disputes in the private sector. He explained that molecular markers were used to check purity of officially certified seed lots before and after its entering into the market. He further reported that comparisons of DNA-profiles were also carried out in seed of registered varieties. He added that microsatellite markers had been developed for pepper, watermelon, melon, rice and Chinese cabbage, which covered a large part of domestic varieties. An expert from the Korea Forest Seed and Variety Service (KFS) reported that, according the Seed Industry Law, N° 8597, revised on August 3, 2007; KFS had become the authority for granting plant breeder's rights in the forestry sector, including trees, several fruit crops, ornamental plants and mushrooms. For that purpose, KFS had established the Korea Forest Seed and Variety Center (KFSV). He explained that KFSV had two departments, one responsible for plant variety protection and the other responsible for forest seed and seedling management. KFSV was also responsible for forest genetic resources management, including ex-situ conservation and DNA profiling for forest plant varies and mushrooms.

62. Experts from <u>Spain</u> reported that cereals and vegetables were the species with the largest number of varieties in his country and new techniques would be necessary for DUS examination in these species. It was explained that Spain cooperated with the CPVO in several projects, in particular in a project for an option 1 (a) approach for disease resistance in tomato, jointly with France and the Netherlands; and for an option 2 approach in sweet pepper and melon, which could be reported to the BMT at its twelfth session.

63. An expert from the <u>United Kingdom</u> reported that the United Kingdom had been involved in several projects in cooperation with the CPVO, which would be presented during the BMT session.

64. The representative of the Food and Agriculture Organization of the United Nations (FAO) elaborated on the continued collaboration with UPOV, including through providing technical assistance to member countries in the application of UPOV guidelines for DUS testing. She emphasized the need for developing national capacities in use of biomolecular techniques through increasing North-South and South-South exchange and cooperation. She also reported on the ongoing preparation of the second Report on the State of the World's Plant Genetic Resources for Food and Agriculture (SoW) that would be presented to the Commission on Genetic Resources for Food and Agriculture at its twelfth session in October

2009. The SoW was envisaged to have eight comprehensive chapters covering all aspects of policy, technical, social and economic aspects as relevant for plant genetic resources and food security. It would be based on country reports and identify the emerging trends that provide elements for updating of the Global Plan of Action on Conservation and Utilisation of Plant Genetic Resources for Food and Agriculture.

65. The representative of the International Community of Breeders of Asexually Reproduced Ornamental and Fruit-Tree Varieties (CIOPORA) reported that a project for the development of molecular markers for *Prunus* spp. had started with the aim of selecting 60 markers for each species for the management of germplasm collections of these species.

66. The representative of the International Seed Federation <u>(ISF)</u> reported that ISF had established a working group on molecular markers for variety testing which would hold its first meeting on the afternoon of September 18, after the BMT session. He reported that there was a general agreement of ISF members with respect to Option 1(a) and Option 2; however, there had been no agreement with respect to Option 3. The working group would review the situation and would establish written position.

67. The representative of the European Seed Association (ESA) explained that he had already reported on the matter of essentially derived variety in potato. ESA also contributed to the discussions in ISF.

Report of work on molecular techniques on a crop-by-crop basis

(a) <u>Vegetatively Propagated Crops</u>

Use of a Microsatellite-Based System for the Construction of a Reference Collection in Grapevine

68. The BMT received a presentation by Mr. Javier Ibañez (Spain), based on document BMT/11/8, a copy of which is provided as document BMT/11/8 Add..

69. Mrs. Marisé Borja (ISF) wondered if the varieties and microsatellites included in the study in Spain could be augmented with varieties and microsatellites from other countries. Mr. Ibañez explained that some of the 9 microsatellites used in his study were the same as the microsatellites which had been included in an *Office International de la Vigne et du Vin* (OIV) study.

70. Mrs. Beate Rücker (Germany) noted that the project was using molecular markers to check the identity of varieties and wondered if the identity was checked only on the basis of molecular markers, or whether the morphology was also used to check the variety identity. Mr. Ibañez explained that all varieties were described morphologically.

71. Mr. Joël Guiard (France) noted that many synonyms had been identified by ampelography and wondered if those had been confirmed by the microsatellites. Mr. Ibañez confirmed that they had, but that further synonyms had been found. In reply to a further question from Mr. Guiard, Mr. Ibañez explained that he had not studied the ampelography of those new synonym varieties, but that other institutes had made that analysis.

72. The Chairman wondered if the approach proposed for maize in documents BMT/10/14 and BMT-TWA/2/11 might be a way forward for grapevine. Mr. Ibañez noted that, apart from mutation varieties, no pairs of varieties had been found with differences in less than 2 alleles. However, there was no correlation between microsatellite differences and morphological differences which meant that it did not mean that such a situation could not occur in future.

73. The Chairman wondered if the approach proposed for maize in documents BMT/10/14 and BMT-TWA/2/11 might be a way forward for maize and for vegetatively propagated crops in general. Mrs. Beate Rücker (Germany) considered that it would be dangerous to consider such an approach in a general way and observed that it was a question of balancing risks. In that respect, she noted that the molecular marker information had been used to identify additional varieties for comparison for distinctness. She noted that distinctness was not a problem in grapevine.

74. Mr. Ben Vosman (Netherlands) recalled that, at a previous BMT session, an approach had been put forward for the use of 2 alleles to establish distinctness in rose varieties, which would not have resulted in any different decisions on distinctness compared to decisions based on morphology. Mr. Huib Ghijsen (ISF) agreed that distinctness was not a problem in seedling varieties of such vegetatively propagated crops and considered that it would only be necessary to identify mutant varieties by means of the DNA-profile for comparison by morphology. Mr. Joël Guiard (France) observed that it was necessary to respect the UPOV Convention and not to take decisions on distinctness based on molecular markers which were not linked to the phenotype; however, molecular markers could play a role in the management of reference collections.

Construction of an Integrated Microsatellite and Key Morphological Characteristic Database of Potato Varieties on the EU Common Catalogue Part 1: Discussion of Morphological and Molecular Data

75. The BMT received a presentation by Ms. Lysbeth Hof (Netherlands), based on document BMT/11/9 Rev., a copy of which is provided as document BMT/11/9 Add.

76. Ms. Hof explained that, in the case of the pair of varieties with 0.91 similarity, it had been possible to check the parentage of the varieties and to confirm that it was consistent that one of the varieties was a parent of the other. In response to a question from Mr. Huib Ghijsen (ISF), Ms. Hof confirmed that the varieties were distinct.

77. Mr. Tetsuya Kimura (Japan) sought clarification on why it had not been possible to identify 2 of the samples. Ms. Hof explained that the profiles had matched with 2 different varieties which were morphologically very similar.

78. Mr. David Calvache (Spain) noted that potato was a tetraploid and wondered if there was information on whether differences arose from the same alleles and the same markers. Ms. Hof reported that that aspect had not been checked except for the very similar pairs, where it had been found that the differences arose from different markers.

79. Mr. Bert Scholte (ESA) reported that ESA would consider the information from the study in relation to its work on essentially derived varieties, but requested information on how the project might go forward. Ms. Hof explained that the next step was still under

consideration. An approach similar to that proposed for maize, or by using a combination of molecular and morphological information, was under discussion. However, she noted that, in the meantime, the database was already proving very useful for variety identification purposes.

80. In response to a question by Mr. Joël Guiard (France) on the stability of the molecular markers, Ms. Hof reported that all samples had arisen from the single sources mentioned in the document.

81. Mr. Ben Vosman (Netherlands) requested information on whether the 8 unexpected pairs of varieties with 100% similarity had been granted plant breeders' rights (PBRs) in the same country or not. Ms. Hof reported that 2 of the pairs had been granted PBRs in the same country with a considerable time between the grants, whilst the others had been granted PBRs in different countries. Mrs. Beate Rücker (Germany) recalled that the aim of the project had not been to question decisions on PBRs. She reported that in the case of the pair of varieties for which she had knowledge, the two varieties had been distinct and, even though the applications had been made a long time apart, the two varieties had been compared in the DUS growing trial. However, she explained that it would be useful to have molecular marker information indicating similar varieties in advance of the growing trial and that information could help in respect of the quality control and insurance afforded to the DUS trial.

Construction of an Integrated Microsatellite and Key Morphological Characteristic Database of Potato Varieties on the EU Common Catalogue Part 2: The Database

82. The BMT received a presentation by Mr. Alex Reid (United Kingdom), based on document BMT/11/10 Rev., a copy of which is provided in document BMT/11/10 Add..

83. Mr. Reid reported that the database developed in the project was restricted to varieties on the European Union Common Catalogue: the Scottish Agricultural Science Agency (SASA) had a larger database, of around 1,400 varieties, containing additional varieties.

Use of a Molecular Marker-Based System for Identification of Varieties Within the Genus Eucalyptus

84. The BMT received a presentation by Mrs. Gisele Ventura Garcia Grilli (Brazil), based on document BMT/11/27.

85. In response to a question from Mrs. Marisé Borja (ISF), Mr. Luís Gustavo Asp Pacheco (Brazil) explained that the examination of DUS in Brazil was on the basis of morphological characteristics: the project had been investigating the use of molecular markers for variety identification. In that respect, he reported that a project on the *Eucalyptus* genus had studied over 400 SSR and SNP markers. However, 25 markers were sufficient to identify all varieties.

86. The Office explained that the draft Test Guidelines for Eucalyptus, prepared by experts from Brazil and considered by the Technical Working Party for Ornamental Plants and Forest Trees (TWO) at its forty-first session, held in Wageningen, Netherlands, from June 9 to 13, 2008, contained an annex of "molecular descriptors for the identification of clones and varieties of *Eucalyptus*". The TWO had suggested that the BMT should be invited to consider

the molecular markers included in that annex. The BMT noted that it was invited to consider the technical aspects of those molecular markers, but that the possible inclusion of such information on the Test Guidelines was a matter for the TWO and the Technical Committee.

(b) <u>Self-pollinated crops</u>

Development and Evaluation of Molecular Markers Linked to Disease Resistance Genes for Tomato DUS Testing (Option 1(a))

87. The BMT received a presentation by Mr. Ben Vosman (Netherlands), based on document BMT/11/6, a copy of which is provided in document BMT/11/6 Add..

88. Mrs. Lysbeth Hof (Netherlands) requested information on the cases where the pathogenesis assays did not match the molecular marker assays and wondered if there had been a check on the repeatability of the different laboratories. Mr. Vosman reported that the material in the pathogenesis assays might have been different to that used for the molecular marker assays and explained that a separate project was needed to investigate that aspect.

89. Mr. Marcel Bruins (ISF) expressed concern at situations where the molecular marker assay indicated that the variety was resistant, but the pathogenesis assay showed that the variety was susceptible: he recalled that it was essential for there to be a 100% correlation between the pathogenesis assays and the molecular marker assays in order to fit within an Option 1(a) approach. With regard to discrepancies between the two types of assays, Mr. Vosman considered that the molecular marker assays were more reliable than the pathogenesis assays. He noted that it was always clear whether the gene was present, but the resistance and susceptibility in pathogenesis assays, particularly for fungal diseases, was not always clear.

90. Mrs. Marisé Borja (ISF) observed that the molecular marker assays were based on DNA and noted that it would be possible for the gene to be present without expression. Mr. Vosman agreed that the source of the discrepancies would need to be investigated and considered that that was unlikely and that the discrepancies were a result of problems in the pathogenesis assays. He recalled that there was a perfect correlation for the Nematode (Mi) and TMV resistance genes.

91. Mr. Pere Arús (ISF) requested information on whether the molecular markers were covered by patents and whether a license was needed. Mr. Vosman explained that he was not aware of any patent protection and that the work fell into the category of research.

92. Mr. Joël Guiard (France) agreed that it was not appropriate to imply that disease resistance was a simple resistant / susceptible characteristic if there were different degrees of resistance, or if there was partial resistance, for example due to multigenic control. In addition, the discrepancies between the pathogenesis assays and the molecular marker assays could result in a decision to delete the characteristic concerned because it was not sufficiently reliable for use as a DUS characteristic. Ms. Elizabeth Jones (ISF) noted that background changes to the DNA could result in changes to the expression of the gene.

93. The BMT noted that an approach under Option 1(a) was conditional on the assumptions set out in document TC/38/14-CAJ/45/5 being met. It was also noted that it would be a matter for the relevant authority to consider if those assumptions had been met.

94. Mr. Huib Ghijsen (ISF) expressed concern at the use of markers which were covered by patents and proposed that only methods which were freely available should be used. The Office recalled that guidance on the use of patented methods in DUS testing had been addressed in document TGP/7/1 "Development of Test Guidelines", Guidance Note GN14.

95. Mrs. Anne Weitz (European Community) noted that there had been a lot of work on the project but there was further work to be done. In particular she noted that not all the assumptions of an Option 1(a) approach had been fulfilled and clarified that the intention was to develop this project in line with an Option 1(a) approach.

Functional SNP Markers for the Vernalization Requirements in Barley: An Option 1 Approach

96. The BMT received a presentation by Ms. Carol Norris (United Kingdom), based on document BMT/11/17, a copy of which is provided as document BMT/11/17 Add..

97. The Chairman noted that the project clearly fell within an Option 1(a) approach and wondered how the work might be developed in DUS testing. Mr. Andrew Mitchell (United Kingdom) explained that it would be necessary to establish that all the assumptions for an Option 1(a) approach were fulfilled and then to decide on whether the approach would offer a cost benefit in DUS testing.

98. Mr. Joël Guiard (France) wondered if it would be possible to extend the approach to wheat. Ms. Norris explained that, although that had not been feasible in the past, there were indications that it might be possible due to new development in molecular techniques.

Combining Molecular Distances to Morphological Characteristics for the Management of Field Comparisons in Spring Barley

99. The BMT received a presentation by Ms. Cécile Collonnier (GEVES), based on document BMT/11/21, a copy of which is provided in document BMT/11/21 Add..

100. Mrs. Beate Rücker (Germany) explained that the situation in barley was quite different from maize, because varieties of barley were, in general, morphologically very similar. Therefore, care was needed.

101. Mr. David Calvache (Spain) wondered why bulk samples of 20 seeds were used and how it would be possible to consider uniformity in bulk samples. Ms. Collonnier explained that the bulking was necessary in order to minimize costs. She explained that it was possible to detect one off-type plant in a sample of 20 plants.

102. In response to a question on whether the benefits of such an approach for barley would be as great as for Maize, Ms. Collonnier explained that, due to the lower morphological variability compared to maize, they were not expecting the same gain as in field comparisons. Mrs. Joëlle Lallemand (France) added that that point had to be evaluated.

(c) <u>Crops with varieties of different propagation types</u>

A Research Project Co-Financed by CPVO: "Management of Winter Oilseed Rape Reference Collections

103. The BMT received a presentation by Ms. Carol Norris (United Kingdom) and Ms. Cécile Collonnier (France), based on document BMT/11/11, a copy of which is provided as document BMT/11/11 Add.. Ms. Norris explained that the project was complete.

104. Mrs. Anne Weitz (European Community) noted that the project had demonstrated that the expectations of an Option 2 approach had not been met. A next step might be to consider an approach similar to that proposed for maize; however, she clarified that the original project was complete.

105. Ms. Collonnier reported that the work which she had presented on the use of molecular distances in combination with GAIA for the management of WOSR reference collections would continue in France. Ms. Norris explained that she would discuss possible next steps with the other partners in the project.

International guidelines on molecular methodologies

106. The Chairman introduced document BMT/11/4. He reported that ISTA were unable at attend the BMT session but had provided some information: there was no formal collaboration arrangements between the International Seed Testing Association (ISTA) and the Organization for Economic Co-operation and Development (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.

ISO TC34 SC16 (a central body for international harmonization and standardization of biomolecular methods applied to food and seeds)

107. The BMT received a presentation by ISO based on document BMT/11/25.

108. A new subcommittee "TC34/SC16" had been established by the ISO Technical Management Board in April, 2008, for international harmonization and standardization of bio-molecular methods applied to foods and seeds. Among the three Working Groups of TC34/SC16, Working Group 2 (WG 2: Varietal identification) appeared to be relevant for the work of the BMT. WG 2 is envisioned as using biomolecular markers to determine organism identity. Such work might include:

1) Determination of performance and quality criteria for the use of microsatellites, SNPs and other DNA-and protein-based molecular markers for cultivar identification and germplasm screening.

2) Determination and description of standard marker sets for regional and quality criteria for crop plants, fruits and vegetables.

Codex work on DNA-based methodology

109. The BMT received 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.

<u>Guidelines for DNA-Profiling:</u> Molecular Marker Selection and Database Construction "BMT Guidelines"

110. The BMT considered documents BMT/11/3 and BMT Guidelines(proj.12), and made the following recommendations with regard to document BMT Guidelines(proj.12):

Section Ato read "The purpose of this document (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 pecific precautions need to be taken to ensure quality entry into a database."Section Ato delete the second paragraphSection B111.1to delete "(f) cost" as the BMT Guidelines should focus on quality 1.3 (first)1.3 (second)to be renumbered etc.[] (1.5)to be numbered etc.[] (1.6)to read "useful level of polymorphism" and reference to specific techniques to be deleted2.1 (a)to retain the word "between" and delete the word "across"2.1 (b)to retain the words "as far as possible"2.1.1 (c)to be deleted on the basis that it will not always be appropriate2.1.2The second sentence to read: "They have been used and analyzed in different laboratories, and under specific experimental conditions are generally robust and repeatable."2.2.1.3 (d)to read: "effective separation between the various alleles in suitable detection systems;"2.2.2to delete [Number of loci] as the purpose of the markers may vary, e.g. in the case of Option 1(a), the number of loci is not relevant.		
Section B1.1to read: "Important criteria for choosing a methodology are: []"1.1to delete "(f) cost" as the BMT Guidelines should focus on quality1.3 (first)to delete the fourth sentence starting "Such a database can"1.3 (second)to be renumbered etc.[] (1.5)to be numbered 1.5 and to delete the comment in the square brackets in relation to RAPDs, AFLP, etc2.1 (a)to read "useful level of polymorphism" and reference to specific techniques to be deleted2.1 (b)to retain the word "between" and delete the word "across"2.1 (d)to retain the words "as far as possible"2.1 (e)to be deleted on the basis that it will not always be appropriate2.2.1.2The second sentence to read: "They have been used and analyzed in different laboratories, and under specific experimental conditions are generally robust and repeatable."2.2.1.3 (d)to read: "effective separation between the various alleles in suitable detection systems;"2.2.2to delete [Number of loci] as the purpose of the markers may vary, e.g.	Section A	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
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	2.2.1.3 (d)	
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3	The second sentence to read: "UPOV has developed guidance for the use of products or methodologies which are the subject of intellectual property rights and this guidance should be followed for the purposes of these guidelines." The BMT proposed that the Council should consider the text included in document TGP/7/1, GN 14 in parallel with the BMT Guidelines and delete this sentence if that text is not acceptable.
4.1	the last sentence to read: "The plant material from which the samples are taken should be traceable in case some of the samples subsequently prove not to be representative of the variety.
4.3	to add the following sentences: "With regard to being representative of the variety, consideration should be given to the features of propagation (see the General Introduction). The size of the sample should be determined taking into account suitable statistical procedures."
4.3.1 to 4.3.4.	to be deleted
5.2.1	to replace the words "agree on certain" by "consider"
5.2.1 first (new).	to delete the words "standardization of"
5.2.1 second (new).	to be deleted
5.2.2	to delete the text in square brackets
5.3.1	to delete the words in the square brackets, as this is covered by the first sentence of Section 5.3.1
5.3.3	to delete "(systematic errors)"
6.8	to delete "[Crop database]"
7 (new)	to be deleted
Glossary	to delete the explanations of PIC and FP, corresponding the deletion of PIC and FP from Section B 2.1.(a)

Practical exercise in the development of an exchangeable database of molecular data of plant varieties

111. The BMT received a presentation by the Office of the Union, based on document BMT/11/5.

112. The BMT agreed that it would be more appropriate to change the title of the agenda item to "Development of common database structure for molecular data".

Statistical methods for data produced by biochemical and molecular techniques

113. The Chairman noted that no papers had been presented for the item on statistical methods for data produced by biochemical and molecular techniques.

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114. 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 Technical Working Party on Automation and Computer Programs (TWC) might wish to consider that matter.

Recommendations on the establishment of new crop specific subgroups

- 115. The BMT noted the following plans for meetings of the existing Crop Subgroups:
 - Crop Subgroup for Maize: to hold a meeting in Autumn/Winter 2009, tentatively in conjunction with the maize and sorghum breeders' meeting in the United States of America;
 - Crop Subgroup for Potato: to consider a future meeting according to developments in on-going projects reported at the BMT session;
 - Crop Subgroup for Soybean: Mr. Marcelo Labarta (Argentina), Chairman of the Crop Subgroup for Soybean, to discuss the need for a meeting with experts from Brazil; and
 - Crop Subgroup for Wheat and Barley: subject to sufficient papers, to consider a meeting in conjunction with the twelfth session of the BMT.

116. The BMT noted that Ms. Francoise Blouet was no longer in a position to act as Chairperson of the Crop Subgroup for Oilseed Rape. It was noted that, if a meeting of the Crop Subgroup for Oilseed Rape became necessary, the Technical Working Party for Agricultural Crops (TWA) would be invited to propose a new Chairperson to the Technical Committee.

117. Mr. Pere Arús (ISF) noted that there was important work being done on certain temperate fruit crops, recalling in particular that the CPVO was co-funding a project on Peach. He wondered how that work might be considered by an appropriate crop subgroup. The Office explained that the intention was to create crop subgroups where specific proposals were at a point for consideration by the relevant DUS experts, breeders and molecular experts.

118. The BMT agreed that there were benefits in holding crop subgroup meetings in conjunction with the BMT session: such an arrangement was more cost- and time-efficient for participants and also provided the benefit that the participants in the crop subgroups were able to hear about developments in other crops.

Date and place of next session

119. In response to the invitation received from the Government of Canada, the BMT agreed to hold its twelfth session in Ottawa, Canada from May 11 to 13, 2010, with the preparatory workshop to be held on May 10, 2010.

Future program

120. During its twelfth session, the BMT planned to discuss the following items:

- 1. Opening of the session
- 2. Adoption of the agenda
- 3. Reports on developments in UPOV concerning biochemical and molecular techniques

- 4. Reports on the work of the Crop Subgroups
- 5. Short presentations on new developments in biochemical and molecular techniques by DUS experts, biochemical and molecular specialists, plant breeders and relevant international organizations
- 6. Report of work on molecular techniques on a crop-by-crop basis:
 - (a) vegetatively propagated crops
 - (b) self-pollinated crops
 - (c) cross-pollinated crops
- 7. International guidelines on molecular methodologies
- 8. Development of common database structure for molecular data
- 9. Methods for analysis of molecular data
- 10. The use of molecular techniques in examining essential derivation
- 11. The use of molecular techniques in variety identification
- 12. Recommendations on the establishment of new crop specific subgroups
- 13. Date and place of next session
- 14. Future program
- 15. Report of the session (if time permits)
- 16. Closing of the session

121. The BMT agreed that, in order to encourage the presentation of information in relation to the use of molecular techniques in examining essential derivation and in variety identification, it would be appropriate to dedicate a specific day to items 10 and 11 at the twelfth session of the BMT. In particular, breeders and other experts would be offered the possibility to attend for that specific day.

Medal

122. Mr. Henk Bonthuis was awarded a UPOV bronze medal in recognition of his chairmanship of the BMT from 2006 to 2008.

Visit

123. On the afternoon of September 18, the BMT visited "*Finca El Encin*" an experimental farm or estate belonging to the Madrid Institute for Rural, Agrarian and Food Research and Development (IMIDRA). BMT participants had the opportunity to visit the ampelographic museum; the national collection of grapevine varieties, formed by 3,259 accessions; and the laboratory on molecular biology.

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

[Annexes follow]

BMT/11/29

ANNEX I

LIST OF PARTICIPANTS

I. PARTICIPANTS

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[Annex II follows]

BMT/11/29

ANNEX II

Welcome Address made by

Mrs. Alicia Villauriz,

General Secretary of Rural Affairs, Ministry of Environment and Rural and Marine Affairs

Mr. Henk Bonthuis, Chairman of the Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular (BMT), Mr Rolf Jördens, Vice Secretary-General of UPOV, Mr. Button, Technical Director of UPOV, Mr. Lavignolle, Mr. Tabata and distinguished participants,

I am pleased to welcome you to this Eleventh Session of the BMT. Sessions of the Technical Working Party for Agricultural Crops (TWA), the Technical Working Party for Vegetables (TWV), the Technical Working Party for Fruit Crops (TWF), and the Technical Working Party on Automation and Computer Programs (TWC), have already been hosted in Spain.

As a member of UPOV, the Government of Spain will continue to play a leading role in fulfilling its obligations as a member State and in actively protecting intellectual property rights of new varieties.

As such, Spain has been a very active member of the organization, and cooperates with the UPOV Office on the organization of Courses and Seminars on Plant Breeder's Rights. This year, from 17 to 21 of November, the "Seventh Training Course on Protection of New Varieties of Plants" for Latin-American countries will be held in Santa Cruz de la Sierra (Bolivia) in cooperation with the Spanish Agency for International Cooperation and Development (AECID).

Plant breeding is a key factor for the development of the horticultural, agricultural, forest and fruit sectors. New varieties of plants are a key tool for the farmers to improve both product quality and yield.

During this week, developments since the last session of the BMT in Seoul will be discussed to see how they can contribute in the examination of new varieties of plants for the purposes of granting plant breeder's rights. It may be possible to improve the examination in different ways, e.g. in reducing the time of the examination, or in the consideration of a larger number of varieties of common knowledge, thereby increasing the quality of the examination of distinctness, uniformity and stability, and strengthening the plant breeder's rights system under the UPOV Convention.

Participants in this BMT Session who will join us in the technical visit to the Madrid Institute for Rural, Agrarian and Food Research and Development (IMIDRA), on Thursday will have the opportunity to visit:

<u>The Ampelographic Museum</u>: an open-air museum exhibiting the best known and most popular wine and table grape varieties in Spain, as well as other lesser-known varieties, foreign varieties, rootstocks, etc.

<u>The National Collection of Grapevine Varieties</u> that was started at the end of the 19th century to counteract the grape phylloxera epidemic which was ravaging the European vineyards; and

<u>The Molecular Biology Laboratory</u> where molecular markers have been used for a long time to identify varieties of grapevine and other species.

I am sure you will enjoy it.

Finally, I would like to thank Mr. Henk Bonthuis, Chairman of the BMT, Mr. Rolf Jördens, Mr. Button, Mr. Lavignolle and Mr. Tabata from the UPOV Office and the staff of the Spanish Plant Variety Office (OEVV) for organizing this meeting, and I wish all of you a good stay in Spain and best results of the work developed during these days.

Thank you for your attention.

[End of Annex II and of document]