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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

Seventh Session
Hanover, Germany, November 21 to 23, 2001

REPORT OF DISCUSSIONS AND DEVELOPMENTS IN UPOV REGARDING POSSIBLE USE OF MOLECULAR TECHNIQUES IN DUS TESTING

prepared by the Office of the Union

Developments in 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 (hereinafter referred to as "BMT") was established by the Council at its 26th ordinary session held in Geneva on October 29, 1992, at the recommendation of the Technical Committee in its twenty-eighth session, held in Geneva from October 21 to 23, 1992.

2. The BMT held its first session in Geneva on April 19 and 20, 1993. It has held five sessions as follows:

2nd session: Versailles, France, March 21 to 23, 1994
3rd session: Wageningen, Netherlands, September 19 to 21, 1995
4th session: Cambridge, United Kingdom, March 11 to 13, 1997
5th session: Beltsville, United States of America, September 28 to 30, 1998
6th session: Angers, France, March 1 to 3, 2000

3. Annex I to this document contains a list of presentations on the use of DNA-profiling techniques for various crops.

4. At its sixth session, the BMT thought that further progress in the consideration of the use of DNA profiling techniques in the DUS testing could be achieved through intensive discussion in small groups established on a species-by-species basis. It would be also expected that, once a few species were chosen, member States would coordinate their studies and exchange necessary information. Therefore, it was proposed to establish *ad hoc* crop subgroups, formed jointly by crop experts and biomolecular technicians, for further studies on the possible use of molecular techniques in DUS testing. The Technical Committee, at its 36th session in April 2000, agreed to this proposal.

Work Program of the *ad hoc* Subgroups

5. Subgroups were established for Maize, Oilseed Rape, Rose, Tomato and Wheat and meetings were held during February/March 2001.

6. Each subgroup was invited to consider the potential for use of molecular techniques on the basis of a "work program" developed by the Technical Committee (Annex II to this document) and an "issues paper" (document BMT/6/14), prepared by the Office of the Union in consultation with the Chairman of the BMT and the Chairmen of the Subgroups.

7. On the basis of the above papers, the subgroups considered:

- where there is most need for such techniques
- various possible models for the way in which molecular techniques might be introduced for DUS testing.

8. A summary of the outcome of the subgroup meetings is reproduced in Annex III to this document while the interim report of the subgroups is available in document BMT/7/2.

9. The Technical Working Party for Vegetables (TWV), at its thirty-fifth session held in Salerno, Italy, from June 25 to 29, 2001, recommended that the Tomato subgroup's work should be continued and extended to cover vegetable species other than Tomato where work on molecular techniques was being undertaken. Members of the TWV agreed to notify the Office of UPOV where work was planned and in addition to submit papers to the next session of the BMT.

10. The Technical Working Party for Agricultural Crops (TWA), at its thirtieth session held in Texcoco, Mexico, from September 3 to 7, 2001, considered it would be useful to introduce a subgroup for a vegetatively propagated agricultural crop and suggested that Sugar Cane or Potatoes might be appropriate. It was noted that a European Union project on potatoes was due to commence shortly but would not produce any results for two to three years. As a first step, the Office of the Union requested that members encourage the submission of papers, covering work on molecular techniques for these crops, to the forthcoming session of the BMT.

11. The Technical Working Party for Ornamental Plants and Forest Trees (TWO), at its thirty-fourth session held in Nagano, Japan, from September 24 to 28, 2001, agreed, as part of a study on molecular techniques, to provide the Netherlands (by November 9, 2001) with information on any pairs of Rose varieties which had been found to be not distinct in a DUS examination. Any such pairs of varieties would be examined to see if they were distinguishable using molecular characteristics.

12. The Technical Working Party for Fruit Crops (TWF), at its thirty-second session held in Valencia, Spain, from October 1 to 5, 2001 reaffirmed its support for the establishment of an *ad hoc* crop subgroup for Peach. The TWF also wished to consider the possibility of establishing a subgroup for Citrus and suggested this might be combined with the Peach subgroup under a single Chairman. The first step would be the presentation of a paper(s) on molecular characteristics for Peach and Citrus at the BMT. The BMT could then decide if there was sufficient relevant information on which to base a crop sub-group

Subgroup Established by the Technical Committee (TC) and the Administrative and Legal Committee (CAJ)

13. At its sixth session, the BMT further noted that different opinions existed among its members in the areas as follows:

(a) Interpretation of "the expression of the characteristics resulting from a given genotype or combination of genotypes": The BMT revisited the question of interpretation, which was still divided into two positions. Several experts insisted that the wording implied "phenotype." Therefore, differences in molecular markers possibly resulting from differences in non-expressed parts of DNA could not establish distinctness alone. In response to this interpretation, the Vice Secretary-General of UPOV reported the view expressed in the CAJ that these words do not necessarily mean "phenotype." The same language would be satisfied if a characteristic is simply inherited. The decision on the use of molecular markers for the assessment of distinctness should be based upon technical consideration within the scope of the Convention.

(b) Minimum distance: With respect to the concept of a "minimum distance," the BMT noted two different views:

(i) One view was that the concept of a minimum distance had reduced in significance after the adoption of the 1991 Act and the introduction of the essential derivation concept. All clear differences satisfying uniformity and stability criteria, irrespective of their degree and the nature of the characteristics, should be accepted as "clearly distinguishable." In practice, the differences had been very small in some cases, e.g. in single-gene controlled characteristics for disease resistance and flower color.

(ii) Another view was that the concept of a minimum distance should be taken into account, before the introduction of new characteristics, to ensure the quality of protection. The introduction of the essential derivation concept should not influence what is "clearly distinguishable." All small differences, such as one allele difference in DNA-profiling, should not be regarded as "clearly distinguishable."

(c) Supporting evidence: The BMT discussed the introduction of molecular characteristics as supporting evidence. The legal status of supporting evidence characteristics was questioned. Some delegates suggested they should be independent characteristics used as a last resort only.

14. Following the request of the BMT, the Technical Committee (TC), at its thirty-sixth session held from April 3 to 5, 2000, discussed the issues and noted the existence of differing interpretations and understandings on the above points amongst member States and also

between legal and technical experts. The TC therefore proposed a small *ad hoc* meeting composed of technical and legal experts with a view to establishing a basis for common understanding and interpretation of these fundamental questions before further discussion in its next session and in the Administrative and Legal Committee (CAJ).

15. At its fourty-second session held on October 23 and 24, 2000, the CAJ agreed to establish an *ad hoc* subgroup as suggested by the TC and requested that the Office of the Union propose terms of reference for this *ad hoc* subgroup.

16. At its fourty-third session held on April 5, 2001, the CAJ adopted the terms of reference as follows:

(a) The subgroup 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:

(i) conformity with the UPOV Convention, and

(ii) 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.

(b) In conducting its assessment, the subgroup may refer specific aspects to the Committee or the Technical Committee for clarification or further information as considered appropriate.

(c) The subgroup will report its assessment, as set out in paragraph (a) above, to the Committee, but this assessment will not be binding for the position of the Committee.

17. It was noted that the above terms of reference would shortly be updated with a report, by the Office of the Union, of the first meetings of the *ad hoc* crop subgroups. This would outline the initial discussions, prior to consideration by the BMT and Technical Committee, on possible application models for the utilization of biochemical and molecular techniques in the examination of Distinctness, Uniformity and Stability.

18. The establishment of the ad hoc group allows the Technical Committee and the BMT to focus their work on the development of technical prospects rather than seek to answer wider issues before these technical prospects can be developed.

Possible Work Areas of the BMT in future session

19. As indicated above, there are now several bodies working on different aspects of the use of biochemical and molecular techniques for DUS testing and related questions as follows:

➢ CAJ/TC ad hoc subgroup:	– conformity with the UPOV Convention; – potential impact on the strength of protection;
➢ Technical Committee	– Establishment of crop subgroups;
➢ Technical Working Parties and Technical Committee	– Examination of suitability of possible characteristics from a technical perspective according to the General Introduction;
➢ Crop subgroups:	– Crop specific application of molecular techniques in DUS testing.

20. It may be useful for the BMT to identify clearly its proper field of work in order that the different bodies will work in a mutually supportive manner, and avoid any duplication of work. The following areas are not covered by other bodies:

- ➢ general developments in biochemical and molecular techniques;
- ➢ harmonization of protocols for the use of molecular techniques, including the establishment of guidelines for the use of molecular techniques;
- ➢ recommendation, where sufficient information has been accumulated, to establish crop subgroups;
- ➢ analysis and processing of molecular information, including management of database.

21. The "Work Program (Annex II to this document)," which has been elaborated primarily for the crop subgroups, illustrates the range of work to be considered in relation to the use of biochemical and molecular techniques in DUS testing. "Work Program" item 1 "Molecular methods available and suitability for use" corresponds to the work area mentioned above and may be considered to identify the role of the BMT. In addition, under item 4, "Assessment of Essential Derivation" and "Use for Identification" might be addressed by the BMT, which could provide a forum to exchange views.

22. The application of molecular techniques in UPOV Test Guidelines may follow the following procedures:

(a) Once the development of a specific biochemical or molecular technique for a particular crop provided a basis for its consideration for use in DUS testing, this would be considered by a crop subgroup.

(b) Any proposal made by a crop subgroup for the use of a biochemical or molecular characteristic for a particular crop would be considered by the relevant Technical Working Party, Technical Committee and the *ad hoc* TC/CAJ subgroup.

23. Furthermore, it should be noted that the Technical Committee is currently revising the General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of New Varieties of Plants. The new General Introduction will be complemented with a series of associated documents, of which document TGP/15, to be entitled "New Types of Characteristics," will contain a general guidance of the application of DNA profiling techniques for DUS testing. The elaboration of such a document will be an important role of the BMT.

Possible Interim Conclusion of the BMT

24. As some 10 years have passed since UPOV started to consider the possible use of DUS profiling techniques for DUS testing and the new procedures for the adoption of a DNA profiling technique as UPOV characteristic is now in place, the BMT may wish to make specific recommendations to the Technical Committee on its role in the examination of biochemical and molecular techniques for their possible application in DUS testing.

[Annex I follows]

ANNEX I

Presentations on DNA Profiling Techniques Applied to in Different Crops Species

Sessions	DNA-Profiling Techniques	Crops	Document	Report from
BMT 2	RAPD, STS	Citrus	BMT/2/3	Australia
BMT 2	PCR-based methods	Maize	BMT/2/4	France
BMT 2	SSR, RFLP, RAPD	Soy beans	BMT/2/6	USA
BMT 2	RFLP	Maize	BMT/2/7	France
BMT 2	RFLP, RAPD	Oilseed Rape	BMT/2/8	UK
BMT 3	RAPD	Ryegrass	BMT/3/3	Belgium
BMT 3	RFLP, RAPD	Oilseed Rape, Barley	BMT/3/4	UK
BMT 3	RAPD	Alfalfa	BMT/3/9	France
BMT 3	RFLP	Sunflower	BMT/3/10	France
BMT 3	RAPD	Hydrangea	BMT/3/11	France
BMT 3	RAPD	Conifer	BMT/3/12	France
BMT 3	RAPD	Peach	BMT/3/13	France
BMT 3	RAPD	Strawberry	BMT/3/14	Israel
BMT 3	RAPD, SSR	Potato	BMT/3/15	UK
BMT 3	RAPD	Lolium	BMT/3/16	Australia
BMT 4	SSR	Maize	BMT/4/2	ASSINSEL
BMT 4	RAPD	Ryegrass-Fescue	BMT/4/3	Czech Republic
BMT 4	AFLP	Ryegrass	BMT/4/4	Belgium
BMT 4	RAPD, AFLP	Pot Azalea	BMT/4/5	Belgium
BMT 4	AFLP	Rice	BMT/4/10	Japan
BMT 4	STS, SSR	Oilseed Rape	BMT/4/11	France
BMT 4	Patanin Polymorphism	Potato	BMT/4/12	Germany
BMT 4	Microsatellite	Carnation	BMT/4/15	Netherlands
BMT 4	RAPD, AFLP	Peach	BMT/4/16	France
BMT 4	STS	Ryegrass	BMT/4/18	France
BMT 4	Microsatellite	Oilseed Rape	BMT/4/20	UK
BMT 5	AFLP	Ryegrass	BMT/5/4	UK
BMT 5	AFLP	Rape Seed	BMT/5/5	France
BMT 5	AFLP	Rose	BMT/5/6	France
BMT 5	RFLP	Maize	BMT/5/7	France
BMT 5	AFLP	Strawberry	BMT/5/9	France
BMT 5	AFLP	Strawberry	BMT/5/10	Japan
BMT 5	STS	Ryegrass	BMT/5/11	France
BMT 5	AFLP, RAPD, Microsatellite	Tomato	BMT/5/13	ASSINSEL
BMT 5	AFLP	Azalea, Flax, Linseed	BMT/5/16	Belgium
BMT 6	ISSR, ISTR	Chrysanthemum	BMT/6/2	UK, USA
BMT 6	AFLP	Sugar Beet	BMT/6/3	Belgium
BMT 6	Microsatellite	Wheat, Oilseed Rape	BMT/6/4	UK
BMT 6	SSR	Maize Inbred Lines	BMT/6/5	USA
BMT 6	AFLP	Ryegrass	BMT/6/7	UK, BE, NL
BMT 6	RAPD	Grapevine	BMT/6/8	Spain
BMT 6	AFLP	Rape Seed Inbred Lines	BMT/6/9	France
BMT 6	Microsatellite	Pear	BMT/6/11	Japan

[Annex II follows]

ANNEX II

Work Program of the *Ad hoc* Crop Subgroups on Molecular Techniques

Item	Questions
1. Molecular methods available and suitability for use	(a) Capacity for discriminating varieties (polymorphism) (b) Availability of appropriate markers (or easiness for developing appropriate markers) (c) Cost & availability - accessibility of technology (patents & licenses) - cost of equipment & licensing - cost of materials - safety issues (e.g. radioactivity) (d) Repeatability / Consistency of results from repeated sampling of a variety : - repeatability on material from different plants - repeatability on material from different tissues - repeatability on material from different growth or physiological stages and / or grown in different environments (e) Reliability and agreement between laboratories and with different equipment (f) Means of improving repeatability / consistency
2. DUS Issues (a) Distinctness	(a) Variability between varieties - differences in closely related varieties (e.g. number of bands and molecular distance calculations) - are all existing varieties distinguishable ? - are robust genetic distance methods available?
(b) Uniformity	(b) Variation within existing varieties - level of uniformity in relation to current uniformity standards for the crop - possibilities for operating with different uniformity criteria whilst still retaining current levels of distinctness
(c) Stability	(c) Variability of data from different years, seed generations and seed sources (Stability of DNA)
(d) Influence of different methods on levels of DUS	(d) Influences on levels of distinctness and uniformity - of the choice of molecular marker sets - of the choice of genetic distance methods
3. Relationships to phenotype	(a) Linkage of bands to phenotype (b) Correlation of DUS of traditional characteristics with findings on DUS from molecular data (c) Potential for identifying molecular markers with links to phenotype (d) Comparison of genetic distances using molecular data and traditional characteristics (phenotypic distance)
4. Potential applications (discussing possible application models and criteria for the use)	(a) Establishment of DUS (or supporting evidence for conventional characteristics?) (b) Management of Reference Collections (c) Assessment of Essential Derivation (d) Use for identification (inclusion in variety description)
5. Possible impacts of the introduction and unsolved problems	(a) Unsolved problems /necessary work (b) Possible impacts of the introduction on the protection system

[Annex III follows]

ANNEX III

Summary of the Outcome of the Crop Subgroup Meetings

The crop subgroups considered where there is most need for molecular techniques and various possible models for the way in which such techniques might be introduced for DUS testing.

I. Need for Molecular Characteristics

1. There was a broad consensus amongst participants of the subgroups that the greatest need for the development of molecular characteristics is in the "management of reference collections." The term "management of reference collections" encompasses the need to establish distinctness from any other variety whose existence is a matter of common knowledge and the need to organize, in an effective way, the growing trial of candidate varieties and other reference varieties which have previously not been established to be distinct from the candidate varieties. The potential advantage of molecular characteristics is that information obtained from different DUS examiners will be directly comparable and could be used by other parties for considering distinctness against candidate varieties. This would allow the screening of a larger collection of varieties than currently included in physical reference collections, and by the use of these characteristics for establishing distinctness prior to the growing trial ("pre-screening") could significantly reduce both the number of reference varieties which need to be included in the growing trial and the number of traditional characteristics which need to be examined for distinctness.

2. It was noted that the process of pre-screening could require a greater difference between varieties than the minimum distance for distinctness used in a growing trial, since it was only the first step in determining distinctness. It was considered that this greater difference ("minimum distance plus") would allow the introduction of a suitable safety margin for molecular characteristics if they were used in this way. Experience gained over time may then allow this safety margin to be reduced.

II. Models for the Possible Introduction of Molecular Techniques in DUS Testing

3. In considering the possible use of molecular techniques, or any characteristic, it is necessary to consider how they might be used to examine Distinctness, Uniformity and Stability.

Distinctness, including use in "Pre-screening"

4. The discussions in the subgroups reviewed technical developments in relation to three different general approaches, which might be considered for the introduction of these techniques. Each of these was developed with regard to the existing level of difference required for distinctness of plant varieties, or "minimum distance":

OPTION 1: Molecular Characteristics as Predictors of Traditional Characteristics

(a) It was generally agreed that molecular characteristics which directly and consistently predict distinctness for traditional characteristics (e.g. gene specific markers for herbicide tolerance or disease resistance) presented no major concerns regarding possible erosion of the "minimum distance." It was noted that, at present, there are only a limited number of molecular characteristics with such linkage. It was also noted that the reliability of the linkage would need to be kept under constant review.

(b) An alternative is to identify a set of molecular characteristics which can be used reliably to estimate a traditional characteristic; for example, quantitative trait loci, although this is not the only possible example. Having estimated the traditional characteristic in this way, the information could then be used as a basis for clearly distinguishing varieties.

OPTION 2: Calibration of Molecular Characteristics against Traditional Characteristics

5. It was considered that concerns regarding the possible erosion of minimum distance might be addressed by calibrating the minimum distance required for distinctness using molecular characteristics against the minimum distance established by traditional characteristics, to ensure that there would be no significant change as a result of the introduction of the former. It was also considered necessary to conduct an analysis, prior to any decision on its introduction, to review any change in the nature of decisions which may result and consider the impact on the effectiveness of plant variety protection.

OPTION 3: Development of a New System followed by Impact Analysis

6. The final approach considered by the subgroups was the development, from scratch, of a system for determining distinctness in a technically robust way (and also in accordance with the UPOV Convention). Having developed such a model system, this would be analyzed (e.g. by a review of possible differences in decisions compared to the existing system) for its impact on the effectiveness of plant variety protection. Consideration would then need to be given as to whether such changes, if any, were acceptable when considered alongside any other possible benefits or disadvantages.

Uniformity and Stability

7. The current subgroups consider self-pollinated or vegetatively propagated species for which, broadly speaking, the current uniformity requirements are based on uniformity in an absolute sense, rather than cross-pollinated species, for which uniformity is assessed in relative terms.

8. The work in the subgroups demonstrated that when existing protected varieties (i.e. uniform for traditional characteristics) are examined there is often, at least to some extent, some lack of uniformity for molecular characteristics. When considering the possible introduction of molecular characteristics it would be necessary to consider if there should be a requirement for such characteristics to be uniform in absolute terms, as for traditional characteristics, or if relative uniformity would be acceptable. It was noted that requiring higher standards of uniformity could, in some cases, have a negative impact on variety performance.

9. At present there is insufficient information to establish whether it would be simple and practical for breeders to establish uniformity and maintain this (i.e. stability) for molecular characteristics. The general consensus at the meetings was that, in principle, a lack of absolute uniformity should not necessarily prevent the use of these characteristics if satisfactory guidelines are developed. However, it was noted that lack of absolute uniformity would diminish the power of discrimination for the characteristics.

[End of Annex III and of document]