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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

Second Session

Versailles, France, March 21 to 23, 1994

REPORT

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

Opening of the Session

1. The Working Group on Biochemical and Molecular Techniques and DNA Profiling in particular, (hereinafter referred to as "the Working Group") held its second session in Versailles, France, from March 21 to 23, 1994. The list of participants is reproduced in Annex I to this report.
2. The Chairman of INRA's Versailles Research Center, Mr. Frantz Rapilly, the Director of GEVES, Mr. Lefort, and the Deputy Director, Mr. Guiard, welcomed the participants to the INRA station at Versailles. The session was opened by Mr. Guiard in his capacity as Chairman of the Working Group.

Adoption of the Agenda

3. The Working Group unanimously adopted the Agenda as reproduced in document BMT/2/1. The Chairman pointed out that seven new documents had been prepared (BMT/2/2 to 8) for the present session. In addition, the following other documents could be of help in the discussions: BMT/1/3 and 4, and TC/24/4, 5 and 7.

4. The Chairman referred to the main aim of the work, namely to study DNA Profiling and to coordinate the development of methods (also including other biochemical and molecular techniques). Therefore, apart from the species mentioned in the Agenda and the two main methods, RFLP's and RAPD, further species and methods should also be discussed in the future. Special attention should be given during the discussions to the reproducibility of results and to genetic background knowledge of the correlation between the results and the phenotypic expression. Discussions should also concern the use of the results, whether they could be used for DUS testing and, if so, whether in parallel with traditional characteristics, as a supplement or as a substitute or whether for identification purposes only. He proposed that discussions under the next item should deal with the technical aspects only and the consequences of possible introduction be left to a later part of the discussions.

Presentation of Documents

Tomato

5. Mr. Ben Vosman (Netherlands) introduced document BMT/2/2 on the use of molecular techniques for the identification of tomato cultivars and highlighted the differences between the RFLP probes, RAPD's and microsatellites or Oligo-nucleotide probes.

Citrus

6. Mr. Rod Peakall (Australia) introduced document BMT/2/3 on the application of DNA profiling to the determination of distinctness between varieties in Citrus and implications for varietal identification in other plant species, limiting himself under the present agenda item to the first part of the document highlighting the differences between the RFLP's and PCR based RAPD and STS.

Maize

7. Mrs. Joelle Lallemand (France) introduced document BMT/2/4 on molecular markers in maize mentioning as main methods studied those on RFLP's, RAPD, AFLP and microsatellites. She also referred to an additional list of publications available during the session and reproduced as Annex II to this report.

8. Mr. Alain Murigneux (ASSINSEL) introduced document BMT/2/7 on maize fingerprinting: improvement of the RFLP protocol and selection for probe quality, reporting on a project initiated by the Comité technique permanent de la sélection des plantes cultivées (CTPS) with partnership of GEVES-INRA and SEPROMA. The biochemical analysis and interpretation of DNA profiles have been produced by BIOCEM investigating the production of data for establishing the genetic distance and several parameters playing a major role in the quality and quantity of test results.

Soybean

9. The Working Party saw a video recording of a seminar held in the United States of America on the techniques of simple sequence repeats (SSR) or

microsatellites highlighting the advantages of that method over other methods. (Persons interested in more information on that recording should address themselves to Mr. Atchley, USA).

10. Mr. Alan A. Atchley (USA) afterwards introduced document BMT/2/6 on the biochemical and molecular techniques and DNA profiling: preliminary assessment and comparing of methods as applied to soybeans. He added that at present distinction of soybean varieties was made mainly on the basis of characteristics on disease resistance and on morphological characteristics.

Oilseed Rape and Barley

11. Mr. James Reeves (United Kingdom) introduced document BMT/2/8 on the use of DNA profiling for distinctness, uniformity and stability testing which explained the research going on in the United Kingdom on the developing and evaluation of methods for assessing the extent of DNA polymorphism in oilseed rape and barley, concentrating on RFLP's but also studying PCR-based techniques (RAPD).

Other Species

12. Mr. Rod Peakall (Australia) introduced the second part of document BMT/2/3 concentrating on the analysis of the data received and calculation of a genetic distance. He concluded that, depending on the information available at present for a given species, different methods would be optimal for the different species. However, STS microsatellites might be the most promising method in future.

The Use of DNA Profiling Methods by Expert Witnesses in Disputes on Essential Derivation

13. Although the agenda had planned to first discuss the use of DNA profiling methods for DUS testing, the discussions immediately led to the use of them for the proof of essential derivation and thus it was decided to go ahead with those discussion first.

14. Mr. Bernard Le Buanec (ASSINSEL) reported that, inside ASSINSEL, the wish had been expressed to keep the criteria of distinctness, uniformity and stability completely separated from those for essential derivation. If possible, the same should also apply for the tools used to define those criteria. There was a risk that when the same tools were applied to both criteria a risk of confusion would arise. The DNA profiling techniques are primarily tools to establish a genetic link between varieties and to trace parentage.

15. Mr. Avner Bar-Hen (France) introduced document BMT/2/5 on the basis and uses of distances for varietal characterization and explained different statistical tools to measure distances. He concluded that it was important, however, before applying any method that the crop expert should clearly define:

- (i) what he wanted to measure,
- (ii) how he wanted to do that, and
- (iii) what precision he would require.

16. In the discussion, that followed it was stated that different methods would lead to different results with respect to the distances. UPOV needed to

establish guidelines to explain certain criteria giving advice on the use of certain methods for the calculation of data or how to discard certain methods.

17. Mr. Stephen Smith (ASSINSEL) explained the understanding of the American Seed Trade Association (ASTA) of essential derivation in maize varieties. In order to prevent erosion of protection there should be a certain boundary between the original variety and an essentially derived variety (edv). He outlined current proposals of the ASTA where a variety with 90% similarity or more should be considered an essentially derived variety (edv) while below 75% of similarity a variety would not be considered an edv.

18. Mr. Barry Greengrass (UPOV) recalled that the Diplomatic Conference which adopted the 1991 Act of the UPOV Convention had asked the UPOV Secretariat to establish Guidelines on essential derivation and that the Office had prepared a discussion document on the subject which had been discussed in a meeting with the professional organizations. The Administrative and Legal Committee of UPOV had decided (and its decision had been endorsed by the Council) in the immediate future, however, not to proceed further in the preparation of Guidelines. All agreed that the edv issue was not related to the procedure for granting protection but to another procedure whereby a breeder either in informal registration or the court may seek to establish that particular variety is an edv of his protected variety.

19. Breeders know when they are doing real breeding work, however, there remained uncertainty as long as the threshold that would be acceptable was not known. Guidance was needed for the breeders how to interpret the criterium of essential derivation. Although the UPOV Convention contained examples of breeding methods which may lead to an edv, it was not considered useful by the breeders to add further examples. Breeders preferred to search for objective assessments of the genetic distance, crop by crop, to discuss the thresholds for each crop and try to reach a common agreement among themselves. Guidance on the methods to be used to assess threshold could be useful. The advantages and disadvantages of each of the methods, their limits and the method of calculating and interpreting the results should be discussed and fixed crop by crop. It was generally accepted that certain markers known to have a good coverage of the whole genome should be given a completely different weight from an equivalent number for which that knowledge was not available and which covered a small part of the genome only. Greater weight should also be given to markers of known genetic functions as opposed to those for which it was unknown.

Possibilities and Consequences of the Introduction of DNA Profiling Methods for DUS Testing

20. The Chairman pointed out that in order to be acceptable for use in the testing of distinction, uniformity and stability, the DNA profiling method had to fulfil the same requirements as any other new characteristic before it could be accepted. It would have to be a robust and standardized method and leading to comparable results. It must be precise and it must be possible to reproduce the results between years and between labs in different countries.

21. Mr. Le Buanec (ASSINSEL) reported that, in principle, ASSINSEL had none or few problems with the use of DNA profiling techniques for establishing essential derivation. For the testing of distinctness a morphological (or physiological) marker would be much better adapted as it referred to the expression of the genotype as spelled out in the UPOV Convention. In the case

of use for distinctness, referred to by the Chairman, uniformity and stability were also required. In the case of DNA profiling it was easy to show a difference in the DNA. It would, however, be difficult for many species and for many markers to prove uniformity and stability. The problem may be overcome in the future, but at present it was a real problem. ASSINSEL would not exclude that, for some crops the use of DNA profiling might be useful, but suggested that this should be discussed crop by crop.

22. In the discussions several further criteria were mentioned which the methods would meet with difficulty. A large number of additional characteristics resulting from the application of the method would be accepted in one go and would lead to a reduction of the minimum distance required between varieties. It was understood that that distance should not be reduced as a result of the increased power to distinguish. The tool and the power of the tool should be seen separately.

23. It was suggested that it would always be possible to find some difference with DNA markers. It was generally accepted that this would mean that any new variety would be distinct and granted a new title of protection. That could lead to the erosion of the present system, something which nobody wanted.

24. The use of DNA profiles as markers of, for example, certain disease resistances which otherwise are costly to check, was not contested. In this case, the resistance characteristic was used for distinctness and the DNA profile was only a replacement of the normal test.

25. Different opinions were expressed on whether, for example, in the case of two male sterile varieties, which otherwise were not distinguishable, the proof by DNA profiling that male sterility resulted from different genetic mechanisms would be sufficient to grant two separate rights.

26. When looking at the results of some DNA methods one may be looking only at the content of the genome with no knowledge of its significance. A gene may be proved to be present but may not express itself. Would it be justified to grant a separate right if in the phenotype, and thus in the field, and in the use of the variety there was no difference at all?

27. Several experts expressed, although being contested by others, the idea that, although at present not being able to use DNA profiling for DUS testing, it could be used as complementary information. DNA profiles could identify genotypes which had been proved to be distinct by other means and could give much information which could be used to choose the best reference variety. They could be used in the grouping of varieties without themselves forming grouping characteristics. The decision would continue to be made on the basis of the expression of the genotype, e.g. morphological or physiological characteristics. There existed thus two sets of characteristics, one used for the establishing of distinctness and another set of additional characteristics used only for identification. The DNA profile would thus just be a help and not the basis for the establishing of distinctness.

28. Other experts claimed that even if only used to identify a genotype which had proved to be distinct by other means, a characteristic had to fulfil the requirement of homogeneity and stability. These two criteria, however, were not fulfilled at present by most of the DNA profiles.

29. It was proposed to list the reasons in favor of the inclusion of DNA profiles in the testing of DUS. However, as the breeders had not reached a final conclusion among themselves, the drawing up of such a list was considered premature and was postponed to the next session of the Working Group.

30. The question of homogeneity had not as yet been studied sufficiently and it would be premature to make any judgement. It would need special study with respect to synthetic varieties. Numerous further studies would still be necessary. That would also raise the question of the consequences of the use of DNA profiling and the extra burden for small breeders who do not so far use it and thus do not select for those characteristics. They would be obliged to start with the methods and make their varieties homogeneous. That meant that breeding and maintaining costs would increase considerably.

Future Program, Date and Place of the Next Session

31. The Working Group agreed that further sessions were necessary in order to continue discussions and make progress. At the invitation of experts from the Netherlands, it agreed to hold its third session in Wageningen (Netherlands) from September 19 to 21, 1995. The session would start in the morning of September 19 and close at noon on September 21, 1995.

32. The Working Group agreed to collect more information on a larger number of crops with different ways of propagation and also to cover the area of ornamental species not previously covered. The documents to be prepared by different experts should, if possible, for each given crop:

- (i) list the different methods under study,
- (ii) list the questions and problems arising,
- (iii) assess the objectives for the species concerned,
- (iv) compare and evaluate the methods, taking into special account knowledge of the genetic control of the markers used, repeatability inside one laboratory and between laboratories,
- (v) consider the availability of the method to everybody (especially if the method was patented),
- (vi) consider the technical costs involved,
- (vii) evaluate the aspect of uniformity and stability through a plant to plant comparison and whether the method might be useful for DUS purposes and or for proving essential derivation,
- (viii) propose the standardization of the method considered best for that species.

33. It was finally agreed that several documents would be prepared for the next session with a deadline at the end of June 1995 to enable distribution in good time before the next session.

34. To improve a better understanding of the different methods and to use the same terms in the above documents and in the discussions during the next session, it was agreed to try to reach agreement on names and definitions of the different methods. The experts from Belgium offered to prepare for the beginning of March 95 a document listing the methods, giving the main principles on which they were based, the different names used, and making proposals for a common name to be used by the BMT.

35. The question of statistics and the statistician's requirement for well-defined questions were repeated. Every expert should, at national level, contact the local statistician and formulate with him for each type of study (DUS, essential derivation) the questions concerning the motivation of the study and the means envisaged to answer the question posed, eg. what should be measured, how it should be measured and with what precision. Only once these questions were settled could one look for harmonized statistical methods for the different purposes. The experts from France offered to prepare a paper on the analysis of distance. The TWC should also be informed, however, on the outcome of the discussions and place the item on its agenda.

36. The expert from ASSINSEL would try to prepare a paper on the position of the breeder vis-à-vis the DNA profiling methods for DUS tests and for the establishment of essential derivation and another paper on the technical costs of the application of the methods and their accessibility, especially vis-à-vis methods protected by patents.

37. As a result of the above, the agenda for the coming session of the BMT would comprise the following items:

- (i) Definition of methods of DNA profiling (BE to prepare a paper);
- (ii) Documents on certain species ;

Apple (only problems arising and objections, no results of methods)	GB	to prepare a paper
Barley	GB	"
Hydrangea (mainly RAPD)	FR	"
Lolium (mainly RAPD + STS)	AU + BE	"
Lucerne	FR	"
Maize (if possible)	ASSINSEL	"
Oak (marker)	FR	"
Oilseed Rape	GB	"
Pinus maritimus (proteins)	FR	"
Poplar (if possible)	BE	"
Prunus (isozymes)	FR	"
Sunflower	FR	"
Tomato	ASSINSEL	"

- (iii) Statistical Aspects of DNA profiling including analysis of distance (FR to prepare a paper);
- (iv) Technical costs and access to the method of DNA profiling (ASSINSEL to prepare a document);
- (v) Position of the breeders vis-a-vis DNA profiling (ASSINSEL to prepare a document);
- (vi) Possibilities and consequences of the introduction of DNA profiling methods for DUS testing;
- (vii) The use of DNA profiling methods by expert witnesses in disputes on essential derivation.

Visits

38. In the afternoon of March 21, 1994, the Working Party visited part of the research installations of INRA, Versailles, where research was in progress regarding RFLP's and PCR's. In the afternoon of March 22, 1994, the Working Party was given a guided tour through the Genethon Human Genome Research Centre at Orsay near Paris and the INRA farm at Moulon where it received information on breeding aims and the use of electrophoresis and DNA profiling in support of those aims.

39. This report has been adopted by correspondence.

[Two Annexes follow]

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[Annex II follows]

MOLECULAR MARKERS IN MAIZE (REFERENCES)

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