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UPOV

BMT/3/18
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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANT

GENEVA

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

Third Session
Wageningen, Netherlands, September 19 to 21, 1995

REPORT

adopted by the Working Group

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 third session in Wageningen, Netherlands, from September 19 to 21, 1995. The list of participants is reproduced in Annex I to this report.

2. Mr. N.G. Hogenboom (Netherlands), Director of the Agricultural Research Department (DLO-NL) of the Centre for Plant Breeding and Reproduction (CPRO-DLO), welcomed the participants to Wageningen, Netherlands. The session was opened by Mr. J. Guiard (France), Chairman of the Working Group.

Adoption of the Agenda

3. The Working Group unanimously adopted the Agenda as reproduced in document BMT/3/1 after having agreed to discuss item 5 after item 2, item 4 after item 6, and to change the order of the reports in item 3 as follows: Lolium, Barley, Oilseed Rape, Lucerne, Sunflower, Potato, Tomato, Strawberry, Peach, Hydrangea, *Pinus maritimus*. The Chairman pointed out that several new documents had been prepared for the present session, which were distributed immediately before the session and were given the document numbers BMT/3/12 to 16.

4. The Chairman recalled that the aim of the session was not to have an exhaustive review of the different methods applied to the different species but to look at improvements achieved. It was not possible to ignore these new tools. One had to carefully check their possible use for DUS testing, not as a substitute but as a supplement. Before reaching that stage, however, conditions under which such use might be possible would have to be discussed and agreed. He recalled the possible use of the techniques for checking essential derivation and referred to paragraphs 19 and 21 of the report of the second session (document BMT/2/9) reproducing the position of the breeder as follows:

> "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 criterion of essential derivation. Although the UPOV Convention contained examples of breeding methods which may lead to an essentially derived variety (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 thresholds 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."
>
> .....
>
> "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."

5. The Chairman further stated that the situation as indicated in paragraph 4 of the above report had not changed and in order to remind the Working Group read out the paragraph as follows:

> "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, RFLPs 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."

The Chairman proposed to follow the same procedure which had been approved by the Working Group.

6. Some experts made reference to the publication in the Gazette from Australia on the granting of protection of a *Banksia coccinea* variety in Australia with the help of the RAPD method. Mr. Peakall (Australia) clarified that in his opinion in that case the RAPD results had been only supplementary information but not decisive for making the grant.

Definition and Nomenclature of Methods of DNA-Profiling.

7. Mr. M. De Loose (Belgium) introduced document BMT/3/3 on the "Identification Methods Based on Molecular Techniques." He explained the different terms as molecular markers, protein polymorphisms, highlighted some basic principles (DNA synthesis, probes, restriction enzymes), the cloning, the Polymerase Chain Reaction (PCR), the detection of RFLPs, and the detection of restriction site polymorphisms after DNA amplification. He further explained the Restriction Fragment Length Polymorphism (RFLP), restriction digest after selective amplification (Cleaved Amplified Polymorphic Sequences (CAPS)), amplification of anonymous sequences using one short primer; Random Amplified Polymorphic DNA (RAPD), selective amplification after restriction digest; Selected Restriction Fragment Amplification (SRFA) or Amplified Fragment Length Polymorphisms (AFLP) and Microsatellite or Variable Number or Tandem Repeats (VNTR).

8. The explanation of the different methods was followed by a discussion on whether it was possible to know if the markers were equally distributed over the genome or not. This question was very important as it would have a big effect on the genetic difference between two varieties whether the differences found were spread over the whole genome or were close together in one part only.

Short Presentation of Research Results of Different Species

9. The Working Group noted several research results reported by the following individual experts and reproduced in the following documents:

(a) Mr. K. van Laecke (Belgium) reported on the "Evaluation of RAPD-Markers for the Identification of Ryegrass Varieties" (document BMT/3/3);

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(b) Mr. R. Peakall (Australia) reported on "An Analysis of Genetic Variability Within and Among Lolium Varieties: Implications of DNA-profiling for the Identification of Outcrossing Varieties" (document BMT/3/16);

(c) Mr. J. Reeves (United Kingdom) reported on "The Use of DNA-Based Markers for Distinctness, Uniformity and Stability Testing in Oilseed Rape and Barley" (document BMT/3/4);

(d) Mrs. J. Lallemand (France) reported on "The Use of PCR Markers for Variety Testing in Alfalfa" (document BMT/3/9);

(e) Mrs. J. Lallemand (France) also reported on the "Evaluation of the Potential of RFLPs for the Study of Distinctness, Uniformity and Stability in Sunflower" (document BMT/3/10);

(f) Mr. K. O'Donnell (United Kingdom) reported on the "Use of Molecular Markers for Distinguishing Potato Varieties"(document BMT/3/15);

(g) Mr. M. van Grinsven (ASSINSEL) reported on "A Model Study in Tomato to Assess the Usability of Molecular Marker in Determining Essential Derivation" (document BMT/3/5) and on "EDV and the Impact of Molecular Markers" (document BMT/3/5 Add.);

(h) In the absence of Mr. M. Zur (Israel) Mr. Guiard (France) introduced document BMT/3/14 on "Strawberry-Cultivar Identification Using Randomly Amplified Polymorphic DNA (RAPD) Markers";

(i) Mr. J. Guiard (France) also reported on "The Use of RAPD Markers to Determine the Genetic Diversity of Peach Varieties" (document BMT/3/13);

(j) Mrs. J. Lallemand (France) reported on "Varietal Identification of *Hydrangea* Using RAPD Marker" (document BMT/3/11);

(k) Mrs. J. Dattee (France) reported on "The 'RAPD-Megagametophyte' Approach: An Efficient Technique for Genetic Analysis in Conifer Species," on the "Genomic Mapping in *Pinus Pinaster* (Maritime Pine) Using RAPD and Protein Markers," and on the "Genomic Analysis in Maritime Pine (*Pinus Pinaster*). Comparison of two RAPD Maps Using Selfed and Open-Pollinated Seeds of the Same Individual" (document BMT/3/12).

Technical Costs and Access to the Method of DNA-Profiling

10. Mr. Smith, ASSINSEL, introduced document BMT/3/7 on the "Availability and Costs of Molecular Markers," highlighting the different methods (RFLP, RAPD, SSR (Simple Sequence Repeats), DAF (DNA application fingerprinting) AP-PCR (Arbitrarily Primed PCR) and AFLP), their patent position, their availability and the expected costs per data point for one polymorphic locus screened per one variety.

Statistical Aspects of DNA-Profiling Including Analysis of Distance

11. Mrs. C. Dillmann (France) explained in detail document BMT/3/6 on "The Estimation of Molecular Genetic Distances in Maize or DUS and ED Protocols: Optimization of the Information and new Approaches of Kinship" with the help of several further diagrams and tables shown as transparencies and reproduced in document BMT/3/6 Add. Mr. S. Grégoire (France), Chairman of the Technical Working Party on Automation and Computer Programs (TWC), highlighted several points to be observed when applying statistics. The diagrams and tables used in his presentation are produced in document BMT/3/17.

12. The Working Group noted the usefulness of the statistical applications but also realized at the same time the danger of their application if the relationship between the markers was not well understood. Before being able to apply these statistics it was therefore necessary to have a genetic map of the species concerned. Depending on whether a certain number of markers were spread over the genome or grouped closely together in a given part of it, the distance index would be lower and the similarity index higher on the reverse respectively.

13. Some expert remarked that in the past the traditional approach to distinctness and uniformity had been a mono-dimensional one. The observations were done characteristic by characteristic and a variety had to be distinct in one or more characteristics. The use of markers would change that approach. In a dendrogram observations would no longer be made characteristic by characteristic, but all markers would be observed together. It was necessary to understand that new way of thinking, to discuss and understand the statistics involved and the possible consequences if that approach would be accepted not only to prove essential derivation but also for distinctness and uniformity. Thus for the next session some explanations of the different methods and especially of the dendrograms were necessary. The TWC should be asked to provide help in this respect.

Position of the Breeders vis-à-vis DNA-Profiling

14. Mr. C. Grand (ASSINSEL) explained the ASSINSEL Position Paper on Distinction and Derivation as reproduced in document BMT/3/8. He repeated what had already been stated in the previous session that it was importance to keep the criteria of distinctness and derivation separate which would best be done by also keeping separate the methods of observation of distinctness and of derivation.

15. In DUS testing one would search for a difference in the expression of a characteristic, in the checking of essential derivation one would search for conformity, similarity in the largest part of the genome as well as in the phenotypic characteristics. The positive DUS test would give the breeder a right; the test on essential derivation would define the scope of that right. The granting of the right was the responsibility of the national authority; the defense of essential derivation was the responsibility of the owner of the variety. Distinctness should remain a question of difference in mainly morphological and physiological characteristics, essential derivation mainly a question of similarity of the genotype. This was at least the position of ASSINSEL at present; evolution of the methods might, however, require adaptation of that position in the future.

16. The Working Party expressed understanding for the wishes of the breeders. Several experts, however, stated that the consequences of such a separation should be carefully studied and precautions be taken to avoid any misuse. Some methods like polymorphism of isoenzymes were already under extensive study with the aim of using them for the testing of distinctness. The door should be kept open for their use in DUS testing. In special cases their use might be acceptable for DUS. Decisions should be taken case by case. One should, however, also recognize the consequences. Normally attention was paid mainly to the applicant of a candidate variety and on whether it would be unreasonable to reject his application. One should also consider the effect of the decision on the owner of the already existing variety whose rights would be reduced if the candidate was granted a right.

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

17. The Working Group, when discussing the potential of the different techniques for individual species and their possible use for DUS testing, almost always also discussed their possible use for the judgment of essential derivation. The Working Group agreed that, although UPOV had been asked by the Diplomatic Conference in 1991 to establish draft guidelines on essentially derived varieties, the decision on whether an individual variety was essentially derived or not was not for UPOV to take. UPOV should thus not try to fix thresholds for when varieties were to be considered to be essentially derived. It was for breeders to reach a common understanding on this subject on a species-by-species basis. UPOV should, however, discuss which methods or tools might be best adapted to judge essential derivation and the general principles that were involved. The Offices responsible for plant variety protection might also be approached to give advice in the event of court cases on essential derivation, and were called upon to explain the concept to existing and prospective new member States. Therefore it was still necessary to discuss essential derivation inside UPOV.

18. The Working Group stressed that similarity alone was not sufficient to judge essential derivation. It might be that two breeders started independently from the same parents and reached rather similar varieties. Essential derivation only existed if the variety from which the edv was derived ("initial variety") was actually used as breeding material. If the initial variety was not used even a very high degree of similarity would not result in essential derivation. Thus other criteria than the measurement of similarity alone had to be taken into account. It was important that breeders recognize that other factors were relevant to a conclusion concerning essential derivation. Breeders had to consider or reflect on such factors as well (pedigree, parentage, origin, through proof with breeding books, etc.). It was necessary to give the judges in the courts the maximum amount of objective and helpful information before they were obliged to take a decision.

19. Some breeders expressed the view that once a high degree of similarity had been demonstrated by the breeder making a claim of essential derivation, the burden of proof should be reversed. It should be for the producer of the second variety to demonstrate that he had not used that other variety. For that purpose also other means than the DNA markers could be acceptable.

20. Some experts proposed to consider to stop discussions on these tools inside UPOV if it was apparent that they could only be used for the checking of essential derivation, but not for

the testing of distinctness, uniformity and stability. Others thought it highly likely that in the future it might be possible to use the tools also for DUS testing. Particular probes might for example be known to be linked to specific phenotypic traits. It might also be possible to use them not directly for the decision process but as additional information or as a help in the layout of the test fields. It was noted that the language of the Convention provides no support for the idea that genetic probes should be used for essential derivation but not for DUS. The words "characteristics that result from the genotype or combination of genotypes" were used both in relation to the definition of "variety" and "essential derivation."

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

21. Partly immediately after the discussions following the different research reports on individual species, partly in the general discussion after all reports, the Working Group discussed the possibilities and consequences of the introduction of DNA-profiling methods for DUS testing. The discussions centered around several different subjects and the report thus summarized them, grouped according to these subjects.

22. First Impression on Reports: The Working Group noted that most reports had dealt with RAPD, or to a lesser degree with RFLP, but very little information had been given on microsatellites. Part of the reasons for that were limited time and resources in the member States which so far had not allowed sufficient research in microsatellites. However, hope was expressed that by the next session this situation might have changed and reports would deal more extensively with microsatellites. It was also necessary to have a change in the research emphasis and consider more what kind of information the different techniques can give and what would be their possible use.

23. Exchange of Information Between Scientific Researchers and UPOV Plant Experts: The Working Group had resulted in considerable progress in the exchange of information between UPOV crop experts and scientific researchers. The Working Group was a place were both groups of experts met, exchanged ideas and informed each other of their philosophy and their needs. Enormous progress had been achieved between the different sessions of the BMT and now each group understood much better the aims and needs of the other. This exchange had to be continued in the future in the interest of both sides.

24. Genetic Knowledge: The Working Group recalled the decision of UPOV to accept bands in an electroferogram of proteins or enzymes only if good knowledge of the genetic map in connection with those bands was available. The same would also have to apply to DNA markers. This would therefore exclude any quantification of bands if no genetic knowledge was available. It was also important to consider whether monomorphic markers should be given more or less weight than polymorphic markers.

25. Expression of Genes: The Working Group noted that phenotypical differences could be caused not only by the introduction and expression of a particular new gene into the plant genome, but also by blocking expression of an already existing gene. Thus analyzing differences in terms of mere presence or absence of genes might be misleading as absence of an expression would not necessarily mean absence of an action of a gene.

26. Genetically Modified Organism (GMO) varieties: In particular cases like GMO plants, a question arose as to whether it would be recommended to use molecular techniques to distinguish between modified and non-modified varieties where the only detectable difference between both was a particular functional gene. Some experts proposed that an appropriate molecular technique could be useful to check the presence of the new genetic material, but the expression of this gene, through a new observable morphological or physiological characteristic, could render the use of the molecular technique unnecessary.

27. Genetic Distance: One expert wondered why the Working Group had always considered the genetic distance as a measure for essential derivation or possible DUS testing. The term genetic distance did not appear in the UPOV Convention.

28. Repeatability: The Working Group noted that certain methods (e.g. RAPD) showed more problems of repeatability than others. If, however, studied closer and if problem bands were eliminated, good repeatability could be obtained with the remaining bands. Other techniques like microsatellites or AFLP would give higher reliability.

29. Redundancy of Markers: The Working Group noted that in many cases markers duplicated the information of other markers. It was therefore necessary to find a system to eliminate these redundant markers. Where one had a good knowledge of the genetic map it would be possible to look for the best markers to maximize the results with the lowest number of markers and thus decrease the costs of testing using only the most efficient ones. For this purpose, however, it was necessary to have markers which were not correlated and were well spread over the genome.

30. Gel Interpretation Software: The Working Group noted problems in gel interpretation programs with oilseed rape where the visual assessment of the gels did not always agree with the results obtained with the help of the special software, although the differences were consistent. Further research was necessary to better define the banding acceptance criteria.

31. Application to Cross-pollinated Species: The Working Group noted certain research done with cross-pollinated varieties (ryegrass) and difficulties encountered in obtaining repeatable results.

32. Application to Vegetatively Propagated Species: The Working Group noted that in vegetatively propagated species less markers were available. Molecular techniques showed limitations in the case of soma-clonal mutations where despite big phenotypical differences between two varieties the DNA techniques were not able to detect a difference. Back-mutations affecting the uniformity of a variety might similarly not be detected with DNA techniques.

33. Use for Screening of Varieties: The Working Group noted that although all agreed that at present the new methods should not be used for decisions on distinctness, several States used them for pre-screening varieties before doing field tests to find the most similar varieties to be grown side by side with the new candidate. This limited the number of varieties to be grown and reduced cost and labor. One expert wondered whether in a linear approach the point of genetic distance for essential derivation was always at a larger distance from the other variety than the point of sufficient distance to be considered in a separate variety. Should that be the case, DNA-profiling could be used for prescreening of varieties. The answer of some

experts was that distinctness was not a linear function and thus the use of prescreening was not possible. Such use for prescreening would be inconsistent with the suggestions that these methods not be used for distinctness. Normally for screening only the best grouping characteristics would be used, that is to say those characteristics considered best for distinctness, as all varieties eliminated would never be compared with the candidate. Their use for prescreening would *de facto* amount to their use for distinctness testing which was not advocated at the present time. Other experts stated that in addition to their possible use for DUS testing or for a decision on essential derivation, the use of these methods in the most efficient layout of the trials and in the screening of varieties should also be studied. The methods could supply useful information on the origin and background of the variety for an understanding of their likely behavior.

34. Uniformity: The Working Group noted that most reports dealt with the possible use of the techniques for identification and for distinctness purposes but that they lacked information on whether it was possible to check uniformity and stability. The basic rule of UPOV was that no characteristic could be used for distinctness if the variety was not uniform in that characteristic. The Working Group discussed the reasons for the requirement of uniformity. One requirement was to guarantee stability of a variety. Another requirement was to avoid competitors making selections from a non-uniform variety and creating new varieties. A further reason was to avoid problems well known from the so called "umbrella varieties" in vegetables where one umbrella variety covered a large part of the species, thereby reducing the space for new varieties by restricting the space available for a new variety to be protected. If the degree of uniformity was set too strictly, it would allow too many varieties and prejudice the protection system. If it was set too loosely (accepting very heterogeneous varieties) it would limit slots for future protection. It was not clear how to deal with a lack of uniformity in an unexpressed part of the genome. Normally uniformity was only required in characteristics used for distinctness. If a change occurred in the expressed part of the genome it might be detectable by traditional means. A problem was what to do if it occurred in the unexpressed part. Some experts stated that this again suggested that it was not possible to use DNA markers where no genetic knowledge on the expression was available or no correlation to a morphological characteristic existed. If a combination of several small differences in several characteristics was accepted for distinctness purposes, it would be difficult to check uniformity in each of the small difference in characteristics. The combining of characteristics had therefore to be carefully watched. Similar problems would also arise in combining of DNA markers. The Working Group noted with interest but did not endorse the four possible positions mentioned in document BMT/3/4 which reads as follows:

> "..... There are a number of ways of approaching this problem [of the uniformity requirement]:
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> "(i) it could be decided that this lack of uniformity precludes the use of such profiling techniques;
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> "(ii) it could be accepted that the level of non-uniformity exhibited by currently registered cultivars (which would need to be determined systematically and empirically) represented a baseline which candidates in the future would not be allowed to exceed;

"(iii) it could be suggested that from a certain date, all future candidates would have to be uniform in the particular profiling character;

"(iv) it could be accepted that the repeatability (i.e. stability) of the differences between cultivars is more important than the insistence on plant to plant uniformity. Thus if the variability within a cultivar, as estimated whether by single plant analysis or by a bulk analysis, is maintained from generation to generation (is stable) then this could be accepted as evidence of sufficient uniformity within that cultivar. This proposition would be recognizing that the examination of uniformity is at least partly to ensure that the distinguishing features of a cultivar are maintained during multiplication and commercialization. Hence it is stability rather than uniformity *per se* which is essential.

"This last point would represent a change in the philosophy underlying aspects of DUS testing, but might be biologically, as well as practically, desirable. The insistence on complete uniformity of DNA profile within cultivars, in addition to being in all probability difficult to achieve, is also of doubtful biological and agronomic value. Profiling technique are ideally suited to the rapid assessment of stability, since different generations can be screened and compared side by side on the same gene."

35. Proposals for the Technical Committee: Some experts stressed that the discussions in the Working Group should lead to clear proposals formulated for presentation to the Technical Committee to enable a sound discussion of the basic principles of the use of biochemical and molecular techniques in the Technical Committee.

36. Final Conclusions: The Working Group agreed that the new techniques for DNA-profiling were a powerful tool to provide detailed information on the relationship between varieties. They supplied considerable background on a variety and were also very useful for the identification of existing varieties. They would be very useful for the estimation of essential derivation together with other sources of data (e.g. breeding history). The Working Group was, however, not in a position to recommend its use for distinctness purposes. Many questions emerged, especially concerning the genetic map, the link between markers and genes, the link between markers and possible expression of a gene in the phenotype, and the whole question of uniformity. It therefore finally proposed that the Technical Committee not recommend the use of DNA-profiling for DUS purposes before all these open points had been clarified or before harmonized protocols had been established for the use of DNA-profiling (if its use was ever accepted for DUS testing).

37. One expert proposed not to be too strict and to allow their use in very special cases where good genetic knowledge was available and a link of the marker to another traditional characteristic existed (as with the polymorphism of isoenzymes). The majority was, however, opposed to such an advanced step. It stated that in the case mentioned distinctness could be judged on the difference in a traditional characteristic.

38. The Working Group favored the approach of ASSINSEL which was to keep the judgment of essential derivation as far as possible separate from the DUS testing and that the criteria of essential derivation had to be judged species by species. At present information on

DNA-profiling should only be complementary information which may help the expert in the testing but which would not be used for distinctness testing.

39. The Working Group agreed that UPOV should not feel under pressure to accept the new methods just for fear of being regarded as old-fashioned. It had the task of defending the efficacy of the plant variety protection system and of defending it against the introduction of unsuitable tools which might affect its functioning. It had also to remind itself that not all States were on the same level of development in these methods or had the same experience. Non acceptance of methods for DUS testing at the present time was not negativism but was in the interests of the users of the system. It was hoped that before the next session more research into the methods, especially on microsatellites would be completed. Other laboratory methods should also be studied which may be more readily acceptable, for example methods to observe the contents or composition of starch, oil, etc. or other metabolites.

Future Program, Date and Place of the Next Session

40. The Working Group agreed that further sessions were necessary in order to continue discussions. However, more than one year should be foreseen to allow enough progress in the research. At the invitation of experts from the United Kingdom, it agreed to hold its fourth session in Cambridge (United Kingdom) from March 11 to 13, 1997. The session would start in the morning of March 11 and close at noon on March 13, 1997.

41. The Working Group agreed to collect more information on a larger number of ornamental plants and vegetatively propagated species. They should also cover more studies on microsatellites. The documents to be prepared by different experts should, if possible, for each given crop cover the following items

(a) reproducibility of the method,

(b) genetic determination,

(c) costs of the method,

(d) studies on the correlation of genotypic markers with phenotypic expressions (direct link, partial link, causative link or association),

(e) robustness of the method,

(f) knowledge of genetic map of the species,

(g) explanation why the method was considered important,

(h) access to the method (patented or patent pending).

42. It was finally agreed that several documents would be prepared for the next session with a deadline at the end of 1996 to enable distribution in good time before the next session.

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

(a) Short presentation of research results on different species:

- Azalea (Belgium)
- Carnation (Netherlands)
- Lolium (AFLP, CAPS, Belgium and France)
- Maize (microsatellite, ASSINSEL)
- Oilseed Rape (microsatellite, United Kingdom) (Canola, RFLP, ASSINSEL)
- Peach (France)
- Pepper (France)
- Potato (microsatellite, Israel)
- Rice (possibly Japan)
- Rosa (France)

(b) The importance of clear definition of questions to the statisticians (the TWC to prepare BMT/3/17 and an updated document);

(c) The use of DNA-profiling in prescreening as a possible tool in DUS testing (papers prepared for the TWA and TWO);

(d) The interest and value of the dendrogram analysis (the TWC to prepare a document);

(e) The analysis of the molecular variance (AMOVA) (France to prepare a document by April 1996, first for the TWC);

(f) The principal components analysis and other multivarietal statistics (the TWC to prepare a document);

(g) Correlation and causal linkage between DNA markers and morphological traits (France to prepare a paper);

(h) Relation between molecular genetic distance and morphological distance (France to prepare a document on maize, Belgium on ryegrass and the United Kingdom on oilseed rape and cereals);

(i) Position of the breeders vis-à-vis DNA-profiling (ASSINSEL to prepare an updated paper);

(j) Possibilities and consequences of the introduction of DNA-profiling methods for DUS testing;

(k) Control of uniformity in characteristics obtained with biochemical or molecular markers (United Kingdom to prepare a document);

(l) Effect of breeding schemes and parentage on the required distance between varieties (ASSINSEL to prepare a document on maize, soybean, sunflower);

(m) The use of DNA-profiling methods by expert witnesses in disputes on essential derivation. (ASSINSEL to prepare, if possible, a document on its position).

Visits

44. In the afternoon of September 20, 1995, the Working Group visited Keygene. Immediately before its visit it received from Mr. M. Zabeau, Director of Keygene, an introduction to the activities of Keygene, and especially to the Amplified Fragment Length Polymorphism (AFLP) method developed by Keygene. Keygene is a company set up by six (now seven) Dutch plant breeding firms to develop diagnostic tools to better understand the plant genetics and to enable the use of those tools in the breeding of improved varieties. The kit developed by Keygene was considered to be foolproof and could be applied without the need of too detailed knowledge on all details of the method. The quality of the results depended on the quality of the DNA in the individual laboratory. The method had an error of about 10 per cent. In addition to the facilities of Keygene, the Working Group was also shown a demonstration of Keygene's AFLP data analysis software.

45. In the afternoon of September 20, 1995, the Working Group also visited CPRO-DLO, where it heard an introduction to the layout of CPRO-DLO and its activities given by Mr. W. Stiekema, head of the molecular biology laboratory. CPRO-DLO made fundamental research, strategic research and applied research. The Working Group saw in practice gene introduction by bacterium into potato, heard progress made with respect to insect resistance breeding with *Baccillus thuringensis* and the control of flower development in petunia.

46. *The present report has been adopted by correspondence.*

[Annex follows]

ANNEX

LIST OF PARTICIPANTS OF THE THIRD SESSION OF THE UPOV WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA-PROFILING IN PARTICULAR

I. MEMBER STATES

AUSTRALIA

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