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UPOV

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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

First Session
Geneva, April 19 and 20, 1993

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 first session in Geneva, Switzerland, on April 19 and 20, 1993. The list of participants is reproduced in Annex I to this report.

2. The session was opened by Miss Jutta Rasmussen (Denmark), Chairman of the Technical Committee, who welcomed the partipants.

Tasks of the Working Group

3. The Vice Secretary-General of UPOV introduced document BMT/1/2 containing the proposals of the Technical Committee and the decision of the Council with respect to the establishing of the Working Group. The Working Group noted that its main task was to examine the technical methods strictly for the purposes of the UPOV system of plant variety protection and to study the consequences of the introduction of these methods in the testing of varieties in connection with the granting of plant variety protection.

Adoption of the Agenda

4. At the request of Mr. Joël Guiard (France), the Working Party agreed to change the order of the items on the Draft Agenda and to place item 4 (Consequences of the introduction of new methods) after item 6 as it was necessary to hear at least some information on the individual methods in order to be able to discuss the consequences of their introduction.

Biochemical and Molecular Techniques

5. Dr. Thiele-Wittig (UPOV) recalled that this item had mainly been placed on the Agenda in order to ensure that the Working Group would not only deal with DNA-profiling but, as requested by the Council, more generally with all biochemical and molecular techniques.

6. Mrs. Yvette Dattée (France) then gave a short overview of biochemical (protein or isoenzyme electrophoresis) and molecular markers and their use. She explained that biochemical and molecular markers generally revealed small changes of the genome so that a set of markers must be used because each of them individually would be insufficient to reveal a modification of the genome which could be used to establish distinctness. Some of the markers could be easily interpreted in terms of Mendelian inheritance with co-dominance. Further progress in genetic methods with the most important crops would give the chromosomal location of the markers. They would enable the genetic analysis of each varietal structure (pure line, clone, hybrid, synthetic). The advantages expected from biochemical and molecular markers would be as follows: their expression was independent from the environment; they could be used to check the filiation of hybrids; they could be observed early in the development of the plant (saving time and trials facilities) and the genetic basis of the polymorphism could be elucidated.

7. At the proposal of the Chairman, the Working Group agreed to make a small ad hoc survey of the different biochemical and molecular methods at present under study in the different member States. The outcome of that short survey, giving an idea of the present situation in the States represented at the session, is reproduced in Annex II to this report.

DNA-profiling

8. Dr. Matthew Morell (Australia) introduced document TC/28/4 which explained the determination of distinctness, uniformity and stability of varieties using DNA-profiling techniques. The paper listed the advantages of those techniques, compared several methodologies and especially compared the Restriction Fragment Length Polymorphism (RFLP) method with the Random Amplified Polymorphic DNA (RAPD) method.

9. The Chairman referred to document TC/28/7 concerning the Amplified Fragment Length Polymorphism (AFLP) method, submitted to the Office of UPOV by Mr. Martin Clucas, which proposed a practical solution to the measurement of genetic distance and dependency issues. The Working Group noted that it was a patented method more or less similar to the RAPD method. It used restriction enzymes and radiation, gave more bands than the RAPD method, but did not classify them in the same way.

10. The Working Group then discussed at length different elements of the methods and compared them with the traditional methods. During this discussion it was mentioned that the degree of distinctness was difficult to estimate with the new methods, but that certain of the traditional methods would not offer a better answer either. Doubts were raised on the reproducibility of certain methods. If reproducibility could not be ensured, all other advantages that such methods might have would be useless. Some questioned how uniformity would be judged if these methods were used. Others wondered how the DNA profile would be interpreted and whether only those segments where a connection could be made to a phenotypic characteristic should be used, or whether the apparently unexpressed part, and thus the whole genome, could be used for the establishing of distinctness. Some experts were of the view that the proof of the presence of a certain DNA in itself was not enough and that it was important that there be an expression of that certain DNA. In electrophoresis for example, only two of the four categories of proteins under research had recently been adopted by the Subgroup on Cereals, namely only those where it had been possible to connect a certain band of the electrophoretogram with a certain gene locus. Others questioned why the presence of a mere morphological feature of a plant should be thought to be more useful for description/identification purposes than the presence of an apparently nontranslated segment of DNA. The word "fingerprinting" was considered to be misleading by several experts, especially in the case of a synthetic variety. In addition, also using another method one would obtain a different "fingerprint". The first aim was to agree on a standardized method to enable comparison of results between different countries and to avoid differing interpretations. If two varieties showed identical profiles when using a particular limited set of probes, that did not totally prove that they were identical but meant that there was a higher probability that the varieties were the same. Cases were known where mutations of a given variety had, despite a morphological difference, the same profile in response to a particular set of probes. This revealed that the DNA governing the morphological difference was not probed by the set of probes which was used. The breeders should also be asked to what extent they used those methods and what advantages they saw in their use for distinctness purposes. Questions to be solved included whether the method could be used both for identification and distinctness purposes or only for "identififation" and, if used for distinctness, whether it could be used only complementarily, i.e. that it could not be used alone, or whether it could be used alone for the decision on distinctness. A study of specific methods in relation to specific species in order to get a better understanding of the potential of the methods was seen as the best way to advance.

Consequences of the Introduction of New Methods

11. Mr. Guiard (France) introduced document TC/28/5 on identification and distinctness. He stated that, with the new methods, it was always possible to detect small differences between varieties and this would have its consequences for the minimum distance between two varieties. Therefore, it was important to discuss the concept of variety in general and especially the relation between Article 1 of the 1991 Act of the Convention, which gives a definition of variety, and Article 7, which requires a clear difference between varieties as a prerequisite for the granting of plant variety protection. His view was shared by several of the experts present and a discussion followed on how to define a clear difference. It was discussed whether it was possible, for example, to create a hierarchy of characteristics in the sense that certain characteristics controlled by single genes would not be sufficient to establish distinctness and that at least two of them showing a difference were needed;

needed; whether any difference would be acceptable if it was clear and repeatable; whether the acceptance of such differences would bring about plagiaristic approaches which would have to be dealt with by the new criterion of essential derivation. One expert stated that the most important element for distinctness was to establish that a variety was unique, i.e. that it had an identity of its own.

12. The Working Group had a second discussion on the use of particular characteristics or methods and whether they could be used to establish distinctness or whether they could be used only to identify a sample and attribute it to an existing variety. Some experts were of the opinion that there were different criteria for deciding whether to use a method for distinctness or identification purposes in the sense that for identification purposes a smaller difference could be tolerated. Others pointed out that for plant variety protection purposes it is not useful to attempt to distinguish between identification and distinctness. For plant variety protection purposes a variety must be given an identity that can be established for the practical purposes of the plant variety protection system and for this purpose the criteria of distinctness, uniformity and stability are used together.

13. The new methods called for the establishment of what constituted an acceptable difference between two varieties, what constituted a difference between two profiles and how many differences in the profile were necessary for the difference to be clear. In a court case it might be difficult to justify the refusal of rights to a second variety if the applicant could prove a different profile. But it would be very disturbing if just any difference in two profiles would enable another variety to be protected. A good solid identity of the variety should still be required.

14. At present, the number of characteristics was limited partly because of the costs involved and the time to observe them. If, in the future, DNA-profiling were generally accepted and any difference in a profile accepted as a clear distinction difference, that would eliminate the minimum distance requirement which exists not only under the 1978 Act of the Convention, but whose existence is confirmed by the 1991 Act. The Working Group, therefore, had to consider the effect of the introduction of a large number of new characteristics upon the minimum distance concept.

15. Mrs. Dattée (France) introduced BMT/1/3 on the advantages and limits of the use of DNA polymorphism with relation to plant variety protection. She mainly emphasized the second part of that document with respect to the technical constraints of the method, the quality of information and the relevance of molecular markers for plant variety protection. The Working Group discussed the advantages and disadvantages of methods as presented in the table on page 5 of that document. The RFLP monolocus seemed to be the favored method today, but the Working Group noted the rate of change in the technologies and that the validity of the analyses might change.

16. In the ensuing discussions several of the arguments raised beforehand were repeated and discussed further, such as the linking of the DNA profile to a phenotypic characteristic; the need for using only the expressed part of the genome; the effect of the method if used in a complementary manner; the need for harmonization of methods; the costs for the Offices; the costs for the breeders especially during maintenance of the variety; the need to define what was really wanted from the methods; whether it was enough to have one or several differences between two profiles; the need to have a discussion on what the breeder wanted when breeding a new variety. There was an attempt to

give definitions of variety, genotype, genome, genetic markers and their suitability which were, however, difficult to accept. There was again a proposal to look at DNA-profiling in the context of morphological characteristics and use the existing knowledge of those characteristics to get to better understand the new technology.

Future Program, Date and Place of Next Session

17. The Working Group agreed that further sessions were necessary in order to continue discussions on the matter and make progress. In order to have a good preparation of the coming session, the Working Group agreed that it was necessary to get further experience with a limited number of species, followed by a review of the results obtained for each such species in the application of the methods of molecular markers. The Working Group agreed to establish amongst its members four working units for four species with, for each working unit, one country that would be responsible for collecting technical information from other countries and the professional organizations. If available, information on whether the method was able to separate DNA from the expressed or unexpressed part of the genome should also be included. In order to best guide the work of the Working Group, it was agreed to concentrate on two types of methods, namely RFLP-like and RAPD-like methods. If pertinent results from other methods based on PCR or micro-satellites were readily available, they should also be reported on. Information on the reproducibility of the results should be presented by the units to the Working Group, as well as information on genetic mapping or genetic control of DNA polymorphisms and the correlation between polymorphism and phenotypic characteristics.

18. The four working units and the country responsible for collecting the information and preparing a comprehensive document for the next session were as follows:

Working Unit on Citrus - Australia
Working Unit on Maize - France
Working Unit on Soybean - United States
Working Unit on Tomato - The Netherlands

19. The above limitation of the information to be collected was mainly made to enable good progress to be made. If in some countries information was available on other crops that were considered more important for that State or on other methods, that information would also be welcome.

20. In addition to the technical aspects, the discussions during the coming session would have to concentrate on the general principles and the possible use of the technologies. It would have to be discussed whether they could be used for DUS testing and, if so, whether

(i) in parallel with the traditional characteristics
(ii) as a supplement (extra, additional characteristics)
(iii) as a substitute,

or whether they could be used for identification purposes only. Another aspect would be their possible use for the judgement of essential derivation. These general discussions should already be reflected on by the individual units and the responsible States when collecting information.

21. The Technical Working Parties should be informed of the outcome of the first BMT session through the written report on the meeting as well as oral explanations by the Office of UPOV. The attention of the Technical Working Party on Automation and Computer Programs should especially be drawn to possible ways of integrating the results from the present methods with those of DNA-profiling.

22. The Working Group agreed to ask the Technical Committee and the Administrative and Legal Committee for assistance on the following questions:

(a) What was intended in Article 1 of the 1991 Act of the Convention by the term "genotype"? Did it limit the possibilities to the expressed part of the genome?

(b) How to handle the difference of "one or more characteristics" for a clear distinctness (clear distinctness in one characteristic, hierarchy of characteristics depending on their genetic control).

23. At the invitation of the experts from France, the Working Group agreed to hold its next session near Paris in France, from March 21 to 23, 1994, where the practical application of the DNA-profiling methods could be seen in a still to be selected laboratory. The four working units would meet in the afternoon of March 21 at the same place.

New Chairman

24. The Working Group proposed to the Council that it elect Mr. Joël Guiard (France) as Chairman of its coming sessions and thanked Miss Jutta Rasmussen (Denmark) for having presided over the very start of its work in her capacity as Chairman of the Technical Committee.

25. This report has been adopted by correspondence.

[Two annexes follow]

ANNEX I

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André HEITZ, Director-Counsellor
Max-Heinrich THIELE-WITTIG, Senior Counsellor
Makoto TABATA, Senior Program Officer

[Annex II follows]

OVERVIEW OF THE PRESENT SITUATION IN SOME STATES

1. The expert from the Czech Republic reported that he participated mainly as an interested observer. At present, electrophoresis was only used as an intermediate step in the testing of varieties.

2. The expert from Switzerland reported that in his country the involvement in molecular techniques was not with respect to the testing of DUS but in different areas. Thus electrophoresis was used for storage proteins in wheat and maize and RFLPs and RAPD were used in bread wheat. Molecular techniques were used to detect varieties' resistance against rust and in general were used for genomic mapping and determining genetic distances, but by breeders only.

3. The expert from Hungary reported that in the laborary they investigated biochemical polymorphism and isoenzyme and storage protein electrophoresis. They did not use those methods for DUS testing and not yet for the protection of varieties. They needed two to three more years to investigate and make progress and to find the best and cheapest method.

4. The expert from the United Kingdom reported that the new techniques were addressed at two different levels: first, at the technical level and secondly at the practical level of how to apply those methods for protection. At the first level, work was going on with respect to research on most of the techniques. RAPD was studied for use as variety identification and that was part of a general systematic study. So far, the technique was considered to be easy and work was underway to prepare a proposal for its use to estimate the level of discrimination and to study the problems associated with that technique, especially with respect to minimum distance. At the level of application, study was underway on the costs--direct and indirect--and the implications for the breeders, especially with respect to the maintenance of a variety. It was furthermore being studied how to modify the DUS testing in order that the PVP Offices benefit of the new techniques and, finally, how to apply them in general.

5. The expert from The Netherlands reported on research at the CPRO on RFLP, RAPD, micro satellites and PCR. Most methods were used in breeding and for gene mapping. So far, molecular techniques were not used for DUS testing. Electrophoresis in potato was used as a prescreening technique in identifying similar genotypes.

6. The expert from New Zealand reported that the PVRO itself had no experience in these methods. It was aware of the work going on by plant breeders and institutes. The methods were mainly used for gene mapping and assistance in the breeding process, for example for parentage identification. The results were useful for identification but it was sometimes difficult to relate the gene profile to morphological characteristics.

7. The expert from Japan stated that in his country the National Center for Seeds and Seedlings of the MAFF had started studying the methods for the purpose of identification, especially for potato, last year. Another project funded by the MAFF investigating their use in connection with the establishing of essential derivation started this year. Of the DNA profiling methods, especially the RAPD method was studied.

8. The expert from Romania stated that her country applied electrophoresis in the case of maize varieties. In the laboratories intensive studies were going on. However, the methods seemed to be too expensive and therefore would be difficult to apply on a regular basis. They would be applied only in cases of infringements.

9. The expert from Slovakia reported that in the institutes of his country electrophoresis was used as a standard method in cereals and work had started on DNA profiling on RFLP and PCR for oats and peas. They would study also its use to establish minimum distance. In addition, they would study electrophoresis on storage protein and isoenzyme for maize.

10. The expert from Germany reported that in his country they routinely applied electrophoresis of protein for DUS testing as an additional characteristic in cereals. They were studying the possibility of DNA profiling methods for the same purpose. They had started studying the methods step by step and were investigating the extraction, the separation and the staining. They would wait until UPOV agreed which method should be used as it was too expensive to study too many different methods at the same time.

11. The expert from Norway reported that in his country a lot of research was going on but it was difficult to say how far the individual institutes were in their investigations. DNA profiling had not yet reached the stage to allow distinguishing the quality of the different methods. An this early stage, it was necessary to make a cost-benefit study before going too deep into a given method.

12. The expert from France reported that in her country they worked on electrophoresis of storage protein for cereals and isoenzymes for maize. Furthermore, experience with electrophoresis on sunflower, alfalfa and other species had been gained. Molecular techniques would be studied in collaboration with institutes and private companies. At present, studies were underway with respect to RFLP, RAPD and PNTR. There was a joint project between public and private institutes to study the genetic distance between lines and establish a way to measure that distance. A thesis could be expected in about three years' time.

13. The expert from the United States reported that the PVPO did not do its own tests but only reviewed test results submitted to it. They accepted electrophoresis results of storage protein of small grains. As several new methods were developed in different places, it was necessary to establish an accepted method. It was their experience that the RFLP method was not very helpful for distinctness in the case of soybean and, for this species, they were following developments with respect to micro satellites and DNA techniques set up in certain laboratories for the screening of soybean varieties. So far, however, no final results had been obtained. They hoped that UPOV would provide guidance in the search for the right method.

14. The expert from Spain reported that in his Institute DNA profiling was not yet used for DUS testing. The laboratories however used electrophoresis for several cereals and mainly to help confirm results obtained by traditional means. They closely followed the work in research centres of the universities. They were concerned with the cost-benefit ratio and recommended a careful introduction of these methods. There should be a good cooperation between the private companies and the laboratories so that all could benefit.

15. The experts from Australia reported that in their breeders testing scheme the use of these methods had been studied to an advanced stage. The electrophoresis of seed protein was part of the DUS testing. Recently, DNA profiling had been accepted as a supplementary characteristic. Private and public institutes worked together to develop methods which could be applied to the DUS testing. There had been a real explosion in the use of the new technologies in the private sector and therefore they turned to UPOV for advice as to the direction to take and for principles to be developed for the use of these methods.

16. The expert from Canada reported that they had no experience yet with the use of these methods in the testing of varieties. In the public area, however, universities did research on RAPD methods for cultivar identification as a quick and cheap check. With respect to RFLP, the reproducibility across laboratories was a weak point so far. With respect to RAPD, which seemed to be a quick and cost effective method, research was underway for several species. At present, however, they preferred to use these methods only in addition to the traditional methods.

17. The expert from South Africa reported that in the PVR Office of her country electrophoresis was used in cereals, melon and watermelon. Various institutes and private firms investigated these methods in several other areas than plant variety protection. In the Institute for Tropical and Subtropical Crops, work had just started on the use of RAPD in citrus. In avocado and mango electrophoresis of isoenzymes was used. The use of the method in citrus was very important especially for the prescreening of varieties in order to group the right varieties together in view of the cost of the layout of the plantation.

18. The expert from Denmark reported that at present the new technology was not yet used in her Office. Sometimes results from other institutes were taken over. This was especially applicable to results from electrophoresis received from Germany. In each case, however, the breeder was asked whether he was interested to accept that characteristic. If so, he would of course have to keep his variety uniform in that characteristic as well and the characteristic would also be used in connection with certification. Only a few breeders investigated the new methods as they are very costly to handle and many breeders could not afford their use. For Denmark it was therefore important to follow what other UPOV member States were doing and await recommendations for their use before entering further into these methods. The main problem to be discussed was the cost and whether their use should be complementary or not.

19. The expert from COMASSO reported that many breeders used electrophoresis, 1D as well as 2D electrophoresis, in the breeding program. The major companies were also getting acquainted with DNA profiling, however, the cost would limit their use. One special interest for the breeders was the possible use of the method in connection with the criterion of essential derivation.

20. The expert from ASSINSEL reported that there was no special program for investigation into the methods from the side of ASSINSEL. Individual breeders, however, were investigating the different methods. These methods were also largely discussed in working groups. In the United States, ASTA had established guidelines for the use of these methods.

[End of annex and of document]