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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

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

Fourth Session
Cambridge, United Kingdom, March 11 to 13, 1997

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 fourth session in Cambridge, United Kingdom, from March 11 to 13, 1997. The list of participants is reproduced in the Annex to this report.

2. Mr. John MacLeod, Director of the National Institute of Agricultural Botany (NIAB), welcomed the participants to Cambridge, United Kingdom. The session was opened by Mr. Joël Guiard (France), Chairman of the Working Group.

Adoption of the Agenda

3. The Working Group unanimously adopted the Agenda as reproduced in document BMT/4/1 Rev. after having agreed to change the order of sub-items under Item 3 as follows: (a) Potato, (b) Azalea, (c) Carnation, (d) Rice, (e) Peach, (f) Oilseed Rape, (g) Maize, (h) Ryegrass, (i) Tomato.

Short presentation of research results on different species

4. The Chairman referred to the main aim of the work of the Working Group and the conclusions reached at the last session as reproduced in document BMT/3/18, paragraphs 36, 38 and 39.

> "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. [...]. 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)."
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> ....
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> "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."
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> "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 was hoped that before the next session more research into the methods, especially on microsatellites would be completed. [...]"

5. The Chairman further referred to several documents from the Technical Committee, especially the reports on the last two sessions and discussions in the Council, which requested the BMT to concentrate more on methods for DUS testing. He agreed, however, with the Technical Committee that first a method had to be studied and well understood before one could decide on whether it could be used for DUS tests.

6. He also referred to the work in the TWC, to be covered by Item 4 of the Agenda, and on discussions in the TWA on information to be handled under Item 3 (j) (Ryegrass). He repeated the conclusions in that Technical Working Party that one had to be careful to avoid the introduction of new methods creating more problems than it solved. He further referred to the discussions on the expressed and non-expressed part of the genome and proposed to hear a short report from the Vice Secretary-General of UPOV at the end of the present session under Item 11 on the discussions held in the Administrative and Legal Committee.

Potato

7. Mr. Johannes-Peter Ohms (Germany), introduced document BMT/4/12 on the "Use of Patanin Polymorphisms for Distinguishing Potato Varieties."

Azalea

8. Mr. Jan De Riek (Belgium) introduced document BMT/4/5 on the "Identification Strategy Using RAPD and AFLP Markers for Belgian Pot Azaleas," prepared by him together with Jochen Dendauw, Isabel Roldán-Ruiz, Erik Van Bockstaele and Marc De Loose, from the Department of Applied Plant Genetics and Breeding, Centre for Agricultural Research, in Belgium.

Carnation

9. Mr. Ben Vosman (Netherlands) introduced document BMT/4/15 on the "Identification of Carnation Varieties," prepared by him together with Ineke Rus, Rene Smulders and Gerard Bredemeijer from the Centre for Plant Breeding and Reproduction Research (CPRO-DLO) in Wageningen, Netherlands.

Rice

10. Mr. Tomotoshi Shimano introduced document BMT/4/10 on the "Evaluation of the Potential of AFLP Toward the Study of Distinctness in Rice Closely Related Varieties and the Use of RFLP for Variety Testing in Rice," prepared by experts from Japan and a more detailed text distributed during the meeting.

Peach

11. Mrs. Claire Baril (France) introduced document BMT/4/16 on the "Identification of Peach Cultivars Using RAPD and AFLP Markers," prepared by Mr. E. Dirlewanger in INRA, in the *Unité de Recherches sur les Espèces Fruitières et la Vigne*, of the *Centre de Recherches de Bordeaux* in Villenave d'Ornon, France.

Oilseed Rape

12. Mr. Yong Xiang Zhang (France) introduced document BMT/4/11 on the "Development of Sequence-Tagged-Site (STS) Markers and Study of Simple-Sequence-Repeats (SSR) for Variety Testing in Oilseed Rape (*Brassica Napus*)," prepared by him and by Stéphane Fouilloux, Vincent Lonbard, Dominique Brunel, Régine Delourme, Françoise Blouet, and Mireille Bourgoin, in the Laboratory Biochimie of GEVES in Surgères, France.

13. Mr. Robert J. Cooke (United Kingdom) introduced document BMT/4/20 on the "Development of DNA Microsatellites for Distinctness, Uniformity and Stability Testing," prepared by him and David Lee, Michelle Leverington and Wendy Cooper of the NIAB in Cambridge, United Kingdom.

Maize

14. Mr. Stephen Smith (United States) introduced document BMT/4/2 on "An Evaluation of the Utility of SSR LOCI as Molecular Markers in Maize (*Zea mays* L.): Comparisons with Data from RFLPS and Pedigree," prepared by him together with other colleagues from Pioneer Hi-Bred International from the United States of America.

Ryegrass

15. Mr. Marc De Loose (Belgium), introduced document BMT/4/4 on "The Use of DNA-Profiling Techniques for the Identification of Ryegrass Varieties," prepared by him together with Isabel Roldán-Ruiz, Jan De Riek, Jochen Dendauw, Ann Depicker, and Erik Van Bockstaele from the Department of Applied Plant Genetics and Breeding, Centre for Agricultural Research, Belgium.

16. Mrs. Mireille Bourgoin (France) introduced document BMT/4/18 on the "Research of STS Markers for Varietal Identification of Ryegrass," prepared by her together with J. Lallemand, P. Lem at GEVES, France.

17. Mr. Jirí Soucek (Czech Republic), introduced document BMT/4/3 on the "Computer-Aided Fingerprinting of Accessions from Ryegrass-Fescue Complex," prepared by G. Oiffelová and colleagues in the Laboratory of Nitrogen Fixation of the Institute of Plant Molecular Biology of the Academy of Sciences of the Czech Republic.

Tomato

18. Mr. T. Kramer (Netherlands) introduced document BMT/4/17 on the "Conclusions of a Model Study on Essential Derivation Using Tomato as a Crop," prepared by ASSINSEL.

General Discussion

19. In the discussions following each individual report as well as in the discussions after all reports on research results, several main topics were discussed which can be summarized as follows:

Comparison of Methods

20. Many new methods are available and are studied. While during the third session of the BMT the majority of the reports mainly centered on the RAPD (Randomly Amplified Polimorphic DNA) and RFLP (Random Fragment Length Polimorphism) methods, the reports during the present session mainly centered on AFLP's (Amplified Frequent Length Polimorphisms) and especially on the PCR based analysis of molecular markers based on Simple Sequence Repeats (SSR) or microsatellites and Sequence Tagged Sites (STS) or Sequence Tagged Microsatellite Sites (STMS).

21. The RAPD method was obviously left aside with very little interest remaining. Compared with the RAPD method, the AFLP method was considered of better repeatability and more reliability. Its capacity to produce data seemed to have no limits. It could produce new primers. With the RAPD method one did not know from which part of the genome the band resulted, whether from the expressed or the non-expressed part. The same band could also result from different loci.

22. Compared with RFLP's the use of AFLPs and SSRs made it possible to avoid the use of radioactive material and was thus better for the environment. Results from RFLPs were frequently used as a basis for comparison with other techniques. RFLPs and SSRs could cover the whole genome. The SSRs would, however, be more discriminative, more reliable, more repeatable; there already existed good hardware and software for the method, SSRs were repetitive in more than one base pair, and could potentially be standardized more easily.

23. The development of each of the methods as well as the search for new methods is going very fast. It cannot be stopped. In a few years new tools will be available which will require our own techniques to be adapted.

Costs

24. Costs apparently did not pose a problem. The development of primers for microsatellites could be expensive. Often microsatellites discovered on a random basis are used, but as part of the search for new primers, existing databases or literature were searched. Also primers from other species are studied for possible use, especially in species where so far no primers had been developed. Many laboratories are producing new primers. Thus in future microsatellittes would be increasingly usable.

25. The new tools would, however, add to the normal cost of testing and would lead to an increase in testing fees unless at the same time the use of traditional characteristics (morphological and physiological characteristics) decreases. If that had to happen, where would the end point, the limit, be? Would it be possible to stop using morphological or physiological characteristics and to rely exclusively on DNA characteristics?

26. The last mentioned remark was rejected by all experts. Morphological and physiological characteristics would always be needed to be tested. In part, this was true because they would be needed for practical reasons in the handling of the material. Description of the morphological and physiological characteristics would be needed for the

use of the variety in its growing and in certification, and also in part to check the uniformity and stability. The DNA characteristics would remain supplementary to morphological and physiological characteristics.

Use of Data

27. Most reports were silent on the use of data. They also used different terms without definition. Some spoke of distinction, others of separation, identification, discrimination, differentiation, description, some of separation of varieties, others only of separation of species. Others considered its use for prescreening varieties, others for the study of evolution. No one considered the question of uniformity or stability.

Distance Between Varieties

28. While some experts expressed themselves against making a difference between the use of a characteristic for identification and for distinctness testing, the majority saw a difference between the two terms. In normal language or in general terms there may be no difference as in both cases two subjects would be compared to see whether there was a difference or whether they were the same. However, in the framework of UPOV discussions the term "distinct" is reserved for varieties which are sufficiently different to be eligible for protection. If any small difference were acceptable to establish a new variety the two terms would have the same meaning. But this was not the case. Articles 1 and 7 of the 1991 Act of the UPOV Convention makes a clear distinction between plant groupings which are mere "varieties" and plant groupings which constitute "protectable varieties." "Varieties" can exist which are not sufficiently distinct from an existing variety to be protectable. It was suggested that it should be proven that the expression of a certain genetic sequence exists before it can be used for distinctness purposes. A characteristic useful only for identification may be used thereafter to prove that certain plant material belongs to that variety.

29. Several experts warned against the danger of decreasing with these tools the value of distinctness, thereby reducing the minimum distance between varieties as well as the scope of protection. The use of molecular techniques might create more problems than it would solve.

Tasks of the BMT

30. Several experts described the main task of the BMT as the studying and checking of methods for their usefulness in DUS testing. They considered that the BMT had not so far tackled the main tasks as the reports had not approached the question of uniformity and stability. Most reports were silent on the sampling of material. Some spoke of the use of one single plant, others used bulk samples making it impossible to judge uniformity.

31. All agreed that for the next session all reports had to consider the question of variability within a variety as well as variability within a species. These two questions should be included as separate items of the Agenda for the next session.

Effect of the Methods

32. Several experts were worried about the influence of the chosen method on the results. Depending on which method was used, different results could be obtained. It was therefore not enough to develop a good repeatable and reproducible method. For data to be useful one needed to know what the data meant. The interpretation of the data was important.

Knowledge of Genetic Background

33. DNA data can only be interpreted if sound knowledge of the genetic background of the species concerned is available. Any use of data without that knowledge carries many risks of wrong interpretation. That was the reason why in electrophoresis of proteins for certain cereals (maize, wheat, barley), electrophoretic characteristics were only accepted if knowledge of the relevant genetics was available. For wheat, electrophoretic characteristics of glutenin were accepted, but electrophoretic characteristics of gliadins were refused, because it was not possible to genetically interpret the gliadin bands.

34. Before starting a test one had to reflect what was to be done and define the tasks. It was dangerous to apply a method without knowing what the presence or absence of a given band meant. A band could be separately and independently controlled by completely different genes. This was even more important if the method took into account different degrees of presence of a band (weak, strong intensity).

Improvement of Methods

35. Apart from enabling genetic interpretation of its results, a satisfactory method for UPOV purposes needed to be robust, repeatable and precise. It had to recognize individual gene loci. It had to avoid any overloading of the gel that could make certain bands appear or any too low concentration that could make less intense bands disappear. There should be a standardized naming of the alleles and not, as for several methods at present, different naming depending on the gel used or on the laboratory running the test.

36. There should, if possible, be tests for DNA markers in parallel with the traditional morphological and physiological characteristics and the results should be studied for their correlation with respect to the distance of the varieties from each other. It was necessary to compare the classical characteristics with the DNA characteristics and discuss the results with breeders and get their opinion, as they would have to maintain their varieties uniform and stable within the characteristics used for DUS testing.

Statistical Methods

37. The discussions on this Agenda item took place in the John Innes Centre in Norwich and were chaired by Mr. Sylvain Grégoire (France), former Chairman of the Technical Working Party on Automation and Computer Programs (TWC). He reported that the documents BMT/4/7 Rev., BMT/4/8 and BMT/4/9 to be reported upon in the three sub-items

were prepared by their authors for the last TWC session and had been discussed in that Technical Working Party. During those discussions it had been made clear that a better understanding by the experts was necessary to avoid the wrong interpretation of results. He especially referred to the very frequently wrongly interpreted dendrograms. It was necessary to first understand the statistical tools before applying them.

Similarity, Clustering and Dendrograms

38. Mr. John Law (United Kingdom) introduced document BMT/4/8 on "Similarity, Clustering and Dendrograms," prepared by him in the National Institute of Agricultural Botany in Cambridge, United Kingdom.

A Review of Methods for Cluster Analysis of Marker Data

39. Mr. Hans-Peter Piepho (Germany) introduced document BMT/4/7 Rev. on "A Review of Methods for Cluster Analysis of Marker Data," prepared by himself and Dr. F. Laidig from the Bundessortenamt, Hanover, Germany. He regretted that, due to defects in the electronic transmission, several symbols in the statistical formulae had been distorted. For the correct formulae, he referred the audience back to document BMT/4/7 before its revision. [A further revised version is reproduced under document BMT/4/7 Rev. 2].

The Use of the Analysis of Molecular Variance (AMOVA) for Distinction Studies

40. Mrs. Christine Dillmann (France) introduced document BMT/4/9 on "The Use of the Analysis of Molecular Variance (AMOVA) for Distinction Studies," prepared in the *Station de Génétique Végétale* at the Ferme du Moulon, GIF s. Yvette, France.

Discussion on Statistical Methods

41. The Working Group noted that different statistical methods gave different results and that the choice of the measure of distance had an important influence on the results. It was therefore of utmost importance to start all applications of statistical methods by defining clearly the right question and by verifying whether a given method was justified. In this field, close cooperation between the TWC and the BMT was necessary.

42. The Working Group was concerned about the wrong application of dendrograms as explained impressively by Mr. Law with a child's mobile. It agreed that a dendrogram was not an end product but only the first visualization of data. It should not be applied when there was no hierarchical model. A two-dimensional presentation was not supported by the test results. It may thus not show an objective comparison, nor show the shape or the density of clusters. It should only be used where the grouping was known. It was regretted that many scientific publications required authors to submit their results as dendrograms. The TWC was asked to search for tools which could replace misleading dendrograms by other more correct presentations of results.

Correlation and Causal Linkage Between DNA Markers and Morphological Traits and Relationship Between Genetic Distance and Morphological Distance Between Varieties

43. Mrs. Christine Dillmann (France) gave a short report on the work on maize carried out in France in which varieties were screened with DNA markers and, in parallel, with a set of morphological characteristics in the field. Although a correlation could not be established between a given marker and a given morphological characteristic there was good correlation between the total results of all morphological characteristics observed and the total result of all DNA markers.

44. Mr. John Law (United Kingdom) introduced document BMT/4/19 on "Statistical Methods for Assessing and Interpreting Genetic Distance and Genetic Diversity" prepared in the National Institute of Agricultural Botany in Cambridge, United Kingdom.

45. Mrs. Christine Dillmann (France) referred to her report given during the third session of the BMT on the "Estimation of Molecular Genetic Distance in Maize or DUS and ED Protocols" as reproduced in document BMT/3/6 and the discussions on her report as reproduced in document BMT/3/18, paragraphs 11 to 13.

46. Several experts and breeders expressed their concerns on the above methods of comparison. There was no clear correlation between morphological expression and DNA markers. There was a big difference in approach between the use of morphological differences and of genetic differences. These were two different concepts. Molecular markers were not linked with phenotypic expressions and therefore the two concepts should not be mixed even globally as that would give the impression that there was a link.

47. Other experts expressed their view that in certain cases correlations may be established between a certain phenotypic expression, e.g. a resistance to a disease and a given marker, especially if the resistance was introduced in a GMO variety. Others warned again that in those cases a DNA marker may show the presence of the resistance gene but it would give no guarantee whether the gene would also work and express itself in the plant.

48. Other experts stated that if a correlation was proved between a given marker and a specific morphological characteristic, the DNA marker would be used only as a tool to establish the presence of the morphological characteristic.

49. Some experts insisted that despite the above reservations it was interesting to establish whether a global description via DNA markers would give comparable results in separating varieties to a description based on traditional characteristics.

Position of the Breeders vis-à-vis DNA Profiling, the Use of DNA Profiling Methods by Expert Witnesses in Disputes on Essential Derivation and Effect of Different Plant Breeding Schemes with Evaluation of Percentages Between Them

50. Mr. Bernard Le Buanec (ASSINSEL) introduced document BMT/4/6, a "Position Paper on the Use of DNA-Profiling for Assessing Genomic Conformity," adopted by the General Assembly of ASSINSEL on May 24, 1996, and referred also to document BMT/4/17 on "The

Model Study on Essential Derivation of ASSINSEL Using Tomato as a Crop," introduced by Mr. T. Kramer (Netherlands). He repeated again the position of the breeders which was to separate the testing of DUS from the testing of essential derivation and also to use different tools for these two approaches. If one did not separate the tools, sooner or later the two concepts would be mixed and this would weaken the plant variety protection system as a whole. The work of DUS testing was designed to describe the phenotype of the variety and to check whether it fulfilled the minimum distance decided upon in order to enable it to be protected. The concept of essential derivation was not only based on genetic distance but also involved several other questions such as evidence of the use of the other variety to develop the new variety and the aim of the second breeder to come as close as possible to the initial variety, etc. The decision on DUS was taken by the competent national PVR authorities, the decision on the second concept was taken by arbitrators or courts and not by the PVR offices.

51. The Working Group agreed that the question of essential derivation was not for the PVR offices to decide. Several experts stated, however, that courts needed the guidance of technical experts and would probably approach PVR offices for advice. In the Diplomatic Conference, UPOV had thus been requested to establish Guidelines on essential derivation. It was therefore reasonable for UPOV experts to be involved in the establishment of thresholds for essential derivation. Several other experts insisted, however, that the question of essential derivation was in the first instance a question for breeders to agree upon. UPOV experts should be willing to cooperate with breeders but should stick to technical questions only and not get involved in legal discussions.

The Use of DNA-Profiling for Prescreening as a Possible Tool in DUS Testing

52. Mr. Huib Ghijsen (Netherlands) reported on his study on *Poa pratensis*, an apomictic species which could be considered to be vegetatively propagated. The large number of existing varieties called for efforts to reduce the number of reference varieties to be grown in the field. The question was whether it was possible to use DNA profiling or other characteristics not accepted for DUS testing for the prescreening of the whole reference collection in order to avoid growing varieties which were genetically too far from the candidate variety to be compared with it in the field test.

53. While some experts considered *Poa pratensis* to be a very special case in which such tools may be acceptable they warned at the same time against extending such a procedure to other species. Other experts completely rejected the possibility of using for prescreening any characteristic which is not accepted for DUS testing. Prescreening was a kind of grouping as the discarded reference varieties would never be compared with the candidate variety. UPOV had strengthened its requirements for grouping characteristics. Not all characteristics accepted for DUS would be admitted for grouping. Grouping characteristics needed to be absolutely reliable, uniform and stable. When a grouping was made, a variety of one group would never be compared with a variety of another group. Therefore one must be absolutely certain that a variety was not in the wrong group.

54. Other experts stated that in the future for prescreening one had to change the approach. For distinctness a characteristic by characteristic approach was used while in prescreening a

multivariate approach would be adopted using information from traditional morphological characteristics together with new methods.

55. Some breeders warned that while they would not be opposed in principle to prescreening, care had to be taken. In prescreening the requirements for uniformity and stability were not the same.

56. The Working Group finally realized that more discussions were necessary on the question of prescreening and that a paper should explain in detail how groups could be established without the use of DUS characteristics. The Chairman, therefore, asked the experts to offer papers for the next BMT session. He also asked the TWC whether it could offer its help on this subject. It was, however, necessary first to define the problem and to define the parameters for solving the problem. This was very important because some of the solutions might well be outside these parameters. The Chairman stressed that the discussions should not be limited to agricultural species but also cover ornamental species where varieties in many species are propagated vegetatively.

Control of Uniformity in Characteristics Obtained with Biochemical or Molecular Markers

57. Mr. Johannes-Peter Ohms (Germany) introduced document BMT/4/14 on "The Effect of Non-Uniformity and Non-Stability on the Correctness of the Varietal Identification of Seed and Commercial Lots in Cereals," prepared by experts from Germany.

58. Mr. Michael Camlin (United Kingdom) reported on the discussions on uniformity in ryegrass held during the last session of the TWA. He regretted that so far the BMT had only looked into differences between varieties and the variation between varieties but not into uniformity or the variation within a variety. While the question of identification may be discussed without looking at uniformity (and even that was contested by some experts), the question of distinctness could only be looked at together with the question of uniformity. Unfortunately most reports did not tackle uniformity, they either used one single plant or a bulk sample. For the next session, uniformity should be the main question to be studied in relation to DNA markers. This was most important if one moved from vegetatively propagated species to cross-fertilized species such as ryegrass. In these populations with differing genotypes the stability of a variety was normally based on its relative uniformity. Provocatively, he asked the Working Group whether it was possible to ignore a lack of uniformity in molecular markers if the variety proved to be uniform in morphological characteristics. He stressed the need to have some papers on this question for the next session of the BMT.

59. Several experts immediately responded that if UPOV wished to keep its notion of the "characteristic," it had to maintain the requirement that any characteristic used for distinctness must also be checked for its uniformity and stability. If a characteristic was not uniform, it had to be rejected for distinctness. Uniformity was, however, related to the mode of propagation and in cross-fertilized species such as ryegrass only a relative uniformity was required. If one attempted to deviate from this basic rule, one would create more problems for the future than one would solve.

60. The Working Group confirmed that the reports for the present session had left out the question of uniformity and stability. The documents and reports for the next session would have to correct that situation and specifically concentrate on these two requirements.

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

61. Mr. Johannes-Peter Ohms (Germany) introduced document BMT/4/13 on "The Harmonized Presentation and Documentation of Protein and DNA-Polymorphisms as Pre-Condition for the Introduction of Biochemical and Molecular Biological Methods for DUS-Testing," prepared by experts from Germany. The document confirmed the need for good knowledge of genetic control and a clear definition of alleles.

62. The Vice Secretary-General of UPOV whilst explaining that the Secretariat of UPOV did not claim authority to pronounce upon the interpretation of the provisions of the Convention, referred to the language and interrelationships between Article 1, Article 7 and Article 14(5)b of the 1991 Act of the UPOV Convention which had been the subject of discussion in the Administrative and Legal Committee of UPOV on two separate occasions. The subject had been discussed at a joint session of the Administrative and Legal Committee and Technical Committee in April 1993 (see documents CAJ/32/33, TC/29/3 and the report of the session, documents CAJ/32/10 and TC/29/9) and at a session of the Administrative and Legal Committee of UPOV in October 1996 (see document CAJ/36/3 and the report of the session, document CAJ/36/6). The Vice Secretary-General stated that the discussions in the documents and session reports should be studied in detail since they were not readily summarized. However the discussions supported *inter alia* the following propositions:

(a) "Article 1 defined the variety concept, but remained silent on whether or not a variety was eligible for protection; the reference to the genotype was intended to make it clear that the existence of a variety merely presupposed the possibility of defining it according to genetically determined criteria, and not necessarily by characteristics appearing in lists drawn up for the purposes of the grant of breeders' rights. The genotype was neither defined nor even specified in the course of the discussions. There was nevertheless the underlying hypothesis that a variety could not be defined otherwise than by its genes; in that sense, no substantive difference was made between the genotype and the phenotype." (Last three lines of page 3 and first seven lines of page 4 of document CAJ/32/10).

(b) "Article 7 dealt only--and that was already clear from its inclusion in Chapter III--with the circumstances in which a variety may be protected, in view of the fact that it was not eligible for protection by virtue of the mere fact of its being a variety. Article 7 therefore contained stricter conditions than Article 1. To qualify for protection, a variety had to be "clearly" distinguishable. The word "clearly" had not been defined, and it was important to point out that the Diplomatic Conference did not want to introduce specific restrictions. Article 7 did not refer to the characteristics to be taken into account, not even from the point of view of their importance or their essential nature. It was therefore for the examining authority to determine the characteristics or combinations of characteristics that it would use in examination. The Article also did not specify when a difference was clear, so it was for the authority to decide, for instance, whether a single difference was sufficient, assuming that it

was great enough, or alternatively whether one needed only note the existence of a number of differences that were not clear, provided that they could be combined to give a clear difference. The Convention left all these options open." (Paragraph 15(iii), document CAJ/32/10, page 4).

(c) "The words "the expression of the characteristics resulting from a given genotype or combination of genotypes" appearing in Article 1(vi) of the 1991 Act do not conflict with the use of characteristics based upon the features of genetic material (in particular "DNA profiles")." (Paragraph 6(b) of CAJ/36/3, page 6).

(d) "The question of deciding whether a characteristic based upon the features of genetic material and resulting from the use of a well-established method of analysis (a "DNA profile") can be used within the framework of the examination of distinctness should be addressed in each particular case by applying the criteria which have already been established in relation to "traditional" characteristics (including characteristics resulting from the use, for example, of electrophoresis)." (Paragraph 6(c) of CAJ/36/3, page 6).

(e) "The extension of protection to essentially derived varieties ought not to result in a weakening of the criteria for decisions on distinctness." (Paragraph 6(d) of CAJ/36/3, page 6).

(f) "The question whether "directly-read characteristics of the genome" could be taken into account was not settled by the Convention, which did not pronounce on the nature of the characteristics to be considered." (Paragraph 15(b) of CAJ/36/6, page 4).

(g) "The question had to be settled case by case according to the usual criteria, which included the requirement of the clearness of the difference noted and the need to abide by the essential purpose of the protection system." (Paragraph 15(c) of CAJ/36/6, page 4).

(h) "It would in particular be contrary to that purpose (*the essential purpose of the protection system*) to allow the protection of one plant group that was too close to another. It would be wrong to conclude from the position set forth in paragraph 6 of document CAJ/36/3 that the use of biochemical characteristics was sufficient for determining distinctness. The 1991 Act did not rule out the use of new technological solutions, but did not validate those solutions either." (Paragraph 15(d) of CAJ/36/6, page 4).

(i) "It was sometimes suggested that distinctness was associated with the phenotype and the concept of essentially-derived variety with the genotype. The problem was, however, that Article 1(vi) (on the definition of the variety), and Article 14(5)(b) of the 1991 Act used the same terminology." (Paragraph 15(e) of CAJ 36/6, page 4).

63. Perhaps for present purposes the most important views expressed by the Administrative and Legal Committee were:

(a) It was for the Authority to decide whether a single difference were sufficient ... or alternatively whether one needed only note the existence of a number of differences that were not clear, provided they could be combined to give a clear difference. The Committee left these options open in CAJ/32/10 paragraph 15(iii).

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(b) The question had to be settled case by case according to the usual criteria which included the requirement of the clearness of the differences noted and the need to abide by the essential purpose of the protection system (paragraph 15(b), page 4 of CAJ/36/6, and paragraph 15(iii), page 4 of CAJ/32/10).

64. The last two propositions perhaps suggest how to reconcile any eventual use of the new technology with the need to avoid damaging the existing protection system. The use of a minimum number of molecular characteristics, well distributed through the genome would, when compared with some phenotypic characteristics in current use, increase rather than decrease the so-called minimum distance. The closer examination of intra-varietal variability in the next session of the BMT would considerably clarify the impact of using molecular techniques on the UPOV protection system.

65. Breeders and technical experts from national offices who responded to the report by the Vice Secretary-General expressed reservations on the interpretation made by the CAJ. The whole question would need to be carefully discussed again in the Technical Committee and also in the Technical Working Parties and the views of those present during the Diplomatic Conference should be obtained and the preparatory documents as well as the records of the Diplomatic Conference studied in the light of any new insights which emerge as practical work progresses. If, as a result of those discussions and studies, the interpretation of the CAJ was confirmed, an appropriate UPOV approach to these new methods would need to be developed.

Chairmanship

66. As the chairmanship of Mr. Joël Guiard was to end at the next ordinary session of the Council, the Working Group unanimously proposed to prolong the chairmanship of Mr. Guiard to cover at least the next session of the BMT. During that session the progress of the BMT would have to be evaluated and a decision taken on whether the work would require further sessions of BMT as a separate working group or whether the discussions on the subject could be continued in the Technical Committee and the Technical Working Parties.

Future Program, Date and Place of the Next Session

67. The Working Group agreed that at least one further session was necessary in order to continue discussions and make progress. At the invitation of experts from the United States of America, it agreed to hold its fifth session in Beltsville, United States of America, from September 22 to 24, 1998. The session would start in the morning of September 22, 1998, and close at 6 p.m. on September 24, 1998. A request for pre-registration would already be made in May or June 1998.

68. During the session, the Working Group planned to discuss the following items:

(i) Short presentation of research results or their follow up on different species (Chrysanthemum , Peach, etc.)

(ii) Assessment of variability within varieties

(iii) Assessment of variability between varieties

(iv) Statistical methods

(a) Confidence intervals and accuracy of distance estimates
(b) Alternative to dendrograms
(c) Refinement of the analysis of molecular variance (AMOVA) for distinction studies
(d) Combination of information from diverse data types (AFLP, SSR, morphological data, etc.)

(v) Position of breeders vis-à-vis DNA profiling

(vi) The use of DNA profiling methods by expert witnesses in disputes on essential derivation

(vii) The use of DNA profiling for prescreening as a possible tool in DUS testing

(viii) Possibilities and consequences of the introduction of DNA profiling methods for DUS testing

(ix) Definition of variety (reports from the TC and the CAJ)

(x) Future program of the BMT, date and place of the next session if any.

69. The experts were asked to inform the Office of UPOV before the next session of the Technical Committee (April 1998) of the documents they would offer to prepare for a given item of the Agenda. Should the TWC obtain some results under "Statistical methods," the items "Availability of user friendly computer programs," "Library of example data sets" and "Preparation of a statistical monograph" explaining especially when a given statistical method was appropriate or why not and where the risks of method were, could also be included for discussion.

Closing of the Session

70. In closing the session, the Chairman stated, and this was also confirmed by the Working Group, that it had been a good and fruitful session. Numerous subjects had been discussed and a large number of questions raised. The main emphasis of the next session had to focus on uniformity and stability, on the interrelation of the results, on the consequences of the new development on the work in the individual national offices. There was now a better understanding of the methods by the crop experts and a better understanding of the UPOV philosophy and the protection system by the experts from the laboratory. That was one main achievement of this and the previous sessions. Discussions needed to continue between these two groups to avoid one of them going in the wrong direction. In this respect it was important

to clarify and agree on a harmonized interpretation of the definition of variety as specified in the UPOV Convention.

Visits

71. On March 12, 1997, the Working Group visited the John Innes Centre (JIC) at Norwich where it was given information on the activities of the Centre by its Director, Prof. Richard Flavel, and by Dr. Robert Koebner as well as by Dr. Mike Gale on the scientific activities relevant to UPOV and by Mr. Mike Ambrose on the JIC germplasm collection, followed by a visit through the installations.

72. This report has been adopted by correspondence.

[Annex follows]

ANNEX

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[End of Annex and of document]