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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

Sixth Session
Angers, France, March 1 to 3, 2000

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 sixth session in Angers, France, from March 1 to 3, 2000. The list of participants is reproduced in Annex I to this report.

2. Mr. Bart Kiewiet, President, Community Plant Variety Office (CPVO) welcomed the participants to Angers on behalf of the CPVO. He also expressed its pleasure in hosting the Working Group, which performs one of the important tasks of UPOV, discussing the possibilities and impacts of the introduction of new techniques on the UPOV system. He also stated that the European Community intended to become a member of UPOV in the near future.

3. Mr. Greengrass, Vice Secretary-General of UPOV, also expressed his pleasure in the great interest shown in the Working Group including many participants from the outside UPOV circles. He briefly introduced the function of UPOV and the current situation of the UPOV membership to those experts not familiar with UPOV. While 44 countries are UPOV member States, some 60 additional countries are preparing plant variety protection legislation or have already established plant variety protection systems. The outcome of the Working

Group will accordingly influence the technical examination systems for plant variety protection throughout the world.

4. The session was opened by Mr. Michael Camlin (United Kingdom), Chairman of the Working Group. Before opening the session, the Chairman gave an overview of the discussions in the previous session and pointed out the expected points of discussion in the sixth session.

(a) First, he observed that, in view of the continuous progress of DNA profiling techniques, the advantages and limitations of the various techniques were still among the points of discussion. In addition, he pointed out the desirability of constructing a database of DNA profiles of varieties, referring to the problems involved, such as the standardization of DNA profiling techniques.

(b) He emphasized that the most important issue in the sixth session would be the assessment of uniformity and stability of molecular characteristics. This topic has been regarded as the main technical problem for the use of molecular techniques since the fourth session. In the fifth session, the Working Group had noted that rose varieties showed high uniformity in molecular markers. It had also been noted that mutations of phenotypic characteristics in rose varieties could not always be detected by molecular markers. Additional studies had to be made for other species, especially those involving other modes of reproduction. The Working Group had agreed to discuss in the sixth session four approaches concerning the assessment of uniformity for characteristics obtained with molecular markers (paragraph 34 of document BMT/3/18).

(c) Furthermore, with regard to the stability of molecular markers, the Working Group had noted that a high mutation rate might possibly be observed in molecular markers. In this connection, he referred to the concerns of breeders that the introduction of molecular characteristics might create an extra burden for breeders/maintainers.

(d) In relation to statistical methods, the chairman mentioned two problems: the criteria for the choice of marker sets and the correlation between molecular distance and phenotypic distance.

(e) He reminded the participants of the discussions on the four options of the interpretation of the definition of "variety" in relation to the use of molecular information for the judgement of distinctness in an *ad hoc* meeting on February 12, 1998, and of the discussions on "Characteristics Used in Distinctness Test" in the thirty-eighth session of the Administrative and Legal Committee (CAJ). The option supported in the *ad ho*c meeting and in the fifth session of the Working Group, was that information obtained using a molecular tool could not be used alone for a conclusion on distinctness, but only as a complementary aid to confirm a phenotypic difference.

(f) He also referred to the possible use of DNA profiling methods for the assessment of essential derivation. He explained that the task of UPOV is not to determine thresholds for the assessment of essential derivation, but to assess technical tools for the assessment of essential derivation.

(g) With respect to the use of molecular information for the management of reference collections, the Working Group had noted that a poor correlation between molecular distance and phenotypic distance would be a major obstacle to such use. Therefore, the correlation

between molecular distance (markers) and phenotypic distance (characteristics) should be further discussed.

5. Finally, the Chairman expressed and the participants of the Working Group affirmed their appreciation for the great contribution made by the former Chairman, Mr. Guiard (France) to the work of the Committee

Adoption of the Agenda

6. The Working Group unanimously adopted the Agenda as reproduced in document BMT/6/1 after agreeing changes in the order of the items.

Assessment of Variability Within Varieties and Between Varieties, in Particular, Uniformity and Stability in Molecular Markers

7. Mr. Jan De Riek (Belgium) introduced document BMT/6/3 on "Assessment of Distinctness, Uniformity and Stability of Sugar Beet Varieties Based on AFLP Data" prepared by him together with Evelien Calsyn, Isabelle Everaert, Erik Van Bockstaele and Marc De Loose (Belgium).

8. Mr. Robert J. Cooke (United Kingdom) introduced document BMT/6/4 on "Uniformity and Stability of Microsatellite Markers in Wheat and Oilseed Rape" prepared by him together with S. D. Freeman, C. Lowe, J. Jackson, D. Lee, V. J. Lea, P. Donini, J. Batley and K. J. Edwards (United Kingdom).

9. Mr. Martin Heckenberger (Germany) introduced document BMT/6/5 on "Variability within Maize Inbred Lines Determined with SSRs" prepared by him together with M. Bohn, J.S. Ziegle, L. K. Joe, J. D. Hauser and A. E. Melchinger (Germany and the United States of America). The additional results not available in the document, but shown in his presentation, are attached to this document as Annex II.

10. Mrs. Claire Baril (France) introduced document BMT/6/9 on "Usefulness of AFLP Markers to Estimate Homogeneity of Rapeseed Inbred Line Varieties" prepared by her together with V. Lombard, B. Tireau, F. Blouet and D. Zhang (France).

Discussion: Uniformity and Stability

Assessment of Uniformity by Molecular Data

11. Variability within cross-pollinated varieties: The Working Group noted from the extensive study on sugar beet varieties presented by the expert from Belgium, that variability observed by AFLP markers within sugar beet varieties was large, compared with variability between varieties. One expert pointed out the problems in judging distinctness in this type of species, especially taking into account the high variability within varieties. The results of the assignment tests, which identified similar plants from the pooled data of different varieties, attracted much attention. Several experts wondered to what extent similarities observed by AFLP markers corresponded with similarities in phenotypic characteristics.

12. Variability within self-pollinated varieties: The study on maize inbred lines showed variability of molecular markers within inbred lines. One expert questioned the uniformity level of these inbred lines, because the result seemed to differ from the results in the previous sessions on self-pollinated varieties, which had shown high uniformity within varieties. Another expert explained that high polymorphism within varieties was often observed even in self-pollinated varieties, such as lettuce.

13. Correlation between variability in phenotypic characteristics and in molecular markers: French study on inbred lines of Oilseed Rape (BMT/6/9) showed that off-types identified by molecular information corresponded well in most cases with off-types defined by morphological characteristics. The lack of correlation between phenotypic characteristics and molecular data had been regarded as one of the main problems for the use of molecular markers in the past sessions. However, the study which involved molecular distances, a diversity index and a principal component analysis by AFLP markers succeeded in describing variability within varieties, which correlated with phenotypic characteristics. The Working Group noted that the assessment of uniformity by molecular data could be consistent with assessment by phenotypic characteristics in some species with certain marker sets.

14. Investigation of molecular markers showing uniformity within protected varieties: One expert from the United Kingdom referred in his presentation to the assumed problem of molecular markers in relation to the assessment of uniformity. Polymorphism in molecular markers can be observed even within a sufficiently uniform variety. In addition, different molecular markers can show different levels of uniformity in the same variety. However, it was noted that the same situation could be found in some phenotypic characteristics and it should not be assumed that this variability will be the case for all, or even most, molecular markers. He proposed to investigate molecular markers that are not only usefully polymorphic between, but also sufficiently uniform within, existing protected varieties. A preliminary marker set that shows uniform band patterns within a small number of varieties was already in the process of being identified.

15. Some experts questioned the implications of selecting uniform markers. The question could be what kinds of genetic information would be eliminated by such selections. The selections might be biased toward certain information. One possible explanation could be that non-uniform markers observed within a highly uniform variety corresponded with non-coded or non-expressed genetic information. However, one expert from the United Kingdom pointed out that, particularly for self-pollinated crops, uniformity of traditional characteristics results from the overall method of selection and maintenance and not by actively selecting for uniformity of all the individual characteristics on a plant-by-plant basis. In this situation there is no reason why the molecular markers would not be as uniform as other characteristics. The Working Group noted that further studies would be needed in this area.

16. Other experts referred to the risk of selecting molecular markers by the results of a limited number of protected varieties. It was necessary to check whether uniformity in the selected molecular markers could be observed in a large number of varieties, including varieties grown in different regions with different genetic backgrounds if they were to be accepted in the UPOV system. An expert from the Netherlands stated that, in his experience in tomato, a set of molecular markers that showed completely uniform band patterns within all existing varieties was unlikely. Other experts pointed out the chosen molecular markers should not only show uniformity in uniform varieties, but also show non-uniformity in varieties which did not meet the uniformity requirements of the current DUS testing system.

17. The expert from the United Kingdom stressed that his report was preliminary. They planned to analyze a larger number of varieties against the initially selected set of primer pairs and to check whether the same uniformity could be observed in the larger number of varieties.

18. Need for further studies in varieties with different modes of propagation: Several experts suggested that grouping by propagation system in the same species, for example, inbred lines, hybrids with different production systems, and populations in the case of oilseed rape, would be needed for the study of molecular marker uniformity. Different propagation systems might require different approaches, as well as different standards, for the assessment of uniformity by molecular characteristics.

19. Different approaches for the assessment of uniformity in molecular characteristics: The Working Group briefly discussed the five approaches which were originally suggested in the third session and were presented again in the session by the expert from the United Kingdom (BMT/6/4). The five approaches in document BMT/6/4 are as follows:

> "(i) it could be decided that the lack of uniformity precludes the use of certain molecular markers for DUS testing purposes (use of only molecular markers with sufficient uniformity)[1];
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> (ii) it could be accepted that the level of non-uniformity exhibited by currently registered varieties (which would need to be determined systematically and empirically) represented a baseline, which candidate varieties in the future would not be allowed to exceed;
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> (iii) it could be suggested that from a certain date, all future candidates would have to be uniform for a particular selected marker or set of markers;
>
> (iv) it could be suggested that from a certain date, those candidates for which the marker data was the distinctness criterion would have to be uniform for that particular characteristic;
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> (v) it could be accepted that the repeatability (i.e. stability) of the differences between varieties is more important than the insistence on plant to plant uniformity. Thus if the variability within a variety, as estimated either by single plant analysis or by a bulk analysis, is maintained from generation to generation (and is therefore stable), this could be accepted as evidence of sufficient uniformity within that variety."

20. One expert from France stated that approaches to the assessment of uniformity would depend mainly on how molecular techniques are accepted for the assessment of distinctness, for example, as normal UPOV characteristics or as supporting evidence. In his opinion, if molecular techniques were used as supporting evidence, molecular markers could be considered as a tool for identification of varieties rather than that for distinctness. In this case, sufficient uniformity in molecular markers would be required only in a candidate variety and similar varieties to clearly identify them. Uniformity in molecular markers would not need to be observed in all existing varieties.

21. A number of experts also stated that it would be impossible to fix a unique approach for the assessment of uniformity in molecular characteristics, and that approaches and standards

[1] The parenthesis is added for the report.

for the assessment of uniformity would differ, depending on the modes of propagation of varieties, molecular techniques and molecular markers. They should therefore be discussed case by case.

22. However, with respect to the method for assessing uniformity ((i), (ii) or (v)), many experts supported, if feasible, approach (i), the use only of molecular markers that show uniformity in existing protected varieties. The expert from the United Kingdom stated that the choice of the above approaches depended on how quickly molecular techniques would be introduced. Approach (i) would be preferred, but it would take a relatively long time to choose each marker set. An expert from France emphasized that the wording should be not "uniformity" but "sufficient uniformity." Several experts also stated that the Working Group should keep in mind that uniformity did not require absolute uniformity within a variety.

Technical Feasibility of Uniformity Assessment by Molecular Data

23. In the past sessions of the Working Group, the assessment of uniformity had been considered to be the main technical obstacle to the use of molecular markers for DUS testing. However, the presentations given in the session and progress in molecular techniques since the last session seemed to change the views of experts concerning the technical feasibility of uniformity assessment in molecular markers. A majority of experts in the session took the position that the introduction of molecular markers would probably not raise big technical difficulties in assessing and maintaining the uniformity of a variety.

24. For instance, an expert from France expressed optimistic views on assessing and maintaining uniformity for molecular characteristics, based on the experience in the introduction of electrophoretic characteristics. Once the position on the assessment of distinctness were determined, the threshold level of uniformity would possibly be decided without technical difficulties. Once the threshold levels for uniformity were clearly fixed for certain marker sets, all future candidate varieties would be able to follow such uniformity standards. The expert from ASSINSEL basically supported this view, while he stressed the need for consideration of cost aspects of the molecular technique.

Role of Uniformity Criteria

25. Several experts stated that uniformity and stability were less important than distinctness, and that priority should be hereafter given to the discussion of distinctness.

26. The expert from ASSINSEL stated that uniformity criteria was only a tool for making a good decision on distinctness and stability, while it was also important for other reasons, such as for ensuring the purity of varieties. Uniformity made it possible to avoid over-lapping varieties and to achieve clear distinctness. It was also a good indicator for stability.

27. An expert from the United Kingdom pointed out that, on the introduction of new characteristics, the uniformity criterion should be studied with a view to preventing other breeders from selecting a small subgroup of plants from existing protected varieties. He referred to the following paragraph in the revised working document for a New General Introduction to the Assessment of Distinctness, Uniformity and Stability in New Varieties of Plants (TC/36/6):

> "73. With the introduction of new characteristics it may be possible to select different forms within a protected variety. The UPOV protection ensures that nobody can take one of these possible forms and register it as a new variety. This is achieved because no candidate can be distinguished from an existing protected variety by a characteristic that is not uniform in the other variety. Therefore it will prevent the use of new DUS characteristics from eroding the protection of existing varieties. This approach requires reasonable levels of uniformity in the initial varieties of new plant species or types, to ensure that variety development is not inhibited (see Chapter 7.1)."

Position of UPOV Concerning the Application of Molecular Markers for DUS Testing

28. The expert from ASSINSEL repeated several times during the session that UPOV had not made any decision on the application of molecular markers for the assessment of distinctness, uniformity and stability. All discussions in the Working Group were based on the assumption "IF molecular markers were accepted for DUS testing".

29. One expert of the horticultural working groups (TWF, TWO, and TWV) explained their stance concerning the application of molecular markers for DUS testing in horticultural species. He stated that there was no urgent need for the use of molecular markers in horticultural species.

30. On the other hand, one expert stated that the acceptance of new characteristics should be judged on whether they could meet criteria for characteristics in UPOV, regardless of the type of techniques. If new techniques showed clear differences between some varieties and sufficient uniformity within existing varieties, and if the results were repeatable and consistent, they should be accepted as characteristics to be used for DUS testing irrespective of the type of techniques. He further stated that the judgement of "clear difference" and "sufficient uniformity" should be based on statistical analysis. An expert from the United Kingdom emphasized that characteristics should be reliable and not lead to easy plagiarism.

Free Choice or Standardization of Molecular Marker Sets for DUS Testing

31. The Working Group discussed whether molecular marker sets to be used for establishing distinctness needed to be standardized. Some experts insisted that, if molecular techniques were accepted for DUS testing, applicants would be free to use any molecular marker set that met certain criteria for distinctness and showed sufficient uniformity in existing protected varieties and the candidate variety. National offices would not be able to reject marker sets that fulfilled the specified criteria. In addition, one expert wondered, with a view to the rapid progress of molecular techniques and markers, whether molecular marker sets could be fixed at all.

32. The Vice Secretary-General of UPOV expressed his concern about a totally free choice. One of the main tasks of UPOV is the international harmonization of variety testing and variety description among member States. If just any choice of molecular markers were accepted, varieties could not be compared with each other on the basis of the variety description. The standardization of molecular marker sets would be indispensable if they were introduced in DUS testing.

Statistical methods

33. Mr. Javier Ibañez (Spain) introduced his document BMT/6/8 on "RAPDs Mathematical Analysis to Establish Reliability of Variety Assignment in Vegetatively Propagated Species".

34. Mrs. Claire Baril (France) introduced document BMT/6/10 on "The GEVES Software Package for Estimating Genetic Distances Between Varieties, With or Without Linkage Map Information, and Analyzing the Genetic Diversity of a Collection of Varieties Through Molecular Data" prepared by her together with P. Dubreuil and V. Lombard (France).

35. Mr. John Law, Chairman of the Technical Working Party on Automation and Computer Programs (TWC), presented the results of the informal subgroup meeting held during the last session of the TWC.

(a) During its last session, the Working Group had requested the TWC to assess different statistical methods. Mr. Law had emphasized in the TWC the need to create co-operatively for member States and breeders, data sets for varieties including molecular marker data, pedigree data and data concerning morphological characteristics.

(b) However, such a data set had not been established to date, perhaps because of the confidentiality of variety data. The studies of statistical methods were therefore continuing at national level. The lack of assimilated data of a good quality was still the main obstacle to further studies. It would be necessary to establish a system for sharing existing data.

(c) The best statistical method would differ species by species. In particular, more studies were needed for cross-pollinated species.

Discussion on statistical methods

36. Prediction of pedigree relatedness using molecular data or phenotypic characteristics: Several experts reported that phenotypic characteristics were very poor indicators of pedigree relatedness, while molecular data were usually good indicators. Some experts reminded them that pedigree relatedness is important not for protection, but for the judgement of essential derivation.

37. Availability of GEVES Software: Several experts found the GEVES Software introduced in document BMT/6/10 useful and asked whether the software could be made available to experts in the Working Group. It was suggested that those experts who wished to use the Software contact Mr. Joël Guiard (France).

38. Improvement of the precision of molecular distances: The expert from France explained that knowledge of the distribution of markers on a linkage map improved the precision (standard deviation) of genetic distance between varieties. However, another expert insisted, based on her results with similar research, that such an improvement might not always be expected.

Possibilities and consequences of the introduction of DNA profiling methods for DUS testing and Position of the breeders vis-à-vis DNA profiling

39. Mr. Bernard Le Buanec (ASSINSEL) introduced discussion paper BMT/6/6 on "DUS Testing: Phenotype vs Genotype" prepared by him.

40. Mr. Joel Guiard (France) briefly introduced the draft document for TGP/13 entitled "Genetic labeling: a support for decision-making about distinctness", which will be submitted for the coming session (thirty-sixth session) of the Technical Committee. This draft document is available as a part of document TC/36/7. The main points of his presentation were as follows:

(a) There are cases where, although a clear difference between a candidate variety and other varieties could be observed in performance characteristics, such as yield and quality, distinctness could not be established on the basis of the phenotypic characteristics in the UPOV Test Guidelines. These cases occur especially in species where few phenotypic characteristics meet the criteria of UPOV and are available for the judgement of distinctness.

(b) Characteristics resulting from "genetic labeling", which describe the genetic structure, rather than the specific phenotypic information, could serve to help decision making as supporting evidence in the above cases, if they met certain conditions.

Discussion

Role of the Working Group

41. The Vice Secretary-General of UPOV emphasized the importance of making real progress in the discussion of the BMT towards the establishment of principles for the use of molecular techniques in DUS testing. There would be less urgent need in the member States with government testing for the introduction of molecular techniques. However, as the UPOV membership expanded worldwide, member States which adopted breeder-testing systems had increased. He pointed out the possibility that in the future some member States, especially those with breeder testing systems, might start to allow the use of molecular techniques for establishing distinctness upon request from an applicant. The Working Group should therefore speed up its discussion to avoid the situation that some member States go their own way without awaiting the establishment of UPOV principles.

42. The expert from ASSINSEL pointed out that the official stance of UPOV had not been decided yet and should not be prejudged. He stated that, however, it did not mean that ASSINSEL rejected the future use of molecular markers for DUS testing and that ASSINSEL hoped progress for the discussion of the Working Group. The expert from France insisted that the role of the Working Group was not only to discuss how to use molecular techniques in DUS testing, but also to analyze and explain possible positive and negative consequences of the introduction of molecular techniques in DUS testing.

Phenotype vs Genotype

43. The Working Group discussed the interpretation of the wording "the expression of the characteristics resulting from a given genotype or combination of genotype" in Article 1(vi)

of the 1991 Act of the UPOV Convention. On one hand, several experts insisted that the purport of the wording should be "phenotypes". The expert from ASSINSEL stated that in his opinion the wording had been clearly intended to mean phenotypes in the preparation of the 1991 Act.

44. With this interpretation in conjunction with Article 7, a possible conclusion would be that the use of characteristics other than phenotypic characteristics could not be accepted for the judgement of distinctness. At this stage, molecular characteristics could not be regarded as phenotypic characteristics, because the linkage between phenotypic and molecular information had not been well established, and because some information given by molecular techniques might not relate to any phenotypic information. Therefore, differences in molecular markers possibly resulting from differences in non-coding parts of DNA could not alone establish distinctness between two varieties. If this interpretation were strictly applied, molecular techniques would not be used alone for the judgement of distinctness without the revision of the Convention.

45. The Vice Secretary-General of UPOV reminded the Working Group that the Administrative and Legal Committee of UPOV (CAJ) had expressed the view that the wording does not necessarily mean "phenotypes". The same language may simply mean that a characteristic must be inherited. No discussion of the subject can be found in the records of the 1991 Diplomatic Conference. The CAJ was of the view that the language of the 1991 Act of the Convention does not require or forbid the use of molecular markers for the judgement of distinctness. Technical circles must recommend whether it is desirable to use such techniques in the light of the overall functioning and objectives of the Convention. His intervention was based on the following propositions in the CAJ (Paragraph 15 of CAJ/36/6):

(a) "Expression of characteristics" should not be understood in the genetic sense. A "characteristic" was an element, in the abstract, of the description of a variety, and the "expression" was the specific form that the element assumed; for instance, the words applied equally well to the length of a stem as they did to a gene (expression being the allele in that case).

(b) 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.

(c) 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.

(d) It would in particular be contrary to that purpose 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.

(e) 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.

Minimum distance

46. The Working Group also discussed the concept of "minimum distance" and the impact of the introduction of molecular techniques on "minimum distance". The expert from ASSINSEL posed the problem that, if molecular characteristics were accepted for DUS testing, one molecular band difference might be regarded as "clearly distinguishable" in Article 7 of the 1991 Act. Is that what we want? He stressed the need for defining a new concept of "minimum distance" for molecular characteristics, e.g., the number of markers needed to establish distinctness and the necessary quality of the markers. The Chairman questioned how the minimum distance (threshold level for assessing distinctness) was defined for molecular characteristics, considering the fact that single-gene controlled characteristics, such as disease resistance and flower color, could establish distinctness in the current system.

47. One view was that the concept of the minimum distance had reduced significance after the adoption of the 1991 Act. The Vice Secretary-General of UPOV noted that a very small difference, such as a point mutation, could establish distinctness in many species. This was taken by ornamental breeders to be a weakness of the UPOV system. However, the introduction of the essential derivation concept by the 1991 Act had enabled breeders to defend their interests in such cases. The essential derivation concept had released national offices from the most extreme forms of minimum distance dilemma. One expert also stated that the minimum distance had been simply a concept and had never been clearly defined. In practice, the minimum distance had in some cases been almost zero.

48. Another view was that, on judging distinctness, the concept of minimum distance should be taken into consideration in order to ensure the quality of protection. If the concept of the minimum distance were to be nullified, and if all small differences could be accepted as the basis for distinctness, the breeder would have to make use of essential derivation in every case. The introduction of the essential derivation concept should not influence the concept of minimum distance. In addition, the quality and meaning of protection would be significantly degraded, and the existing protection framework would be broken down. The creation of new varieties would become extremely easy, and the value of protection might be almost nothing. The expert from ASSINSEL stated that breeders might not wish to face such a situation.

New approach for the assessment of distinctness

49. The expert from France proposed a new approach, assessing distinctness not on a characteristic-by-characteristic basis, but by the combination of characteristics. In other words, distinctness would be assessed by the distance between varieties derived from the totality of differences of all characteristics, such as a molecular distance. This approach could be regarded as a true "minimum distance" approach. The minimum distance would be meaningful and not approach zero. This approach would enable the avoidance of granting protection for varieties which did not deserve protection, such as a variety derived by a mutation in a single gene from an existing variety, and the maintenance of the quality of protection. However, he stressed the need for further studies on this approach and proposed to seek the possibility of using molecular characteristics only as supporting evidence until enough information needed for the new approach is accumulated. This proposal attracted much attention from the experts as a possible future approach, although it would result in a significant change in the approach to the judgement of distinctness.

Supporting evidence

50. The Working Group also discussed the use of molecular characteristics as supporting evidence for the assessment of distinctness. The expert from the United Kingdom questioned the status of supporting evidence characteristics. If the final decision on the distinctness of the variety was based on whether molecular characteristics showed a clear difference or not, molecular characteristics would play the same role that normal UPOV characteristics did in the decision making process. In addition, he observed that the use of molecular techniques as supporting evidence for performance characteristics proposed by the expert from France would fully open the door to performance characteristics for the establishment of distinctness, which, as such, might result in a significant change in the current protection system.

51. The expert from ASSINSEL stated that ASSINSEL has already been in a position to accept supporting evidence characteristics. However, the use of supporting evidence characteristics should be limited to the cases where testing experts are strongly convinced of the distinctness of varieties by the results in the field trial. If the testing experts have no clear conviction based on the field trial, the supporting evidence characteristics should not be used at all. The status of "supporting evidence" characteristics was therefore clearly different from that of normal UPOV characteristics.

52. He also stated that the use of molecular characteristics as supporting evidence characteristics might not be a big problem for ASSINSEL. The important question was whether molecular characteristics should be introduced into the judgement of distinctness, uniformity and stability as normal UPOV characteristics in the future.

Transitional Period

53. The expert from ASSINSEL anticipated in his discussion paper that, if molecular markers were to be introduced into DUS testing in the future, the protection system might be significantly changed. He therefore stressed the need for special care during a transitional period to effectively protect the rights of the breeders already protected by the present system, even after the implementation of a new protection system.

54. One expert stated that, even if we decided to allow the use of molecular characteristics, phenotypic characteristics would not be totally replaced by molecular characteristics. A possible, realistic approach would be to introduce molecular characteristics as additional normal characteristics or supporting evidence characteristics in the current system in the same way as for electrophoresis characteristics. The question "how molecular characteristics could fit in with the current system?" should be discussed.

55. The expert from the CPVO also stated that, before discussing any transitional period, the Working Group should discuss to what extent and how molecular techniques could be introduced in DUS testing.

Stability

56. The expert from ASSINSEL also expressed its serious concerns with stability. As relatively high mutation rates on molecular markers had been reported in the last session, the

maintenance of molecular characteristics over the protection period for the stability criteria might present new burdens to breeders/maintainers. Should a mutation in one marker be considered as the loss of stability? One possible solution might be to establish a sub-threshold for stability in molecular characteristics, taking into account its possible impacts on the maintenance practices of breeders/maintainers.

The use of DNA-profiling as a possible tool for management of reference collections in DUS testing

57. Mr. John Law (United Kingdom) introduced document BMT/6/2 on "Most Similar Variety Comparisons in Chrysanthemums" prepared by him together with J. C. Reeves, J. Jackson, P. Donini, R. J. Cooke (United Kingdom) and J. S. C. Smith (United States of America).

58. Mr. Joël Guiard (France) briefly introduced the draft document for TGP/4 entitled "Management of reference collection", which will be submitted for the coming session (thirty-sixth session) of the Technical Committee. This draft document is available as a part of document TC/36/7. The main points of his presentation were as follows:

(a) The management of reference collections is becoming more and more difficult as the UPOV membership expands worldwide, and whilst the number of varieties increases continuously. The establishment of a new effective management method is therefore indispensable in order to maintain the efficacy of and confidence in technical examination by minimizing the risk of overlooking existing varieties similar to a candidate variety and to reduce the costs for maintaining reference collections.

(b) For instance, the physical collection of propagating material should be continuously maintained, in principle, in each member State. Moreover, it is necessary to exchange variety descriptions amongst member States. However, the comparability of variety descriptions in phenotypic characteristics prepared by different countries would be limited in some species because of genotype x environment interaction.

(c) He therefore proposed to consider the introduction of molecular techniques for the characterization of varieties. One of the key problems in this regard may be the lack of good correlation between phenotypic distance and molecular distance. He accordingly proposed to investigate the possibility of combining molecular data with phenotypic data to offset their respective shortcomings.

59. Comparison of different distance estimators: In relation to the presentation made by the expert from the United Kingdom, several experts pointed out some problems in comparing figures derived from different distance estimators. In particular, the direct comparison between phenotypic distances and the molecular distances might be misleading. Figures of molecular distances could be compared only with those derived by the same molecular distance estimator for the same species.

60. The Working Group was not able to give enough attention to the discussion on this item because of the lack of time.

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

61. Mr. Trevor J. Gilliland (United Kingdom) introduced document BMT/6/7 on "Assessing Genetic Conformity Between Varieties of Ryegrass" prepared by him together with E. Calsyn, M. De Loose, I. Roldán-Ruiz (Belgium), R. Coll (United Kingdom), and M. J. T. Van Eijk (Netherlands).

62. The Working Group discussed the use of DNA profiling methods for assessing essential derivation in conjunction with the discussion for DUS testing.

63. Distinctness and essential derivation: The expert from ASSINSEL emphasized that the notions of distinctness and essential derivation should be clearly kept separate. If molecular techniques were to be accepted for both assessing distinctness and judging essential derivation, there would need to be two different thresholds. The expert from the United Kingdom stated that the judgement of essential derivation would not be based only on characteristics used for distinctness. Much wider information could be used to judge essential derivation. In addition, one expert reminded the Working Group that genetic conformity was not the only criteria for the judgement of distinctness.

64. Alternative approaches: One expert proposed an alternative approach for the judgement of essential derivation by molecular techniques. Essential derivation could be judged based on whether a variety maintained certain unique sets of molecular marker patterns of the initial variety that could not have been obtained by independent breeding. Several experts questioned the feasibility of identifying such sets, although the proposed approach was conceptually agreeable. Another problem could be that two independent breeding activities with the same initial variety might result in two different varieties which share a unique set of molecular markers. They would not be essentially derived.

65. Continuation of discussion on essential derivation: The Working Group also discussed whether it should continue the discussion on essential derivation in its next session. One expert stated that, to date, essential derivation had not been clearly defined for its application in practice, even about 9 years after the introduction of this concept in the 1991 Act. He emphasized the importance of clarifying the legal definition of essential derivation rather than discussing possible approaches by molecular markers in the BMT.

66. The expert from ASSINSEL stated that, in the Diplomatic Conference, UPOV had been requested to establish guidelines on essential derivation. The discussion on essential derivation in the Working Group could be considered as a part of activities of UPOV to establish such guidelines. The Working Group should focus on technical aspects, for example, identifying the methods and tools necessary to assess essential derivation and providing technical information on how to use molecular markers to assess genetic conformity.

67. The Working Group agreed to continue discussions on the assessment of essential derivation in the next session.

Short presentations of biochemical and molecular techniques: new techniques, advantages and limitations of various techniques (this item could be illustrated with experimental data obtained in different species)

68. Mr. Tetsuya Kimura (Japan) introduced document BMT/6/11 on "Microsatellite Markers of *Pyrus* spp.: Identification of Pear Accessions by Apple SSRs and Similarity Between Pear and Apples" prepared by him together with Toshiya Yamamoto and Yoshiyuki Ban (Japan). He illustrated an example of the application of microsatellite markers available in the major species for closely related species.

69. Mr. Ben Vosman (Netherlands) introduced document BMT/6/12 on the "Standardization of Molecular Marker Systems for Variety Testing" prepared by him together with R. Cooke, M. Ganal, R. Peeters, P. Issac, M. Röder, J. Jackson, S. Rendell, M. Dijcks, Y. Kleyn, D. Visser, K. Wendehake, T. Areshchenkova, V. Korzun, M. Amelaine, V. Wickaert and G. Bredemeijer (France, Germany, the Netherlands, and the United Kingdom). He presented the results of an on-going EU project that aims to demonstrate the technical viability of STMS markers for variety identification. The project had attempted to identify good marker sets for tomato and wheat and to standardize the methodology in different laboratories. He reported that there were only a limited number of existing molecular markers which fulfilled all of the criteria specified by the project, that is that markers be freely available (not patented), highly polymorphic, mapped, evenly distributed over the genome, suitable for multiplexing and, easy to score and reproducible in different laboratories. The main problem is that many markers are not freely available. The first comparison of data of the selected markers generated by the participating laboratories revealed a high degree of uniformity. He also analyzed possible reasons for observed discrepancies among the laboratories. They would eventually test 500 varieties of each species and construct databases.

70. Mr. Kader Fatimi (Celera AgGen/Agrogene, France) made a presentation on "Reliability and Reproducibility of SSRs Markers in Maize". The summary of his presentation is attached to this report as Annex III. He introduced the SSR marker system which his company had put into service and explained its reliability and reproducibility.

71. Mr. Dmitry Dorokhov (Russian Federation) gave a presentation on "Russian Potato Cultivars – Identification by Protein and DNA Profiling". The summary of his presentation is attached to this document as Annex IV. He explained the study on the variety identification of 50 potato varieties by protein, isozymes and RAPD markers.

Access to Data, Construction of Databases

72. The Working Group noted from the above presentations that molecular techniques were entering into a new phase, from the phase of research and development to the phase of application in practice. It also noted that a large number of DNA profiles of varieties had been produced by different projects and by different institutions. In view of this situation, the Working Group discussed problems associated with the access and sharing of existing DNA profiles and other data, and the construction of databases.

73. Access to DNA profiles: The access to DNA profiles of varieties that had already existed or will be produced, would become an issue for the further studies and future application of molecular techniques. The accumulation of DNA profile data of varieties and the construction of databases were progressed in isolation by different public projects, public

institutes and private companies. The access to such data and databases seemed to be generally limited to the members of the projects at this stage.

74. Construction of a central DNA profile database: The ideal solution is the construction of a central DNA database of variety profiles. Some experts thought that UPOV should take the initiative in constructing such a central database sharing existing DNA profiles. The Working Group recognized the need for discussing possible principles and frameworks for constructing an open-system for sharing DNA profile data. A problem would be the standardization of molecular markers. One expert doubted whether molecular markers could be standardized in view of the rapid progress in molecular techniques and markers.

75. Access to other variety information: Several experts reported that breeders were generally very cautious about providing detailed information on varieties to the public. For example, in the case of the EU project, all seed samples had been handled not with variety denominations, but with special codes. As a result, the DNA profiles obtained in the project could not be analyzed in conjunction with phenotypic data and by reference to the mode of propagation. In this connection, the expert from the Netherlands proposed to investigate the possibility of utilizing, for the further studies of molecular techniques, propagating material that is submitted to the national office for protection or listing.

76. Database of phenotypic variety descriptions: The expert from ASSINSEL insisted that UPOV should first construct the database of phenotypic variety descriptions rather than that of DNA profiles. The database of phenotypic variety descriptions would be very useful not only for national offices, but also for breeders. The Vice Secretary-General of UPOV stated that the inclusion of technical information into UPOV ROM was currently under discussion in the Technical Working Parties and the Technical Committee. Several experts explained that the main problem would be the heavy workload needed for processing data in a standardized format by national offices.

77. Ownership of variety descriptions: One expert questioned the ownership of variety descriptions and other data of protected varieties. In other words, could variety descriptions be freely used by the national office and be placed in public domain, for example in an open database? The Working Group realized that it would be a sensitive question. The expert from the CPVO stated that variety descriptions and testing reports of varieties for which application were filed with the CPVO belonged to the CPVO. The expert from ASSINSEL suggested that the applicants had paid testing fees, and that the applicants therefore could assert their rights over the variety descriptions. One official of the UPOV Office stressed that variety description might have a different status to that of testing reports.

Future Program, Date and Place of the Next Session

78. The Working Group agreed that it would be difficult to make real progress if the current format of the session were simply continued in the next session. An expert from France highlighted three different levels of existing problems: (i) legal interpretation of the Convention and general principles; (ii) problems that could be currently discussed only on a species by species basis: e.g., possible approaches for the assessment of distinctness and uniformity by molecular markers; (iii) the evaluation of different molecular and statistical techniques. He also stressed that generalization would be required after discussion on a species-by-species basis of specific problems, in order to establish general principles for the use of molecular techniques in DUS testing.

79. The expert from ASSINSEL reconfirmed the current position of ASSINSEL; they were not in favor of the use of molecular characteristics as independent characteristics. However, if molecular characteristics could fulfill all the requirements of normal characteristics, breeders would not see any problems in their use.

80. Legal and policy questions: First, the Working Group agreed that there were still lots of fundamental unsolved questions in relation to the use of molecular markers in DUS testing, such as the interpretation of the wording of Article 1 (iv) (phenotype vs. genotype), minimum distance, supporting evidence characteristics, transitional problems, the management of reference collections and databases. Several experts stated that these legal- or policy-type problems should be discussed not only by technical experts, but also by legal experts, policy makers and breeders, in other appropriate forum, the Technical Committee (TC), the Administrative and Legal Committee (CAJ), or/and a special separate working group. The results of the discussions on the fundamental questions might influence the whole framework of the protection system as well as the application of molecular markers in DUS testing. The Working Group decided to report the result of its discussions to the TC and CAJ for their consideration.

81. *Ad hoc* crop subgroups: Secondly, the Working Group agreed that real progress could not be expected without intensive discussion in small groups on individual species. Possible approaches and their problems could be discussed only on a species-by-species basis. The extensive studies of many existing varieties of individual species would be needed to make real progress. It could be also expected that, once a few species were chosen, member States would coordinate their studies and exchange necessary information.

82. Some experts proposed discussion of individual species in the Technical Working Parties, whilst others preferred to set up *ad hoc* subgroups for selected species. Most experts thought that discussions on molecular techniques had not yet reached the stage of discussion in the Technical Working Parties. Possible approaches for the introduction of molecular techniques, their potential impacts and problems had not yet been clearly defined. The Working Group therefore decided to establish separate *ad hoc* crop subgroups. On the other hand, it recognized that testing experts in the Technical Working Party should be involved with the discussion in the *ad hoc* crop subgroups. It agreed that the chairmen of the *ad hoc* crop subgroups should be chosen from experts in the Technical Working Party in question. The role of the *ad hoc* crop subgroups would not be to make any decisions, but to prepare documents that could be a basis of further discussions in the Working Group, the Technical Working Parties and the Technical Committee. The Working Group confirmed that the Technical Working Parties should be the decision-making bodies for the introduction of new characteristics into DUS testing for each species.

83. The Chairman suggested that each subgroup could meet once in year 2000. The official of the UPOV Office also suggested that the main tasks of the subgroups were (i) to analyze existing results of DNA profiling studies, (ii) to attempt to construct possible models for the assessment of DUS, the effective management of reference collection, and/or the judgement tool of essential derivation, and (iii) to identify unsolved problems for their application in practice and the possible impacts of the introduction on the protection system. The documentation established by the subgroups would be indispensable for making progress in the next session of the Working Group.

84. The Working Group discussed the selection of species for the subgroups. A majority of experts supported two criteria, (i) the need for the introduction of molecular techniques in DUS testing (species for which a limited number of characteristics are available and species which urgently need effective methods for the management of reference collection) and (ii) the availability of DNA profiling data and on-going studies. It had noted that horticultural Working Parties (TWV, TWF, TWO) had insisted that there were no urgent needs for the introduction of molecular techniques in DUS testing of horticultural species. However, several experts stated that there would be potential needs for molecular techniques, especially in the management of reference collection. The Working Group therefore decided to include horticultural species. In the light of the above criteria, it chose the following five species:

(a) Oilseed rape
(b) Wheat
(c) Maize
(d) Rose
(e) Tomato

85. The Chairman stressed that the selection of species did not mean that studies on other species would not be needed nor discussed in the next session. He urged the need for further progress on more species, for example, rice and lettuce.

86. Role of the Working Group: Many experts emphasized the importance of continuing the Working Group as it was the only forum where testing experts, molecular scientists, statisticians and breeders were able to exchange their views and opinions on the use of molecular techniques in DUS testing as well as essential derivation. The Vice Secretary-General of UPOV suggested that the future sessions of the Working Group be made more open to those outside of the habitual UPOV circle and that it shift to an open-scientific forum to some extent.

87. Next session of the Working Group: The experts from Germany offered to host the seventh session. The Working Group accepted that offer and agreed to hold its seventh session in Hanover, Germany, in the middle of October 2001.

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

(a) Reports on the discussion in the Technical Committee and the Administrative and Legal Committee (presentation of the UPOV Office)

(b) Short presentation on biochemical and molecular techniques: new techniques, advantages and limits of different techniques (this item could be illustrated with experimental data obtained in a range of different species)

(c) Possibilities and consequences of the introduction of DNA profiling methods in DUS testing (reports from *ad hoc* crop subgroups for Maize, Oilseed rape, Rose, Wheat and Tomato)

  (i) Assessment of distinctness, uniformity and stability
  (ii) Management of reference collection
  (iii) Examining essential derivation

(d) Assessment of variability within varieties and between varieties

(e) Construction and standardization of databases of DNA profiles of varieties

(f) Statistical methods (this item could be illustrated with experimental data obtained in different species)

(i) Combination of information from diverse data types (AFLP, SSR, morphological data, etc.)
(ii) Comparison of genetic distances with phenotypic distances
(iii) Confidence intervals and improvement of precision of distance estimates

(g) The use of DNA profiling as a possible tool for management of reference collections in DUS testing

(h) The use of DNA profiling methods in examining essential derivation

(i) Future program, date and place of the next session

Visits

89. During the session, the Working Group visited the Angers Center of the *Institut National de la Recherche Agronomique* (INRA) and was briefed on the activities of the Plant Pathology and Phytobacteriology Division, and in particular, on the application of molecular markers in plant pathology. It was given lectures on the following subjects: (i) biodiversity of plant pathogenic bacteria, (ii) molecular marker assisted detection of plant pathogenic bacteria in seeds and (iii) genetic linkage of and molecular markers for resistance genes.

90. The Working Group also visited the *Insitut National D'Horticulture* (INH) and was briefed on the organization of INH and the activities of the Applied Horticultural Biology Research Unit. It was given lectures on morphological characterization and molecular studies of the reference collection of *Hydrangea* and two presentations on molecular studies.

91. After explaining the use of AFLP markers and isozymes for characterizing *Hydrangea*, Mrs. Joelle Lallemand, BioGEVES, made a presentation on the results on the application of ISSRs markers to Poplar, Pea, Ryegrass, Rose, Sunflower, etc. She emphasized several advantages of ISSR markers: high repeatability, no digestion process, no need for prior knowledge of target sequences and free availability (no patent in 5'end).

92. Ms. Mathilde Briard, INH, presented national genetic resources for *Daucus carota* and other *Daucus* and gave detailed information about the analysis of an optimum sample number for accurate evaluation of accessions using AFLPs and a statistical sequential approach. Ms. Valerie Le Clerc, INH, also gave a presentation entitled "Molecular Markers and Variety Identification with Carrot Varieties", which explained the results of the application of ISSR markers for the identification of a broad range of carrot varieties.

93. The Working Group visited the Community Plant Variety Office (CPVO) and was given presentations on the CPVO and the Community plant variety rights system and an overview of the technical examination system.

94. This report has been adopted by correspondence.

[Annexes follow]

ANNEX I

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[Annex II follows]

ANNEX II

Slide 1

Variability within maize inbred lines determined with SSRs

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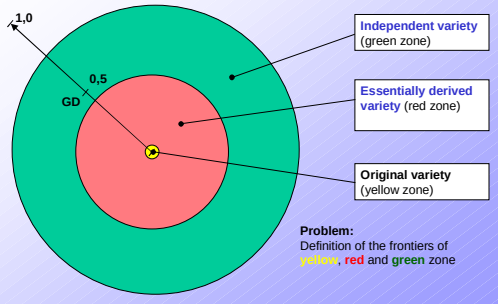
Slide 2

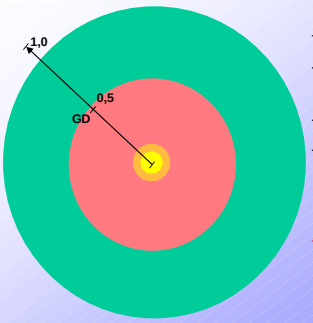
Essentially derived varieties (EDVs)



Problem:
Definition of the frontiers of yellow, red and green zone

Slide 3

Problems



- **which Marker-system(s)?**
- **Marker-data reliable enough?**
- **only Marker-data?**
- **no frontiers but grey-areas?**

¬ **Stability analysis**

Slide 4

Material & Methods

- 9 **maize lines**
- 2 – 5 **accessions** per line
  - Different **breedes, years** or breeding **generations**
- 100 **SSR markers** evenly distributed across the maize genome
- Detection of **heterozygosity** and **fragment size differences**
- Genotyping of **doubled haploids**

Slide 5

Lines & accessions

Maize line	Heterotic Pool	Number of accessions	Type of accessions
1721	Dent	**5**	1988, 1994, 1998, 2 breeders
5113	Flint	**3**	1991, 1998, different breeders
5248	Flint	**4**	1994, 1998, 2 breeders
5271	Flint	**4**	1994, 1998, 2 breeders
2065	Flint	**2**	1991, 1998
1105	Flint	**4**	1970, 1980, 1991, 1996
F012	Flint	**3**	F_6 , 2 different F_{10} plants
S002	Dent	**3**	F_6 , 2 different F_{10} plants
P006	Dent	**3**	F_6 , 2 different F_{10} plants

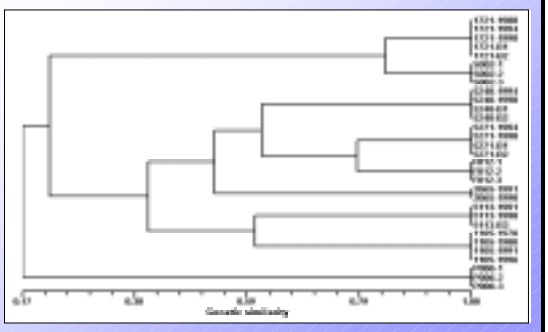
Slide 6

Observed Results

- Totally **392 SSR fragments** identified
- **1 – 8 fragments per marker**, average (avg.) of 4
- **PIC-Value** varied from **0.03** to **0.77**; average of **0.54**
- Only **2** markers **monomorphic**

Slide 7

Expected Results



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Results

- **33** markers showed **heterozygosity** for at least one accession
- **0** to **14 heterozygous accessions** per SSR (avg. 0.87)
- **0.5** to **5.7 heterozygous marker loci per accession** (avg. 2.6) within a line
- **Genetic similarity** within accession sets varied from **0.91** to **0.99**
- **Genetic variability** mainly caused by **heterozygosity**
- In addition, SSR fragments with small **size differences** were detected (1 to 3 bp differences).

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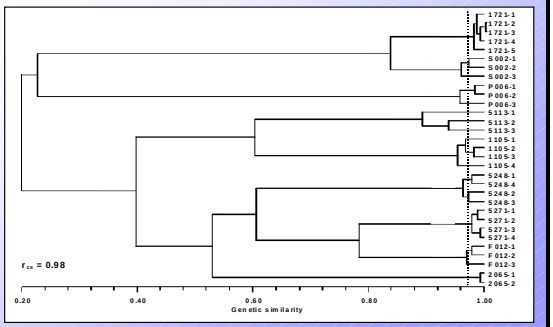
Observed Results



Slide 10

1bp-differences

Genotype	Marker	Scored Size	Exact Size	Peak height
1105-1	MC 1046	172	172.5	1484
1105-2	MC 1046	172	172.3	1830
1105-3	MC 1046	172	172.4	1345
1105-4	MC 1046	173	172.5	2147
S002-1	MC 1329	109	109.5	889
S002-2	MC 1329	109	109.4	688
S002-3	MC 1329	110	109.6	783
1105-1	MC 1189	227	227.2	2233
1105-2	MC 1189	227	227.2	2203
1105-3	MC 1189	226	227.1	1253
1105-4	MC 1189	227	227.2	2671

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2bp-differences

Genotype	Marker	Scored Size(s)	Exact Size	Peak height
5248-1	MC 1265	.	.	.
5248-2	MC 1265	250	250.6	86
5248-3	MC 1265	248	248.5	138
5248-4	MC 1265	248	248.6	267
1721-1	MC 1784	248/250	247.9/249.9	1308/328
1721-2	MC 1784	248	248.0	2244
1721-3	MC 1784	248	247.9	1815
1721-4	MC 1784	248	247.9	2064
1721-5	MC 1784	248	247.7	957
F012-1	MC 1046	213	213.1	2675
F012-2	MC 1046	211/213	211.0/212.9	862/576
F012-3	MC 1046	213	213.1	810

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Higher differences

Genotype	Marker	Scored Size(s)	Exact Size	Peak height
5248-1	MC 1526	124	124.6	226
5248-2	MC 1526	114	114.6	162
5248-3	MC 1526	114	114.5	332
5248-4	MC 1526	114	114.6	319
1105-1	MC1094	198	197.9	1114
1105-2	MC1094	198	197.9	1333
1105-3	MC1094	198	197.9	1336
1105-4	MC1094	194/198	194.0/197.9	1003/874
1105-1	BNGL 619	242	241.6	615
1105-2	BNGL 619	242	241.6	754
1105-3	BNGL 619	271	270.4	1466
1105-4	BNGL 619	271	270.5	1306

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Heterozygosity

Genotype	Marker	Scored Size(s)	Exact Size	Peak height
1721-1	MC 1131	111/127	111.3/127.4	385/465
1721-2	MC 1131	111	111.3	799
1721-3	MC 1131	111	111.3	633
1721-4	MC 1131	111	111.2	1239
1721-5	MC 1131	111/127	111.3/127.4	533/291
5248-1	MC 1931	176/240	175.8/239.2	814/995
5248-2	MC 1931	176 /240	175.8 /239.2	571/465
5248-3	MC 1931	176	175.8	947
5248-4	MC 1931	176/240	175.8 /239.2	958/362

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Summary 1

Line	Accessions	Fragment size differences 1bp	2bp	3bp	□4bp	Heterozygosity
		No.				
1721	5		1		1	2
5113	3	4	2	1	2	10
5248	4		1			8
5271	4			1	1	4
2065	2					1
1105	4				3	3
F012	3	4				5
S002	3		1		1	4
P006	3	1			1	3

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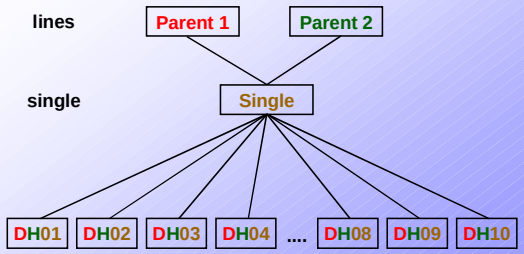
Doubled haploids



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Usual (expected) results

Genotype	Marker	Scored Size(s)	Exact Size	Peak height
Parent 1	MC 1720	257	256.4	1232
Parent 2	MC 1720	245	244.7	1711
Single	MC 1720	257/245	244.7/256.5	727/367
DH01	MC 1720	245	244.7	1698
DH02	MC 1720	245	244.6	2431
DH03	MC 1720	245	244.7	2380
DH04	MC 1720	245	244.7	2256
DH05	MC 1720	245	244.7	1912
DH06	MC 1720	245	244.7	1850
DH07	MC 1720	257	256.4	1317
DH08	MC 1720	257	256.5	1112
DH09	MC 1720	257	256.4	977
DH10	MC 1720	257	256.4	1194

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Unknown alleles

Genotype	Marker	Scored Size(s)	Exact Size	Peak height
Parent 1	PHI099	151	151.5	663
Parent 2	PHI099	153	153.6	966
Single	PHI099	.	.	.
DH01	PHI099	149	149.5	630
DH02	PHI099	149	149.4	574
DH03	PHI099	151	151.5	604
DH04	PHI099	151	151.5	643
DH05	PHI099	149	149.5	712
DH06	PHI099	149	149.4	663
DH07	PHI099	151	151.5	466
DH08	PHI099	151	151.5	730
DH09	PHI099	149	149.4	935
DH10	PHI099	149	149.5	738

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Heterozygosity

Genotype	Marker	Scored Size(s)	Exact Size	Peak height
Parent 1	MC2122	234	234.4	273
Parent 2	MC2122	236/254	236.5/254.4	491/233
Single	MC2122	220/236/254	220.5/236.5/254.4	445/148/616
DH01	MC2122	236/254	236.5/254.5	374/68
DH02	MC2122	236	236.5	308
DH03	MC2122	234	234.4	211
DH04	MC2122	.	.	.
DH05	MC2122	234	234.4	228
DH06	MC2122	234	234.4	348
DH07	MC2122	234	234.4	945
DH08	MC2122	234	234.4	260
DH09	MC2122	236	236.6	813
DH10	MC2122	236	236.5	658

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„Triploids“ (artefacts)

Genotype	Marker	Scored Size(s)	Exact Size	Peak height
Parent 1	MC 2122	234	234.4	273
Parent 2	MC 2122	236/254	236.5/254.4	491/233
Single	MC 2122	220/236/254	220.5/236.5/254.4	445/148/616
Parent 1	MC 1940	216	216.1	2662
Parent 2	MC 1940	255	255.6	614
Single	MC 1940	216/222/255	216.1/221.9/255.6	1212/206/198
Parent 1	MC 1782	240	240.4	2069
Parent 2	MC 1782	265	265.9	633
Single	MC 1782	228/240/265	228.5/240.3/265.8	482/582/212

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Summary 2

- Avg. of **11.6%** of all SSR loci showed unexpected **irregularities** (1% (2065) to 22% (1105))
- **No significant differences** between different **accession types**. (Range from 11% to 12.8%)
- **Scoring problems**
  - Totally 4561 alleles were detected
  - 856 alleles (18.7%) showed a **difference** from detected to scored value of 0.6 bp and higher
    - **390 (8.6%)** alleles showed a difference of **0.6 bp**
    - **466 (10.2%)** alleles showed differences of **0.7** to **0.9 bp**
    - **16 (0.4%)** alleles showed differences of **1 bp** and more

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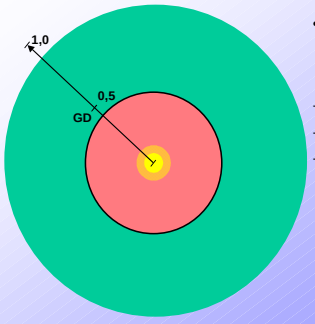
Conclusion



- Yellow zone could reach down to a **GS** of **90 %**

¬ **Scoring problems**
¬ **Duplication/Deletion**
¬ **Heterozygosity**

Further examination necessary (comparison of the observed results with AFLP- & Pedigree-Data)

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[Annex III follows]

ANNEX III

Variability and repeatability of SSR markers in Maize (Zea mays)

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INTRODUCTION

DNA markers are proving to be very useful for introducing new traits into crop plants, or selecting specific traits in segregating populations, identifying and protecting proprietary germplasm.

Microsatellite markers, also known as Simple Sequence Repeats (SSR) are polymorphic, abundant, well-distributed and informative markers in the maize genome. SSRs are PCR-based markers suited for automation and can be combined into «multiplex» systems of multiple markers to further enhance their utility.

PLATFORM TECHNOLOGY

Recently, fluorescent technology has further increased SSRs attractiveness and automated systems are currently available for collecting and recording very precise molecular weight data (PE Biosystems).

Automated detection platforms include internal lane size standard allowing single-base-pair resolution, enabling for the highest degree of discrimination and accuracy (precision sizing to 0.20 bp). In addition, these systems allow context-independent scoring and data merge from different analyses possible. The semi-automated analysis reduces operator error as well as increases data reproducibility

These advantages make SSRs the ideal marker system for use in varietal identification, registration and protection in maize. Given these unique features, Celera AgGen adapted the current fluorescent technology to maize breeder's needs.

MATERIAL & METHODS

SSR Marker Selection

Maize samples were genotyped using fluorescent SSR markers performed by Celera AgGen (Davis, USA) using an automated DNA sequencer (ABI377).

From an initial set of more than 700 SSRs maize markers available, we performed 2 successive preliminary screenings to identify mapped, informative, single copy markers and evenly spaced markers. The initial screening was based on 32 important maize inbred lines of diverse origin, primarily Midwestern corn types (#2 dent). The second screening included a more diverse germplasm base, consisting of 93 inbred lines including elite US and European lines (flint types) chosen on the basis of RFLP profiles to span the full range of corn breeding germplasm.

SSR Marker selection was established on the basis of evenly spaced mapped markers, robust amplification, absence of null alleles (% lines amplified), polymorphism informative content (PIC), reproducibility (among 35 replicates), single-locus amplification and ease of interpretation.

SSR selection resulted in a unique set of 100 Maize SSRs specifically design for maize genotyping. This marker set has at least 2 markers per chromosome arm, 71% coverage of bins, an average PIC value of 0.72 (Table 1). In addition, the majority of these markers (83%) are di-nucletide repeat SSRs.

RESULTS AND INTERPRETATION

SSR analysis displays specific features, among which, stutter. This is an inherent artifact of the PCR amplification process, where 1 and 2 repeat units shorter than the major PCR product is produced. The height of stutter peaks ranges from 3%-70 % of the allelic peak. In addition, for a given locus, stutter peak height generally increases with allele length (more repeat units). However, stutter peak patterns are very reproducible for each specific allele of a given locus.

For instance, the amount of stutter in a single marker was evaluated by looking at 20 maize single seed samples. The -2 base pair (or di-nucleotide repeat) stutter peak was found to be 55.3 % of the allele peak with a mean of 55.3% and a standard deviation of 3.5%. When 10 identical individual maize genotypes were mixed together (bulking of 10 plants in extraction), the percentage of stutter was comparable to the individual seed samples (Mean = 55.4%, S.D. = 2.5%).

When the 2 alleles of an individual start to overlap, the resulting changes in the stutter pattern are predictable and easily interpreted.

The –4 bp stutter peak of allele 1 (A1) overlaps with the peak for allele 2 (A2), resulting in a increase in fluorescence for that allele peak. The characteristic pattern is the two allele peaks are the highest peaks. But the A2 allele peak is taller due to the added fluorescence of the stutter band.

Unlike mammalian SSR, whose size range average 40bp difference in the largest and smallest alleles, the size range of alleles in the maize genome is 100 bp or more. Over this size range, it is characteristic of maize to observe an increase in the amount of stutter, with the increase in number of repeat units.

Stutter patterns are “controlled” by adding sequence to 5’ end of the reverse primer, Sequences selected to promote +A provide greater accuracy (Brownstein, M.J., et al. 1996, BioTechniques 20:1004-1010).

Allele Sizing

Each gel lane contains a size standard, allowing for a calibration curve for every gel lane. Since all samples have the same lane size standard, very precise sizing estimates are obtained and compared among sample even if they were run on different gels. This allows integration of data sets collected over time.

Mo17 repeatability study

The public inbred line Mo17 was amplified in every plate and run on every gel. The data was obtained from repetitions over 22 - 25 gels for 8 randomly selected SSR markers. We observed less than 0.2 bp differences between runs and this size precision was reported with a mean standard deviation of 0.12 bp (Table 2).

Single base pair alleles

Alleles that differ by one base pair (bp) are common in SSR analysis. The single bp variants are usually caused by insertion/deletion of a single bp, usually between the priming site and the repeat unit. These single bp variants are inherited in a Mendelian manner.

For marker MC1523, data indicated that for the 3 most common alleles were 199, 200 and 201 bp in size. The sensitivity of the system (S.D.= 0.1bp) allows for single bp allele bins, and therefore, accurately called the single bp variants. As shown in the traces, some individuals are heterozygous for alleles 1 bp a part (Table 3).

Limits of detection

This experiment was designed to show the sensitivity of the fluorescent detection platform. DNA from 2 different maize inbred lines (B73 and B76) were mixed together in various proportions (0:1, 3:97, 1:9, 1:4, 1:2, 1:1, 33:67), prior to PCR amplification.
The B76PCR amplification product was clearly distinguished even when it represented only 3% of the total maize DNA present. This limit of detection is important when bulked samples, made up from extracting DNA from several maize plants per line, are used for SSR genotyping.

Bulking effects

Maize SSR Genotyping using bulked samples can also be is further complicated by genetic residual heterozygosity. When the analyzed SSR loci are not completely genetically fixed, several “alleles” will be observed.

To that extent, we compared maize SSR genotyping profiles obtained from inbred line Mo17 single seed versus a bulk analysis. Using SSR 1484, we tested 10 single seeds. 7 samples were homozygous for the allele 117 bp and 3 seeds were homozygous for the allele 124 bp. However, when bulked samples of 10 seeds each were used, the resulting genotype is a heterozygote containing alleles 117 and 124 bp. Furthermore, the 124-bp peak is 30% of the total PCR amplification product.

In another experiment using SSR markers MC1456 and MC1502, 9 out of 10 single seeds has an identical profile for both loci (185 and 190 bp, respectively). However, one seed out of 10 had a different allele (187 and 198 bp, respectively). When bulked samples were used the “minor” allele was not detected. This indicated that the “off” genotype is not represented in 1/10 of the bulked seeds. Therefore this genotype is likely to be present in less than 10% of the total seeds in the MO17 bag.

In a repeated study, using SSR marker loci MC1866 and MC1456, using the same 10 single seed DNA samples, we observed that the same single seed is different from the 9 others. However, for marker MC1866, in one of the bulk samples, the “off” allele was detected.

In a companion experiment, 2 single seeds of genotype MO17 were tested with SSR marker MC1065. We observed that one plant was homozygous for allele 104 bp and the other was heterozygous 84/104 bp, respectively.

When 20 single seed were tested for SSR marker MC1182, 11 individuals displayed a homozygous genotype for the 84 bp, 8 were homozygous for the 82 bp and 1 was heterozygous for the 82/84-bp alleles, respectively.

In another experiment, using the same MO17 seed source, 6 bulked samples (10 plants/bulk) were made. Based on the results observed with the MO17 single seed samples, one would expect, to see all bulks genotyped as heterozygous for the 82/84 bp alleles with an expected 1:1 peak height ratio for SSR marker MC1182. However, these 6 bulks displayed all the possible variation. The bulked samples were genotyped either as homozygous for the 82 or the 84-bp allele or heterozygous 82/84 bp alleles.

When larger size bulks of 20, 30 or 50 seeds were used and tested for SSR marker MC1182, comparable results were obtained in terms of variation. Therefore, bulking samples can lead to variation in allele calling.

Allele Calling

In order to evaluate repeatability of allele calling, the traces of 96 samples were superimposed to display the sizing precision and alleles discrimination. Furthermore, following the analysis of more than 1000 maize genotypes, the sizing precision allowed for the definition of pre-defined allele bins. Bin width was defined as 3 times the average standard deviation. The allele frequency histogram shows the allele separation in the pre-defined bins using 96 reference samples.

The allele distribution for Marker BNGL244 accumulated over 8 different maize genotyping projects and representing over 1500 maize lines is reported in Table 4. It appears that 46 different alleles have been identified for this marker. The alleles in bold are the most common alleles. Notice the very low standard deviation on the size calling values.

In addition, the majority of maize lines (87%) were homozygous at the analyzed loci, 12% were heterozygous and in 11 lines we detected 3 alleles (0.6%). These additional alleles could result either from the amplification of two loci, or from 3 alleles present in the 10 original plants used in the genotyping bulk.

In an allele distribution project involving 20 maize inbred lines and analyzed with SSR marker BNGL149, the 179 bp allele was observed in only one of the line tested. Therefore, one might think that this is an artifact. However, although observed only once in the first project, the 179-bp allele was detected again several times in subsequent projects.

Heterozygosity study in Mo17

It is know that the genotype of a single line can change over time. Comparing 2 different sources of the maize public inbred line Mo17 tested this fact. The two maize sources showed an identical marker genotype in 78 of the 91 SSR markers used. For 5 SSR markers, one was homozygous and one heterozygous with an allele in common. For 8 SSR markers, both

sources were homozygous but with different alleles. The Similarity analysis using the Jacquard's index was 0.82.

CONCLUSIONS

Celera AgGen has assembled an optimized panel of 100 Maize SSRs markers design to fully exploit the power and sensitivity of fluorescent SSR technology to accurately identify and protect proprietary germplasm.

This technique reveals single base pair resolution and very good reproducibility. However, automated fluorescent SSR analysis requires attention when dealing with stutter effects, residual heterozygosity, bulking effects and allele size calling.

Table 1: Maize 100 Panel Features

Chromosome	# Markers	Delta cM	PIC	% Relat.
1	12	14,2	0,74	96
2	9	14,4	0,76	94
3	13	9,5	0,72	94
4	13	11,4	0,7	97
5	9	14,4	0,73	98
6	10	11,5	0,75	97
7	8	14,2	0,73	93
8	8	14	0,65	95
9	11	9,4	0,66	95
10	7	17,5	0,75	94
	100	12,7	0,72	95,4

Table 2: MO17 repeatability study

	SSR Marker							
	BNGL244	MC1740	BNGL252	MC1176	MC1523	MC1191	MC1288	MC1079
N	25	24	23	25	24	23	22	22
MEAN	136,74	129,09	164,21	227,80	199,93	214,83	113,37	175,44
ST DEV	0,07	0,07	0,09	0,13	0,20	0,18	0,10	0,10
MIN	136,58	128,99	164,11	227,31	199,45	214,10	113,25	175,06
MAX	136,85	129,23	164,47	227,95	200,36	214,96	113,60	175,57

Table 3: The MC1523 single base pair variants

Allele	N	Average Size (bp)	Std. Dev. (bp)
199	348	199,01	0,10
200	228	199,91	0,08
201	70	200,96	0,08

Table 4: BNGL244 Allele Frequencies

ALLELE	N	Allele Freq.	MEAN	S. DEV	MIN	MAX
129	3	0,002	129,11	0,03	129,08	129,13
136	1	0,001	135,99			
137	59	0,032	136,76	0,06	136,65	366,87
139	1	0,001	138,71			
140	1	0,001	140,53			
143	2	0,001	142,75	0,01	142,75	142,77
144	3	0,002	144,54	0,00	144,54	144,54
147	6	0,003	146,72	0,04	146,67	146,76
151	1	0,001	151,49			
152	6	0,003	152,39	0,11	152,17	152,51
153	13	0,007	152,87	0,09	152,74	153,06
154	10	0,005	154,17	0,25	153,78	154,52
155	2	0,001	154,98	0,21	154,83	155,12
156	4	0,002	156,54	0,02	156,52	156,55
141	44	0,024	140,73	0,05	140,59	140,83
145	**897**	**0,489**	**144,76**	**0,05**	**144,62**	**144,98**
158	**255**	**0,139**	**158,44**	**0,05**	**158,27**	**158,55**
160	71	0,039	160,41	0,05	160,30	160,48
162	36	0,020	162,39	0,04	162,31	162,46
164	16	0,009	164,38	0,05	164,29	164,45
166	1	0,001	166,42			
168	1	0,001	167,79			

[Annex IV follows]

ANNEX IV

RUSSIAN POTATO CULTIVARS, IDENTIFICATION BY PROTEIN AND DNA PROFILING

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INTRODUCTION

Since 1992, the number of domestic and foreign potato cultivars registered in Russian Federation proliferated, but many of them are falsified on market of seeds. Re-organising the potato seeds production and marketing is highly demanded to improve the situation with potato production on the private lands (Anisimov B.V. 2000). In Russian Federation, potato cultivars are separated by morphological characteristics such as flower colour, growth habit, leaf type, disease reaction, sprout and tuber type. However, important limitations to this type of identification exist. Many of the morphological characteristics are subjective and often influence by the environment. Often, these traits cannot be scored simultaneously nor can cultivar identification be performed quickly and efficiently.

A method for plant cultivar identification that would be rapid, reliable, efficient has continually been searched for distinguishing potato cultivars. Bailey (1983) lists basic criteria for markers preferred for cultivar identification. These include (i) distinguishable intercultivar variation, (ii) minimal intra-cultivar variation, (iii) environmental stability, and (iv) experimental reproducibility. Potato cultivars are well suited to biochemical and molecular fingerprinting because each one is a unique genotype reproduced vegetatively and limited intra-cultivar variation may be expected.

Electrophoresis of proteins

Electrophoretic techniques to evaluate soluble protein and enzyme variation are an effective, non-destructive and rapid technique to distinguish potato cultivars (Dunches, and Ludlam 1991). However, tiny differences between sports and line selections in many cases were not observed using these biochemical markers. Despite the fact that number of scorable loci is limited, protein electrophoresis is widely used commercially for potato identification in many countries. As the number of cultivars increases, the probability of having cultivars with matching patterns could be also increased. To overcome the limitations of protein and isozyme fingerprinting, DNA markers have been applied for the same purpose.

DNA markers

Among DNA markers, Random Amplified Polymorphic DNA (RAPDs) (Williams et al., 1990) is more cost effective, technically simple, rapid and requires small amounts of DNA,

comparing to others (RFLPs, AFLPs, SSRs, etc.). Furthermore, RAPDs have demonstrated a greater intra-specific level of polymorphism than the isozyme or some DNA markers in most crop plants. Complete potato cultivar discrimination has been achieved with RAPDs (Demeke et al.,1993; Hosaka et al., 1994).

RAPD discrimination is based upon band presence and absence. RAPD profile can be considered only as unique individual characteristic of the accession obtained by chance. So as, some plants of the studied group can have the same ancestor, they will share more common bands than all others. Due to the heterozygous nature of potato cultivars RAPDs cannot be used to trace potato pedigree. Thus, it may by impossible to conclude from the nature of RAPD or other dominant fragments the phylogenic relation within the group of accessions.

Genetic markers are useful for the protection of plant breeders` rights, monitoring seed production and marketing. Limitation of fingerprint analyses will appear when a cultivar is a close relative of a previously released cultivar, or a result of genetic engineering. But, last particular case can be solved with help of gene-specific PCR markers.

Comparison of Methods.

Unfortunately, at last years the RAPD method was obviously left aside with very little interest remaining. It has been explained by low repeatability of some amplified bands over different laboratories and protocols, and lack of information about origin of the band resulted in RAPD, whether from the expressed or the non-expressed part. The same band could also result from different loci. Despite the higher repeatability and number of polymorphic bands, methods such as AFLP demand more expensive facilities and materials and still remain some disadvantages PCR-based markers.

The new molecular tools would, however, add to the normal cost of testing and would lead to an increase in testing fees. Morphological and physiological characteristics would always be necessary to be tested. Description of the traditional characteristics would be needed for the use of variety in its growing and in certification, and also in part to check the uniformity and stability. The DNA seems to remain supplementary to morphological and physiological characteristics. Cheaper cost and simplicity of RAPD technique seems to be an important advantage for supplementary tests until molecular markers will be accepted as a routine method for plant breeding and variety certification. Despite the low repeatability of some amplified bands over different laboratories and dispute on credibility of this technique, disadvantages of RAPD technique can be eliminated by standardisation of methods for DNA isolation and working protocols for PCR (Paz et al., 1997; Cisneros et al., 1995; Quiros et al., 1993).

Russian Federation has been a member of UPOV since 1998. Since the notion of essential derivation was introduced in the last UPOV convention, the assessment of the genetic relatedness between cultivars has become a critical issue. At present for potato registration and protection of a variety only relies on morphological traits for the establishment of Distinction, Uniformity and Stability (DUS).

Several open points non-clarified yet have explained the fact that molecular methods were not recommended for DUS purposes. They are the absence of harmonised protocols established

for the use of DNA-profiling and lack of standardised statistical tools required to evaluate the utility of molecular data for assessing genetic distance and dependence between cultivars.

MATERIALS AND METHODS

Methods for potato cultivars identification were as described previously (Pisarev et al., 1991; Dorokhov and Kloke, 1995), chosen based on preliminary study of protein, isozymes and RAPD profile polymorphism of Russian potato cultivars (Anisimov et al., 1993). The methods were approved as official methods of potato cultivars testing by the the protein and DNA profiling allow distinguishing even minor differences between related cultivars, originated from somatic clones of the same ancestors. They include analysis of total soluble protein polymorphism revealed with conventional electrophoresis (Pic.1) or iso-electric focussing (Pic. 2), analysis of peroxidase's polymorphism (Pic. 3), and RAPD analysis of DNA (Pic. 4, 5). The methods practical application was made for Genetically Modified plants testing (Pic. 5) and has confirmed the stability of genomic properties of potato plants during *in vitro* propagation. Over 50 Russian and foreign potato cultivars were studied by isozymes, protein and DNA marker techniques, and results showed usefulness of each method. Genetic Jaccard's distance between the potato cultivars calculated based on protein, isozymes and RAPD band similarity, gave almost the same cultivar grouping with distances varied between 0.15 and 0.84 for the most relative and distinct cultivars respectively. The opportunity to make a choice between every available method makes the cultivar identification process highly efficient. A DNA markers-based method is used, as the potato cultivars cannot be reliably distinguished using protein electrophoresis, including identification at all stages of plant development. Monitoring of somatic modifications in genome of propagated *in vitro* and genetically modified potato plants has proved a high efficiency of DNA profiling for genotype identification. It was helpful as well for recognition of interspecific hybrids between potatoes and other *Lycopersicon* (Gavrilenko T.A. et al., Thieme R et al.).

DISSCUSION

The 1991 Act of the UPOV Convention makes a clear distinction between plant grouping which are mere "varieties" and plant grouping, which constitute "prosecutable varieties". "Varieties" can exist which are not sufficiently distinct from an existing variety to be prosecutable. Russian law solely accepted such distinction and maintains two different lists: The State's Register of Protected Breeding Achievements and the State's Register of Breeding Achievements Permitted for Use.

In UPOV protocols the term "distinct" is reserved for varieties which are sufficiently different to be eligible for protection. A characteristic useful only for identification may be used thereafter to prove that certain plant material belongs to that variety. Before molecular markers can be used for distinctness purposes, it should be proven that the expression of a certain genetic sequence exists. Some experts are warning against the danger of decreasing with these tools the value of distinctness, thereby reducing the minimum distance between varieties. They think that use of molecular techniques might create more problems than it would solve. Everyone had to consider the question of variability within a variety as well as variability between varieties. DNA

data can only be interpreted if sound knowledge of the genetic background of the species concerned is available.

Apart from enabling genetic interpretation of its results, a satisfactory method for cultivar identification purposes needed to be robust, repeatable and precise. There should be a standardised naming of the alleles. There was no clear correlation between morphological expression and DNA markers found yet, although many reports on QLT markers for morphological traits were published since 1990. Obviously, any kind of markers can be in use after comprehensive study of its polymorphism, inheritance and association with other types of markers. Accumulation of knowledge and experience in dealing with molecular markers would help to overcome all current disadvantages of RAPD technique. In such case economical characteristic of the method would be very attractive for a routine use.

There should be tests for DNA markers in parallel with the traditional morphological and physiological characteristics. 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 the molecular markers uniform and stable within the characteristics used for DUS testing. Any characteristic used for distinctness must also be checked for its uniformity and stability. In the case of potato a selected characteristic is to be uniform due to vegetative propagation of plant.

New molecular characteristics, if unstable, might force breeders or maintainers of the protected varieties to additional selection work to keep the characteristics stable.

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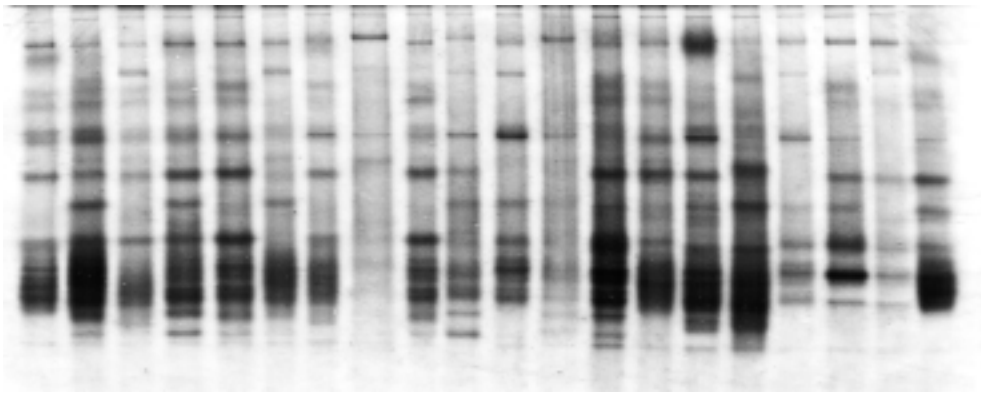
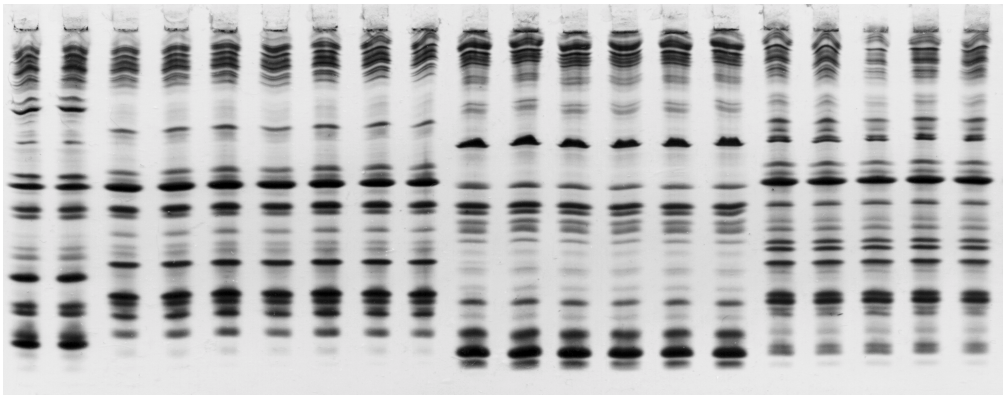
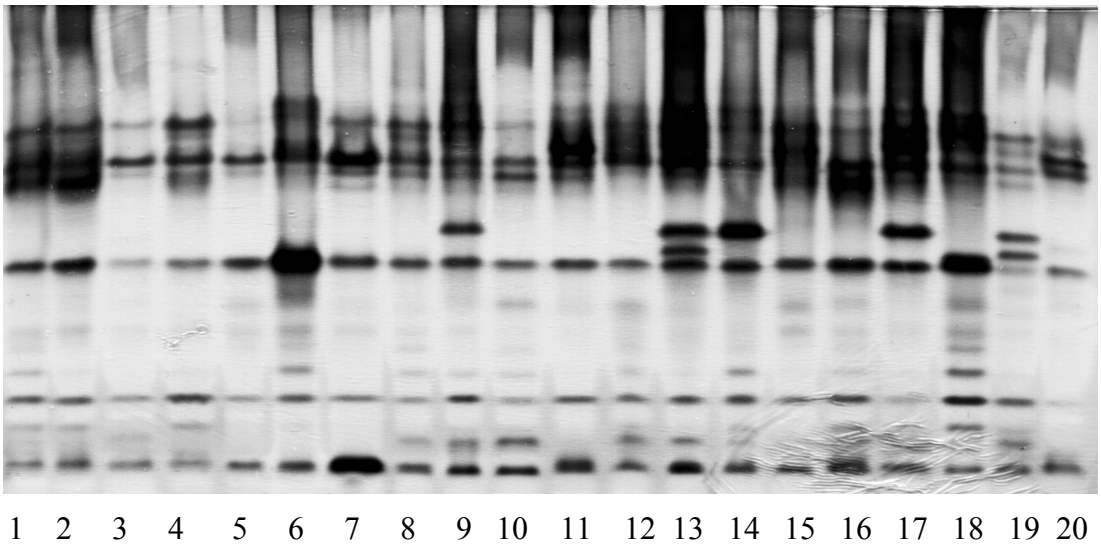


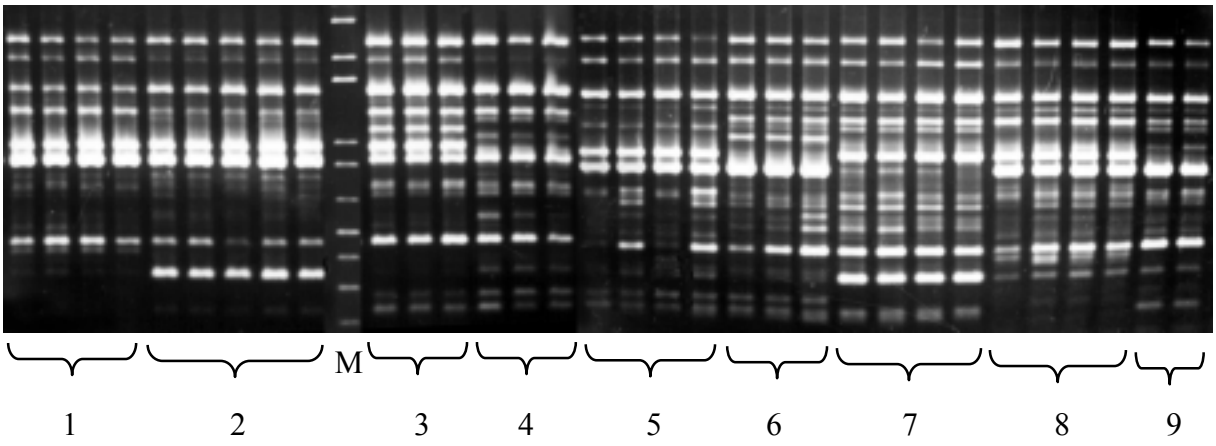
Picture 1. Disc-electrophoresis profile of potato tuber soluble proteins. Lines from left to right: 1, *Elita; 2, Belorusski; 3, Belorusski ranni; 4, Krasnopolski; 5, Volzhanin; 6, Ibier; 7, Iskra, 8, Kristall; 9, Lubitelski; 10, Lugovskoi; 11, Maika; 12, Nadezhda; 13, Nida; 14, Santa; 15, Ramenski; 16, Rezerv; 17, Talovski; 18, Energia; 19, Eva; 20, Viliya*



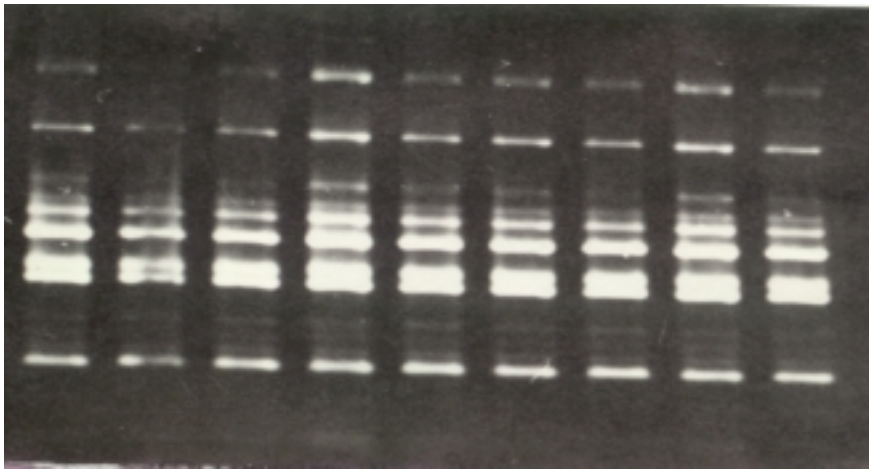
Picture 2. Isoelectric focusing of potato soluble proteins. Lines from left to right: *1,2 –Lorh; 3,…9 – Domodedovski; 10,…15 – Ogonek; 16,…20 – Adretta*



Picture 3. Peroxidase profile of potato cultivars (roots). Lines from left to right: 1, *Malakhit; 2, Nida; 3, Nevski; 4, Nematodoustoichivi; 5, Nestrovski; 6, Novo Usmanski; 7, Orbita; 8, Ogonek; 9, Oressa; 10, Posvit; 11, Maika; 12, Priekkulski ranni; 13, Post 86; 14, Polesski ranni; 15, Polesski rozovii; 16, Rezerv; 17,* Rozovi Izmelet, 18, Ramenski; 19, Stolovi 19; 20, Sedov.



Picture 4. RAPD profile of potato cultivar DNA amplified with random primer OPA-19. Lines 1 -Rozhdestvenski, 2 - Zhukovski, 3 – Nevski, 4 – Golubizna, 5 - Lugovskoi, 6 - Udacha, 7 - Elizaveta, 8 - Resurs, 9 - Pushkinets. M – 1kb Ladder Plus DNA.



c c c c c GM GM GM GM

Picture 5. The uniform RAPD profiles of control (C) and genetically modified (GM) plants of potato cultivar "Russet Burbank" obtained with one of 30 tested primers.

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