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GENEVA

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

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INTERIM REPORT OF THE *AD HOC* CROP SUBGROUPS
ON MOLECULAR TECHNIQUES

prepared by the Office of the Union

Introduction

1. At its thirty-sixth session the Technical Committee agreed to a proposal from the Working Group on Biochemical and Molecular Techniques and DNA Profiling in Particular to establish *ad hoc* crop subgroups on molecular techniques for Maize, Oilseed Rape, Rose, Tomato and Wheat. Accordingly, five *Ad hoc* Crop Subgroups were organized and the following two meetings were held in February and March 2001:

(a) *Maize and Wheat*: NIAB, Cambridge, United Kingdom, February 26 to 28, 2001
(b) *Oilseed Rape, Rose and Tomato*: GEVES, Le Magneraud, France, March 19 to 21, 2001

2. The lists of participants for the Subgroup meetings are attached to this report as Annexes I and II. The list of documents presented, or presentations made during the Subgroup meetings is also attached to this report as Annex III.

3. Each subgroup was invited to consider the potential for the use of molecular techniques on the basis of a work program developed by the Technical Committee and the "Issue Paper" (BMT/6/14) prepared by the Office of the Union in consultation with the Chairpersons of the Subgroups. In particular, each Subgroup discussed the need for the development of molecular techniques in DUS testing and considered various possible application models for molecular techniques, possible impacts of each application model and outstanding technical problems in their potential application.

4. The Subgroups were chaired by the following chairpersons nominated by the Technical Working Party concerned:

(a) Maize: Beate Rücker (Germany, TWA)
(b) Wheat: Michael Camlin (United Kingdom, TWA)
(c) Oilseed Rape: Françoise Blouet (France, TWA)
(d) Rose: Joost Barendrecht (Netherlands, TWO)
(e) Tomato: Richard Brand (France, TWV)

5. This interim report is designed to summarize the main outcome of the Subgroups for discussion in the seventh session of the BMT.

Summary

	Paragraph
1. Existing need for molecular techniques in DUS testing ♦ The current greatest need for the development of molecular techniques was reported to be in "pre-screening" in the process of examining distinctness, rather than the final decision of distinctness. ♦ "Pre-screening" is a part of the process of examining distinctness, (i.e. establishing distinctness between a candidate variety and others prior to a growing trial). Pre-screening could require a greater difference between varieties than differences required for a final decision of distinctness. The introduction of molecular characteristics for pre-screening with this greater difference could allow the introduction of a suitable safety margin for molecular characteristics. ♦ Needs other than DUS testing: Molecular techniques might have potential for use as a tool for the judgement of essential derivation and variety identification	6-14
2. Latest findings of using molecular techniques ♦ Microsatellite markers were considered as the best available technique. Single Nucleotide Polymorphism (SNP) is a new technique at an initial stage of development. ♦ Microsatellite markers showed high discriminating power as well as good repeatability by the standardization of marker sets and methodology ♦ However, molecular markers do not always discriminate all varieties which are distinct using traditional characteristics. Molecular characteristics might therefore be introduced alongside traditional characteristics. ♦ *Future work* – Cooperation in harmonizing microsatellite markers for wheat varieties: The Subgroup agreed to seek the possibility of harmonizing a microsatellite marker set and methodology within UPOV in order to help member States conduct microsatellite studies and establish database for wheat varieties with a harmonized marker set.	15-21

3. Model for the possible introduction of molecular techniques
3.1. Distinctness including "pre-screening"

Option 1: Molecular characteristics as a predictor of traditional characteristics 23-28

- **Option 1 (a): Use of molecular characteristics which are directly linked to traditional characteristics (gene specific markers)**
  - Molecular markers which are directly linked to traditional characteristics might be useful for the examination of traditional characteristics that cannot be consistently or easily observed in the field, or require additional special arrangements (e.g. disease resistance characteristics).
  - *Outstanding issues*: The key for this option is the availability of molecular markers which are directly linked to traditional characteristics. In addition, there would need to be advantages over the traditional examination of the characteristics.

- **Option 1 (b): Use of a set of molecular characteristics which can be used reliably to estimate traditional characteristics; e.g. quantitative trait loci** 29-33
  - A proposal to predict the difference in traditional characteristics by a linear function of a set of molecular characteristics was made.
  - *Outstanding issues*:
    - The usefulness of this option depends greatly on the degree of accuracy in the estimation.
    - Prediction functions differed between different locations and over years due to environment x genotype interactions. For the purpose of pre-screening by molecular characteristics, it is important that one unique prediction function is applicable over years at least for each location, and preferably in different locations
    - Applicability of the prediction for different groups of varieties should be checked using different groups of varieties which might have different genetic backgrounds

Option 2: Calibration of threshold levels for molecular characteristics against the minimum distance in traditional characteristics 34-44

- This option aims to ensure that there would be no significant shift in the typical minimum distances as measured by traditional characteristics
- However, the lack of a clear relationship between molecular marker distances and differences in traditional characteristics will lead to the need to consider how to handle potentially different decisions on distinctness.
- The framework of an impact analysis was developed: the comparison of decisions by traditional characteristics with those by molecular characteristics and the analysis of different decisions using molecular characteristics on the value of protection. The key is whether variety pairs which are not distinct using traditional characteristics are judged as distinct using molecular characteristics and whether such decisions are acceptable for maintaining the value of protection.
- *Outstanding issues*:
  - Comparison of more molecular and traditional characteristics data, especially of varieties not distinct using traditional characteristics
  - Calibration of possible threshold levels and an impact analysis
  - Minimizing unacceptable opposite decisions especially for non-distinct variety pairs in traditional characteristics, e.g. by using molecular markers associated with traditional characteristics

Option 3: Development of new system 45-51

- This approach would mean that clearly distinguishable differences in molecular characteristics would be considered as threshold levels for judging distinctness. The new system should be analyzed e.g. by a review of possible differences in decisions compared to the existing system.

- A proposal for the use of molecular characteristics in the judgement of distinctness in rose varieties was discussed. It was noted that the application for Rose seemed to have less problems than for agricultural crops. Possible reasons are the mode of propagation (vegetative propagation), less potential for an erosion of the minimum distance and better correlation between molecular marker distances and distances in traditional characteristics.
- *Outstanding issues*: Analysis of impacts of new systems on the value of protection

3.2. Uniformity 52-61

- ♦ Variability for molecular characteristics within varieties seems to be higher than that observed in traditional characteristics. The variability within varieties varied among different molecular loci and marker pairs, and among varieties.
- ♦ The use of less uniform molecular characteristics would result in the requirement of a larger threshold level for distinctness (lower discriminating power).
- ♦ *Outstanding issues*:
  - Comparison between variability observed by traditional characteristics and by molecular characteristics
  - Analysis of different types and origins of varieties
  - Establishment of models for the assessment of uniformity
  - Analysis as to whether the selection of uniformity for traditional characteristics will automatically establish uniformity for molecular characteristics and whether uniformity for molecular characteristics can be achieved at an acceptable financial/performance cost
  - Considering whether a threshold level for the assessment of uniformity should be calibrated from the level of variability observed in existing protected varieties.

3.3 Stability 62-67

- ♦ No empirical studies were reported on stability of molecular characteristics. The need to study the stability of molecular characteristics by examining different generations and origins was reaffirmed.
- ♦ It was anticipated that genetic information in coding region was likely to be more stable than in non-coding region.
- ♦ Different degrees of uniformity and stability would be observed for different types of varieties.

4. Possible application for the judgement of essential derivation 68-70

- ♦ The AFLP study of Rose varieties showed a clear-cut difference in molecular marker distances between non-mutant variety pairs and mutant variety pairs. It suggested the possibility of discriminating EDV pairs with non-EDV pairs by molecular techniques.
- ♦ Outstanding issues: It was not clear how molecular characteristics might be used in any judgement on essential derivation. It was reported that several EDV studies were under progress in an EU project

5. Possible application for variety identification 71-72

On the basis of proposals in a working document prepared by CPVO, legal or administrative issues were mainly discussed.

1. Existing need for molecular techniques in DUS testing systems

6. Firstly, each Subgroup discussed existing needs for molecular techniques in DUS testing, assisted by presentations on current DUS testing systems[1].

1.1 Management of reference collections

7. There was a broad consensus amongst participants that the greatest need for the development of molecular techniques is in the "management of reference collections". The UPOV Convention provides that varieties for which breeders' rights are granted must be clearly distinguishable from any other variety whose existence is a matter of common knowledge. The management of reference collections covers the need to manage varieties of common knowledge in the process of establishing distinctness. It includes the collection of variety descriptions and propagating material, the pre-screening of varieties (i.e., establishing distinctness prior to the growing trial) and the effective organization of the growing trial. The potential advantage of molecular characteristics is that information obtained from different DUS examiners would be directly comparable and could be used by other parties for considering distinctness against candidate varieties.

(a) *Reduction of the number of varieties to be included in a growing trial and of the number of traditional characteristics*: Traditional characteristics are influenced by the environment to varying extents. Therefore, the comparison of variety descriptions in traditional characteristics observed at different locations can be used only to a limited extent (e.g. grouping characteristics) for pre-screening. As a result, a large number of varieties need to be included in a field trial for the establishment of distinctness by direct comparison (e.g. 600 maize inbred lines in France). It was considered that the introduction of molecular characteristics might enable more effective pre-screening by increasing the number of varieties that can be clearly distinguished from a candidate variety without direct individual comparison in the field. It could reduce both the number of varieties grown in a field trial and the number of traditional characteristics which need to be examined for distinctness and therefore could lead to a reduction in the cost of DUS examination.

(b) *Expansion of the scope of varieties which are compared in the process of the examination of distinctness*: A huge number of varieties of common knowledge is considered to exist for the species discussed (e.g. over 25,000 modern rose varieties, 10,000 tomato varieties traded in the world). In practice, many member States establish their own national reference collections of varieties in the form of propagating material or/and of variety descriptions, which are useful for the assessment of DUS. However, as shown in the survey on reference collections of spring barley[2], the compositions of reference collections vary among member States and are, in many cases, limited to protected or registered varieties in the relevant country. As UPOV membership expands worldwide and the number of varieties of common knowledge increases, a review of the composition of national reference collections is appropriate. The introduction of molecular characteristics may enable more effective use of variety information obtained in different locations and allow the screening of a larger collection of varieties than currently included in the individual national reference collection.

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[1] See document BMT-TWA/Maize/1/4, BMT-TWA/Oilseed Rape/1/9, BMT-TWV/Tomato/1/3
[2] BMT-TWA/Wheat/1/5

8. Pre-screening vs. final decision of distinctness: "Pre-screening" is a part of the process of examining distinctness and aims to establish distinctness between candidate varieties and other varieties prior to a growing trial. However, it was noted that the process of pre-screening could require a greater difference between varieties than the minimum distance for distinctness used in a growing trial, since it was only the first step in determining distinctness. It was considered that the introduction of molecular characteristics for pre-screening with this greater difference ("minimum distance plus") would allow the introduction of a suitable safety margin for molecular characteristics. Experience gained over time may then allow this safety margin to be reduced.

1.2. Final decision of distinctness

9. Several DUS experts reported that they have not experienced any difficulty in establishing distinctness for new varieties by traditional characteristics. It was reported that only a few new varieties had been rejected due to the lack of distinctness, and in most cases the rejection of applications on technical criteria had resulted from lack of uniformity. Some experts warned that it is not desirable to introduce new characteristics for the purpose of establishing distinctness of varieties which have been judged as non-distinct by traditional characteristics.

10. However, some experts pointed out several potential advantages of the introduction of molecular characteristics for the purpose of establishing distinctness. For example, it was reported that expressions of some traditional characteristics observed in field trials are often different across years or in different locations due to strong environmental x genotype interactions. This inconsistency of some traditional characteristics makes it difficult not only to compare variety descriptions for the purpose of pre-screening, but also to judge final distinctness efficiently. The introduction of molecular characteristics might have the potential to improve efficiency of DUS examination in the case of some species. Furthermore, the introduction of molecular characteristics might enable the deletion of less efficient traditional characteristics, for example, those with low discriminating power and highly susceptible to environmental effects. It could also reduce the repetition needed for field trials and could result in the reduction in the total DUS examination costs and shorten the time taken for DUS testing.

11. However, it was noted that molecular characteristics should not be introduced in a way that would reduce the value of protection and that the greatest need for the use of molecular characteristics is currently for the management of reference collections rather than for the final decision of distinctness.

1.3 Judgement of essential derivation

12. The potential for use of molecular characteristics in the judgement of essential derivation was also discussed in the Subgroups. While molecular techniques were considered to have potential for use as a tool in the judgement of essential derivation, some experts doubted whether essential derivation could be judged only by genetic conformity computed by molecular markers. In particular, they doubted whether derivation from another variety could be proven solely by genetic conformity.

1.4 Variety identification

13. Variety identification by traditional characteristics requires lengthy growing trials. It was considered that molecular characteristics have the potential to provide an easier and quicker alternative method for the identification of varieties and the enforcement of breeders' rights. In particular, it was considered that benefits might exist in species with long growing cycles, such as fruit and ornamental trees. In the Subgroup for Rose, the expectation of breeders for the development of an effective method for the enforcement of breeders' rights was reported, referring to the frequent infringement of breeders' rights for rose varieties. In the Subgroup for Oilseed Rape, breeders found it might be useful to use molecular characteristics for the enforcement of their rights especially with regard to farm saved seed.

14. In the Subgroups, experts from the Community Plant Variety Office presented their working document[3] on the possible use of molecular techniques for the identification of varieties. The proposal considered the use of molecular techniques not only for the identification of varieties, but also for the official post-control of protected varieties. In response to the working document, several experts questioned the necessity for the national authority being involved in the process of variety identification (e.g. certifying a molecular marker profile as a part of an official variety description). It was considered that the involvement of the authority is not required because any relevant information, regardless of the inclusion in DUS examination and official variety description, might be used for the purpose of variety identification and the enforcement of breeders' rights.

2. Latest Findings of Using Molecular Techniques

15. State of art of molecular techniques: In the Subgroups for Maize, Oilseed Rape and Wheat, several experts presented the state of art of molecular techniques and compared different molecular techniques. The following observations were common:

(a) RFLP and RAPD have several significant shortcomings (e.g., repeatability) for practical application for DUS testing;

(b) AFLP has several advantages (e.g., low development costs, highly polymorphic). However, its repeatability is not very high and it is protected by patent.

(c) Microsatellite markers (SSRs) were considered as the best available technique because of its good repeatability, high polymorphism, and easy automation. Availability and accessibility to microsatellite markers varies among species. For example, in the case of Maize, over 1600 public SSR primer pairs were available in MaizeDB. However, many publicly developed primer pairs were not always available for other species.

(d) Single Nucleotide Polymorphism (SNP) is still at an initial stage of development. An expert from Canada reported that 75 to 100 good SNPs for wheat varieties was planned to be developed by using ESTs. It was anticipated that abundant polymorphism might exist in SNPs and that EST-derived SNPs were stable. However, the development cost is usually very high. The Subgroup noted that several SNP studies were to be undertaken.

[3] BMT-TWA/Maize/1/5

16. Detection platform: Development of detection platforms was also reported in the Subgroup. Several experts reported on the application of microarray. It was anticipated that microarrays might be adopted widely in the future because of their convenience and reliability.

17. Molecular studies reported in the Subgroups: Each of the Subgroups noted the results of several molecular marker studies (see BOX 1) which had attempted to distinguish varieties by molecular markers. Most studies, especially microsatellite studies, showed several common features.

BOX 1: Molecular marker studies presented in the Subgroups

Maize: US and UK: microsatellite markers (uniformity study) (BMT-TWA/Maize/1/5)

Oilseed Rape: United Kingdom: 15 microsatellite marker pairs, 10 varieties (BMT-TWA/Oilseed Rape/1/4)

Rose: Netherlands: 23 microsatellite marker pairs, 76 varieties (BMT-TWO/Rose/1/1)
France: 11 AFLP primer pairs, 106 varieties (BMT-TWO/Rose/1/2)
Belgium: AFLP markers (BMT-TWO/Rose/1/3)

Tomato: EU study: 20 microsatellite marker primers, 521 varieties (BMT-TWV/Tomato/1/1)

Wheat: EU study: 20 microsatellite marker pairs, 554 varieties (BMT-TWA/Wheat/1/4)
United Kingdom: microsatellite markers (BMT-TWA/Wheat/1/1)
Australia: microsatellite markers
Belgium: AFLP markers, Microsatellite markers(BMT-TWA/Wheat/1/3)

18. Discriminating power: The discriminating power of microsatellite markers is very high. Each microsatellite marker set could discriminate the majority of varieties. It was reported that only four microsatellite markers could distinguish more than 99% of 250 rose varieties.

19. However, molecular markers provide information only on a slice of the whole genome structure, and they do not necessarily discriminate all varieties which are distinct using traditional characteristics. It has been reported that a few cases of genetically similar, but distinct variety pairs could not be discriminated by molecular techniques as follows:

(a) Rose: Variability originating from mutation could not be detected by microsatellite markers;

(b) Wheat: Some sibling variety pairs could not be distinguished by microsatellite markers

An increase in the number of molecular markers and higher resolution of detection systems might improve the discriminating power of molecular characteristics. However, considering the nature of molecular markers, it is not surprising that they do not detect all the differences between different varieties and it may be more appropriate to consider very similar varieties in a growing trial. Many experts considered that molecular characteristics could be used together with traditional characteristics as the replacement of a part of traditional characteristics or as a tool for pre-screening in the process of judging distinctness by increasing the scope of varieties of common knowledge which can be considered in the process of pre-screening, reducing the number of varieties needed in the growing trial and/or deleting some less efficient traditional characteristics.

20. Repeatability and Consistency: In the Subgroup for Wheat, the EU study presented by an expert from the United Kingdom included the selection of SSRs, the standardization of analysis, the ring-test in five different laboratories and the construction of a database for 554 wheat varieties. It showed good repeatability can be achieved for selected SSRs with a certain level of standardization for the same varieties among the five laboratories, even with different detection platforms. In the Subgroup for Tomato, the EU study of SSRs for over 500 varieties was presented by an expert from the Netherlands. The result was consistent with the study for wheat varieties. The following conclusions were drawn from the studies in order to obtain repeatable results:

(a) The choice of appropriate molecular markers and the standardization of analysis (sample preparation, DNA extraction, PCR, allele calling etc) are critical;

(b) Quality control at each step of analysis (up to the scoring of band patterns) is important;

(c) The level of repeatability varied among varieties and markers. Some markers or varieties appear to have less repeatability than others (however, it would be necessary to investigate other possible source of variability, such as the samples selected for analysis); and

(d) Duplication in different laboratories or different systems was strongly recommended to double-check results.

21. Project - Cooperation in microsatellite studies (Wheat*):* Several different microsatellite marker sets had been developed separately and used for the characterization of a small group of varieties by different groups. Due to the use of different marker sets, existing profiles produced by these different sets cannot be directly compared. However, the EU study had developed a standardized microsatellite marker set for wheat varieties and produced microsatellite profiles for over 500 wheat varieties. The Subgroup for Wheat therefore agreed to seek the possibility of harmonizing a microsatellite marker set for wheat varieties in UPOV to help member States conduct microsatellite studies and establish databases for wheat varieties with a harmonized marker set and methodology. Experts from the United Kingdom[4] agreed to prepare a proposal for the harmonized marker set and methodology in cooperation with the expert from Australia on the basis of the standardized microsatellite marker set developed by the EU study. The Office of UPOV will then distribute the proposal to member States and invite member States to participate in this project.

3. Models for the Possible Introduction of Molecular Techniques in DUS Testing

3.1. Distinctness including "pre-screening" in the process of examining distinctness

22. In the Subgroups, **three possible application models** were discussed for the judgement of distinctness, including "pre-screening" in the process of examining distinctness.

[4] This project will be coordinated by Mr. Robert Cooke, NIAB, UK

- **Option 1**: Molecular Characteristics as a Predictor of Traditional Characteristics
  - **Option 1-a)**: Use of molecular characteristics which are directly linked to traditional characteristics (e.g., gene specific markers)
  - **Option 1-b)**: Use of a set of molecular characteristics which can be used reliably to estimate traditional characteristics; for example, quantitative trait loci
- **Option 2**: Calibration of Molecular Characteristics against Traditional Characteristics
- **Option 3**: Development of New System

Option 1: Molecular Characteristics as a Predictor of Traditional Characteristics

Option 1-a): Use of molecular characteristics which are directly linked to traditional characteristics (e.g. gene specific markers)

23. In this option, distinctness is always based on traditional characteristics. Molecular characteristics will be used only as a predictor of traditional characteristics. It was considered that this option presented no major concerns regarding possible erosion of the "minimum distance" and could already be used in DUS testing. For example, an expert from France reported that molecular markers had been already used for the assessment of a nematode resistance characteristic in the DUS examination of sugar beet varieties.

24. Several experts considered that, as the use of gene specific markers becomes more frequent in the process of breeding (e.g. marker assisted breeding), the introduction of this type of characteristic for DUS examination would be easily accepted by breeders.

25. Several DUS experts considered gene specific markers useful, especially in relation to certain types of traditional characteristics, e.g. disease resistance characteristics, which cannot be consistently observed in the field and require costly special arrangements for the assessment. This option might be considered particularly important for crops like tomato for which many disease resistance characteristics are examined in DUS tests. In addition, molecular techniques might be useful for assessing some important disease resistance characteristics that cannot be examined because of quarantine restriction (e.g. Tomato Yellow Leaf Curl Virus) or lack of appropriate techniques (e.g., powdery mildew and Stemphylium disease resistance).

26. Traditional characteristics controlled by various genetic mechanisms: If two varieties have different genetic mechanisms which lead to the same expression in traditional characteristics, they were not considered to be distinct in this option. The judgement of distinctness on the basis of the presence of certain genes would need to be discussed as a separate issue.

27. Outstanding issue - *Availability*: It was noted that, at present, there are only a limited number of molecular characteristics which are directly linked to traditional characteristics. Progress of ESTs, gene mapping and sequencing is expected to make more information available on the function of genes. However, this option will be limited to traditional characteristics whose expressions are controlled by one or a few genes. In addition, molecular markers need to identify differences in the genes related to differences in traditional characteristics. Moreover, even in very simple cases, more than one gene usually controls the expression of traditional characteristics. For example, there might be suppressor genes which

suppress the expression of genes identified by gene specific markers. Therefore, it was also considered that the reliability of the prediction would need to be kept under constant review.

28. Necessary work: The key of this option is the availability of molecular markers which reliably predict traditional characteristics. In addition, there would need to be advantages over the traditional examination of the characteristic. More information on useful gene specific markers and the reliability of their linkage with expression of traditional characteristics is required for its application.

Option 1-b): Use of a set of molecular characteristics which can be used reliably to estimate traditional characteristics; for example, quantitative trait loci

29. In this option, a set of molecular characteristics associated or linked with traditional characteristics would be used to estimate the expression of a traditional characteristic. Furthermore, it would also be possible to predict total differences in a set of traditional characteristics by using a set of molecular characteristics.

30. Experts from France reported on their current studies in AFLP for maize and oilseed rape with a view to applying this option for pre-screening. The summary of their approach is shown in BOX 2.

31. An expert from France reported in the Subgroup for Oilseed Rape on the study for identifying molecular markers linked to genetic information associated with some traditional characteristics in oilseed rape[5].

BOX 2: Estimation of difference in traditional characteristics by a set of molecular characteristics associated with traditional characteristics

Prediction of difference in traditional characteristics by a linear function of molecular characters

(Difference in traditional characteristics) = $\alpha_1 m_1 + \alpha_2 m_2 + \alpha_3 m_3 + \alpha_4 m_4 + \alpha_5 m_5 + \ldots\ldots + \text{(error)}$

m_1, m_2, m_3, m_4, m_5 ……: molecular characteristics
$\alpha_1, \alpha_2, \alpha_3, \alpha_4, \alpha_5$ ……………: correlation coefficients

Example: **Reported French Study - Maize**[6]

Linear prediction functions of selected QTL against precocity and plant height were estimated by morphological and molecular data of a small group of maize varieties.

Precocity = $\alpha_3 m_3 + \alpha_5 m_5 + \text{(error)}$
Height = $\alpha_2 m_2 + \alpha_6 m_6 + \alpha_7 m_7 + \text{(error)}$

Then, total predicted difference of precocity and height were computed for the pre-screening of varieties.

This approach will be further developed by adding qualitative characteristics as well as other quantitative characteristics.

[5] See document BMT-TWA/Oilseed Rape/1/5
[6] See document BMT-TWA/Oilseed Rape/1/6

32. Outstanding issues: Several outstanding issues were identified in the Subgroups for this option:

(a) *Accuracy of prediction*: The usefulness of this option depends greatly on the degree of accuracy/error in the estimation. A low level of precision of the prediction will require a big safety margin to be taken into account for pre-screening. It would result in the reduction of the number of varieties which can be pre-screened by this option. Some molecular experts reported that QTLs were capable of explaining only a limited percentage of variation in the expression of traditional characteristics. In addition, they warned that the accuracy of the prediction for varieties used in estimating the function is usually overestimated. The accuracy of the prediction must be tested by checking the prediction with varieties that have not been used in estimating the function. Improvement of the accuracy of this option would be achieved by using genetic information closely associated with traditional characteristics and by using more variety information for constructing better prediction functions.

(b) *Influence of different locations or years*: It was reported that the prediction functions and predicted results differed between different locations and over years due to environment x genotype interactions. This implies that different prediction functions are required for different growing trial locations. Accordingly, varieties pre-screened by this system could be different among different locations. However, in order for this approach to be effective, it is important to develop one unique function, at least for each location, that can be used over time for all varieties of common knowledge.

(c) *Applicability of the prediction for different groups of varieties*: Some molecular experts pointed out that the level of the association of quantitative trait loci might vary among different types of varieties (e.g., different origins) because various combinations of genetic information lead to the same expressions of a traditional characteristic. It suggests that the applicability of the prediction system should be checked for different groups of varieties which might have different genetic backgrounds.

33. Future works: The following future work mainly for Maize and Oilseed Rape were reported or proposed in the Subgroups[7]:

(a) Experts from France will seek to improve the precision of the prediction by incorporating more or better molecular information associated with other traditional characteristics, including qualitative characteristics, and by using more variety data;

(b) France has invited other member States to provide data of traditional characteristics for Oilseed rape or Maize varieties to assess environmental influence on the prediction and the usefulness of this prediction system. The Office of UPOV will prepare a circular inviting member States to cooperate with experts to assess the proposed approach. An expert from Germany expressed her interest in cooperating for Oilseed Rape;

(c) It was recommended that this should be tested with other molecular markers such as existing microsatellite data. Experts from France might be able to test this approach if molecular or phenotypic data could be made available to them. Some microsatellite marker

[7] This project will be coordinated by Mrs. Claire Baril, GEVES, France.

data (e.g. Oilseed rape microsatellite data analyzed by experts from the United Kingdom) would be tested in this framework.

Option 2: Calibration of Molecular Characteristics against Traditional Characteristics

34. The function of molecular information produced by molecular markers and the linkage of the molecular information with traditional characteristics are not known in most cases. In addition, most molecular data produced by molecular markers now seems to come from non-cording regions. Options 2 and 3 are approaches for the use of such molecular characteristics for which the linkage with traditional characteristics are not known. The difference between Options 2 and 3 is how to establish the threshold level of distinctness.

35. Option 2 is to calibrate the distance required for distinctness using molecular characteristics against the minimum distance established by traditional characteristics. This Option aims to ensure that there would be no significant shift in minimum distances as measured by traditional characteristics as a result of the introduction of molecular characteristics.

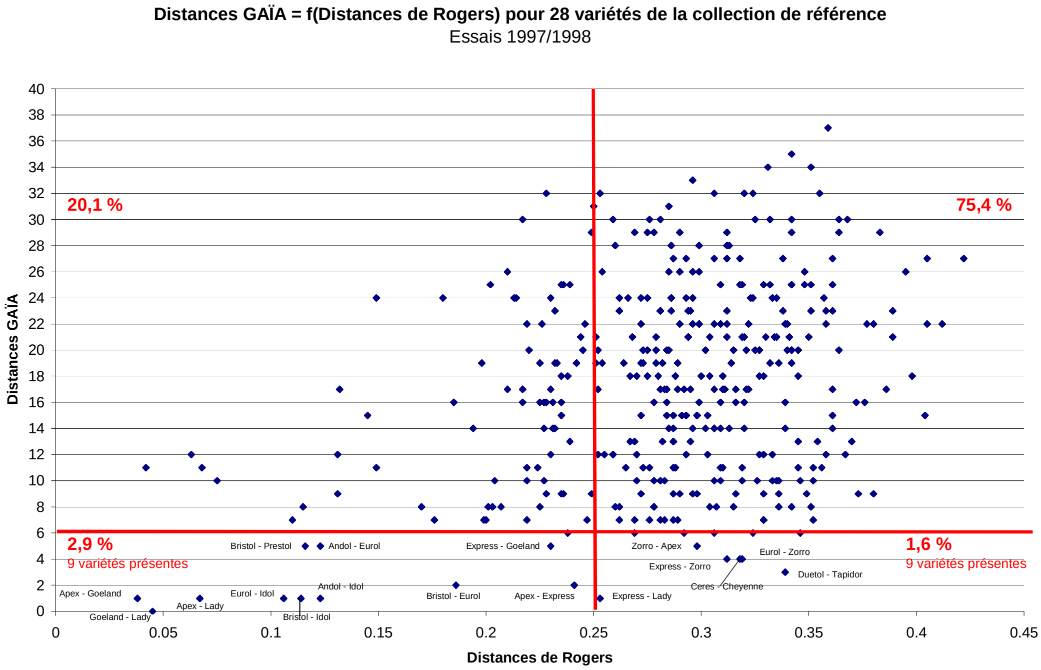
36. The framework proposed in paragraphs 15 to 17 of document BMT/6/14 was considered useful when considering the calibration of possible threshold levels for the judgement of distinctness or for pre-screening.

37. Several molecular experts considered that it is unlikely that there is a sufficient correlation between distance computed by molecular information and difference observed in traditional characteristics to develop a precise calibration.

38. It was reported that a triangular-shape distribution has often been observed in the comparison of distance computed from traditional characteristics against molecular marker distances. This distribution is problematic because large molecular distances do not always mean two varieties are very different in traditional characteristics.

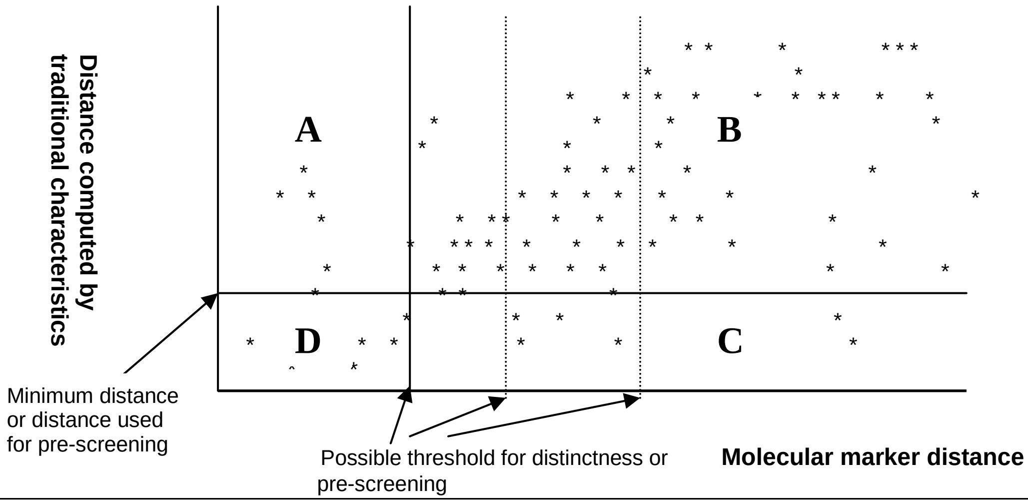
39. Analytical framework: Figure 1 compares molecular marker distances (Roger distances) with GAIA distances (weighted distances developed by GEVES) computed by traditional characteristics for 28 Oilseed Rape varieties. Furthermore, by provisionally setting a threshold level for pre-screening by molecular marker distance (0.25), possible decisions in pre-screening made by molecular marker distances are compared with current decisions made by traditional characteristics (GAIA distance = 6). This comparison clearly illustrated to what extent decisions made by molecular characteristics might be different from decisions made by traditional characteristics. It was also reported that, because of environmental and genetic interactions, the results of this analysis varied among years.

Figure 1. Pair-wise Comparison of (AFLP) molecular marker distances against GAIA distances for 28 Oilseed Rape varieties



40. This framework can be used to analyze possible changes in decisions on distinctness as well as pre-screening.

BOX 3. Framework of "impact analysis", comparisons of decisions by traditional characteristics with those by molecular characteristics



It was considered that, due to the "triangular shape distribution" problem, opposite decisions using molecular marker distances is unlikely to be completely avoidable. The issue is whether opposite decisions for judging distinctness or prescreening especially indicated in Part C (e.g., varieties not distinct using traditional characteristics are judged as distinct by molecular characteristics) are acceptable in maintaining the value of protection.

41. It was suggested that varieties in Part C should be carefully studied because the different decisions in Part C are not necessarily unacceptable. The differences in molecular

characteristics may reflect important differences which are not shown by examining traditional characteristics.

42. In addition, it was noted that there might be a possibility to achieve a better correlation between molecular characteristics and traditional characteristics (in the other words, better (linear-like) shape of distribution than the triangular shape) and to reduce the number of the different decisions.

(a) *Correlation might depend largely on species*: Genetic distances computed by AFLP markers for rose varieties showed much better correlation with distances computed by traditional characteristics. It was considered that, in the case of breeding of agricultural crops, relatively similar breeding goals were achieved in different varieties by using various genotypes. However, in the case of ornamental plant broader differences in traditional characteristics were achieved by various genotypes.

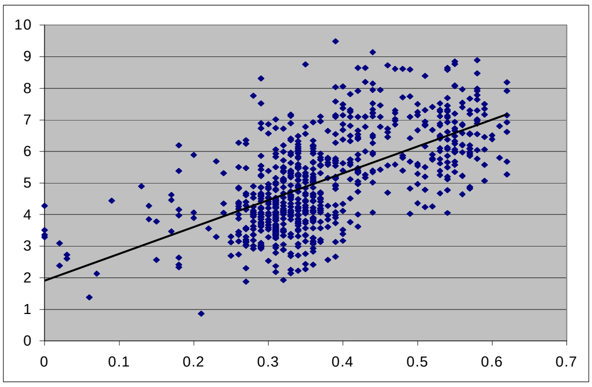


Figure 2. Relation between AFLP and traditional characteristics for 38 rose varieties. (r = 0.62)[8]

(b) *Use of molecular information associated with traditional characteristics*: It was noted that the use of molecular markers associated with traditional characteristics would improve the correlation between differences in molecular characteristics and in traditional characteristics and reduce opposite decisions[9].

43. Outstanding issues: In summary, the following outstanding issues need to be solved for Option 2:

(a) Calibration of possible threshold levels and impact analysis of these possible levels by comparison between decisions by traditional characteristics and those by molecular characteristics;

(b) If necessary (e.g., many different decisions were unacceptable), reduction of different decisions between traditional characteristics and molecular characteristics (Part C), for example, by using molecular markers associated with traditional characteristics.

44. Future work: The following proposals and reports were made during the Subgroups:

(a) *Impact analysis - analysis of more information on both distinct and non-distinct variety pairs*: As explained in paragraph 40, it is important to analyze possible impacts on the value of protection regarding the level of a possible threshold. However, at present, there are only a limited number of comparisons between molecular marker distances and distances computed by traditional characteristics. Further analysis of various types of varieties by both molecular and traditional characteristic data is necessary. In particular, as mentioned earlier,

[8] BMT-TWO/Rose/1/2

[9] An expert from Spain proposed systematic criteria for choosing molecular markers for the similar purpose (BMT-TWA/Maize/1/2). For example, the use of QTL and molecular markers targeted at/around genes linked to qualitative characteristics were proposed.

data of *varieties which are not clearly distinguishable by traditional characteristics (non-distinct variety pairs)* is required

(b) *Use of existing molecular data set (Tomato and Wheat)*: Several microsatellite studies including the EU study on wheat and tomato varieties were reported in the Subgroup. However, most of these results had not been analyzed alongside information on traditional characteristics. Therefore, the Subgroup requested molecular experts concerned to consider the possibility of enabling the molecular data to be compared with traditional characteristics data either by cooperating with DUS experts and national Offices or by making them available to the others with the names of the varieties.

Option 3: Development of New System

45. The final approach considered by the Subgroup was the development, from scratch, of a system for determining distinctness in a technically robust way (which, of course, must be in accordance with the Convention). In other words, clearly distinguishable differences in molecular characteristics are considered as possible threshold levels for judging distinctness irrespective of decisions which would be made on the basis of traditional characteristics. However, in the Subgroups, many experts expressed their fears that this option might reduce the value of protection. Therefore, having developed such a new system, this should be analyzed, e.g., by a review of possible differences in decisions compared to the existing system to consider the impact on the value of protection.

46. Proposal for the introduction of molecular characteristics to Rose DUS testing: In the Subgroup for Rose, an expert from the Netherlands presented his proposal for the possible application of microsatellite markers for establishing distinctness in line with the Option 3. This proposal was based on the following results of microsatellite marker studies in 76 rose varieties:

(a) All tested variety pairs, except for mutant variety pairs, could be discriminated. Different varieties obtained by mutation were not distinguishable by the microsatellite marker sets.

(b) No variability was observed within varieties: All plants of a variety seem to have an identical microsatellite profile.

(c) Microsatellite fingerprinting was highly reproducible.

47. The proposal is summarized in BOX 4. It was proposed that distinctness could be established by any clear difference in molecular characteristics and that the field trial would be conducted for the assessment of uniformity and stability of certain relevant traditional characteristics and also for the assessment of distinctness between a candidate variety and varieties that could not be distinguished from the candidate variety by molecular characteristics.

BOX 4. Proposal for the use of molecular characteristics in the judgement of distinctness in rose varieties[10]

(1) Examination of distinctness
(a) Use of seven polymorphic STMS markers to establish distinctness between a candidate variety and other varieties
⇒ If there are still some varieties which cannot be distinguished from the candidate variety, the second set of seven STMS markers will be used to examine distinctness between the candidate variety and the remaining varieties.
⇒ If there are still some varieties which cannot be distinguished by the second set, those varieties that could not be distinguished by molecular characteristics (these varieties will be possibly identical varieties, sports or other genetically close varieties) will be included in the field trial together with the candidate variety to examine distinctness.

(2) Examination of uniformity and stability
Uniformity and stability of the candidate variety are examined in the field trial.

48. Outstanding issue - Impact analysis: Several experts stressed that this proposal should be implemented only after it is ensured that the introduction of this approach would not have a negative impact on existing protection systems and the value of protection. The Subgroup therefore considered it necessary to conduct an "impact analysis". In particular, variety pairs which are judged as non-distinct using traditional characteristics, but which are clearly distinguishable using molecular characteristics will need to be reviewed.

49. In this connection, the Subgroup for Rose decided to ask member States to provide information on non-distinct Rose variety pairs to the expert from the Netherlands to facilitate the impact analysis of his proposal.

50. Threshold level with safety margin: Because no intra-variety variation is observed in the case of the rose microsatellite study, one band difference might be considered sufficient for clearly distinguishing varieties. One expert suggested considering the possibility of setting a higher threshold level than one band difference for establishing distinctness as a mean of introducing a safety margin. Most variety pairs are considered to be different by several alleles. In this case, even if the threshold level is set at a higher level than one band (e.g., 3 bands differences), the discriminating power of molecular characteristics will not be significantly reduced.

51. Differences between Rose and agricultural crop species: The Subgroup for Rose noted that, in spite of the above-mentioned concerns, the application of molecular techniques for Rose DUS testing may have less objections than for DUS testing for other species. The possible main reasons are as follows:

(a) *Less potential for an erosion of the minimum distance*: Firstly, it was considered that the minimum distance for establishing distinctness in rose varieties is already very small using some traditional characteristics, such as flower color. In some cases, distinctness can be established by relatively small genetic changes, e.g., somaclonal mutation. However, in the case of Rose, molecular characteristics had shown only moderate levels of discriminating powers for mutant varieties, relative to those of traditional characteristics. Accordingly, it

[10] BMT-TWO/Rose/1/1

might be considered that the introduction of molecular characteristics would not substantially reduce the minimum distance from the level established using traditional characteristics and would not substantially impact on the value of protection.

(b) *Vegetative propagation*: The fact that no or very low intra-variety variability has been observed in Rose varieties by molecular characteristics is consistent with the mode of propagation It is unlikely that intra-variety variability will be observed by molecular characteristics for those varieties that are regarded as uniform by traditional characteristics. Therefore, the possibility of selecting a variety from within a protected variety by molecular characteristics is less of a concern.

(c) *Better correlation between molecular marker distances and distances computed by traditional characteristics*: As shown in Figure 3, a relatively good correlation between distances computed by molecular characteristics and distances computed by traditional characteristics is expected for rose varieties. However, it should be checked whether a relatively good correlation is obtained with microsatellite markers for rose varieties.

3.2 Uniformity

52. Microsatellite Studies for Maize, Oilseed Rape, and Wheat: Uniformity studies of microsatellite markers[11] for Wheat, Oilseed Rape and Maize were reported in the Subgroup. All studies observed variability in microsatellite loci within varieties, which is probably higher than those observed in traditional characteristics. In all studies, different levels of intra-variety variability were observed in different varieties and different level of variability were detected in different loci. However, the observed variability levels varied among species according to the level of variability observed by traditional characteristics. For example, in the Wheat study, some microsatellite markers (six out of 23 primer pairs) showed complete uniformity in 20 individuals of 10 tested varieties. In the Oilseed Rape study, higher variability by molecular characteristics was observed within varieties.

53. Selection of molecular bands which are uniform within varieties: One concern for the introduction of molecular characteristics is that molecular characteristics might identify variability within existing protected varieties and enable other breeders to select new varieties from those existing varieties by such molecular characteristics where possibility does not exist. This concern is relevant especially for varieties, (e.g. self-pollinated varieties and vegetatively propagated varieties) which are currently considered to be uniform in an absolute sense. A possible technical solution is to choose molecular markers which produce uniform band pattern within varieties.

54. Use of molecular bands with less uniform nature: However, in the light of the reported preliminary studies of the uniformity studies, it was considered that a set of molecular markers which produce uniform band patterns within varieties might not be always available. Therefore, the Subgroup also discussed the possibility of using molecular bands which show more variability within varieties than that shown by traditional characteristics.

55. It was stressed that the concern explained in paragraph 53 was not uniquely for molecular characteristics, but for the introduction of new characteristics in general. It was therefore considered that this concern should not necessarily impede the use of such

[11] BMT-TWA/Maize/1/3, BMT-TWA/Wheat/1/1, BMT-TWA/Oilseed Rape/1/4

molecular characteristics. The use of molecular characteristics with a less uniform nature would result in the requirement of a larger threshold level for clearly distinguishing varieties. It might mean lower discriminating powers for the purpose of the assessment of distinctness as well as pre-screening. It was also reported that those molecular markers which produce higher uniformity tend to be less polymorphic. The choice of molecular markers should therefore take into account these different factors.

56. The variability observed by microsatellite markers might be explained primarily by lower selection pressure on genetic information (mainly non-coding parts) identified by microsatellite markers, compared to traditional characteristics for two possible reasons: (1) no intentional selection and (2) less linkage to breeding goals. It was also considered that a high resolution capacity of detection systems, a certain type of microsatellite markers (e.g., multiple alleles and unclear banding patterns) and the duplication of genetic information were considered to influence the level of variability observed in molecular characteristics.

57. Influence to breeding or maintenance practices: In the case of electrophoresis characteristics, breeders had quickly adapted their breeding or maintenance practices to the uniformity requirement of new characteristics. Consideration was given to whether this might prove to be the case for molecular characteristics? Therefore, it might be important to analyze possible implications of the introduction of molecular characteristics to breeding and maintenance practices. For example, the question was whether the selection for uniformity in traditional characteristics will automatically establish uniformity for molecular characteristics and to which extent breeding and maintenance practices would need to be changed for achieving uniformity requirement of molecular characteristics. In addition, if purification on the basis of molecular characteristics were required, its practicality would also need to be examined. Moreover, some experts considered that higher requirement for uniformity might suppress variety performance.

58. The assessment of uniformity: Several experts proposed that a threshold level for the assessment of uniformity should be calibrated according to the level of variability observed in existing protected varieties. Some DUS experts emphasized that the introduction of molecular characteristics should not technically create a higher requirement for uniformity than the existing one.

59. The potential for application of off-type approach for molecular characteristics: The AFLP study for Oilseed Rape[12] which had been reported in the sixth session of the BMT showed the possibility of applying the off-type approach by showing that AFLP markers can identify off-types identified using traditional characteristics. However, many experts considered that the same results might not be seen for microsatellite markers. If this is the case, alternative approaches other than off-type approaches for the assessment of uniformity might need to be considered.

60. The assessment of uniformity by molecular characteristics would not routinely identify certain off-types (e.g., mutation) in traditional characteristics. It was therefore considered that, in cases of species like Rose, the assessment of uniformity of other relevant traditional characteristics might be required even after the introduction of molecular characteristics for the assessment of DUS.

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[12] BMT-TWA/Oilseed Rape/1/8

61. Outstanding issues and future work: Three uniformity studies were reported by experts from the United Kingdom to be in progress for Maize, Wheat and Oilseed Rape. In addition, the need for the following works was discussed in the Subgroups:

(a) *Comparison between variability observed by traditional characteristics and by molecular characteristics*: The Subgroups realized the need to compare variability levels observed within varieties by molecular markers with those by traditional characteristics. In particular, the Subgroup for Maize requested the expert who presented the uniformity study for maize to check the type of varieties analyzed and to consider the expected level of uniformity in traditional characteristics.

(b) *Different types of varieties*: In the Subgroup for Oilseed Rape, a breeder emphasized the need to study different types of oilseed rape varieties which show various levels of uniformity in traditional characteristics;

(c) *Model for the assessment of uniformity*: In the Subgroups, no model approaches were proposed for the assessment of uniformity because many uniformity studies had just started. Possible models for the assessment of uniformity need to be developed.

(d) *Implication to breeding and maintenance practices*: As mentioned in Paragraph 57, it would be useful to examine implications of the uniformity requirement of molecular characteristics to breeding and maintenance practices.

(e) *Consideration of intravarietal variability for the judgement of distinctness*: Analysis of differences between varieties has often considered using bulk-samples. The presence of intravarietal variability had not been considered during the discussion on the differences between varieties. Therefore, the judgement of distinctness should be discussed on the basis of plant-by plant data, taking into consideration intravarietal variability.

2.3. Stability

62. In the Subgroups, no empirical studies were presented on stability of molecular characteristics. However, molecular experts discussed these issues on the basis of available information.

63. Mutation rate on microsatellite marker profiles: With respect to the stability of molecular characteristics, two opposite views were expressed. Based on high mutation rates of microsatellite markers reported by human genome studies, some experts considered that molecular characteristics, especially microsatellite marker profiles, might be less stable than traditional characteristics. Other experts expressed optimistic views based on their experiences of molecular studies. An expert from Germany reported an example in which the microsatellite profile of seven wheat accessions out of eight remained unchanged during multiplication of seed samples up to 24 times during 50 years.

64. Location of genetic information: Several molecular experts anticipated that the level of stability might depend on the location of genetic information. It was anticipated that genetic information in coding regions is more stable than in non-coding regions.

65. Different degrees of uniformity and stability for different types of varieties: In the Subgroup for Tomato, it was reported that different stability and uniformity levels might be

observed for different types of tomato varieties. For example, tomato varieties for canning might prove less stable and uniform than other tomato varieties because breeding/selection for the varieties for canning usually ended around F5 generation which is much earlier than in the case of other types of varieties.

66. Genetic drift: DUS experts reported that the drift of expressions of traditional characteristics had been often observed in post control examination and was acceptable to a certain extent. It suggested that certain flexibility might be allowed for the assessment of stability in molecular characteristics in the same way as in traditional characteristics.

67. Future work: The Subgroups affirmed the need to study the stability of molecular characteristics by examining seed samples of different generations and origins. This study is important not only for the assessment of DUS, but also for other purposes of application of molecular characteristics (e.g. judgement of essential derivation and variety identification).

4. Possible application for the judgement of essential derivation

68. Results of the AFLP study on the possible application for the judgement of essential derivation in Rose varieties were reported by an expert from the Netherlands. The study showed a clear-cut difference in molecular marker distances between non-mutant variety pairs and mutant variety pairs. It suggested the possibility of discriminating EDV pairs with non-EDV pairs by molecular techniques.

69. Outstanding issues: Experts raised the following general questions:

(a) It was doubted whether the clear-cut difference between non-EDV pairs and EDV pairs would still be maintained after analyzing more varieties including very similar non-EDV variety pairs;

(b) It was also questioned whether genetic conformity of two varieties can always establish that one variety is derived from another variety. The result of genetic conformity analysis by molecular characteristics might need to be used together with other evidence, such as a breeding history and traditional characteristics.

70. It was reported that several molecular studies including the Tomato study presented were being developed in an EU project for the use of molecular techniques for the judgement of essential derivation.

5. Variety Identification

71. The Subgroups had discussions mainly on legal or administrative issues and on only a few technical issues for this subject.

72. The working document presented by experts from CPVO discussed a possible threshold level for variety identification[13].

[Annex I follows]

[13] BMT-TWA/Maize/1/6

Annex I

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[Annex III follows]

Annex III

List of documents

Document number	Title	Expert presented in the Subgroup
Subgroup for Maize		
BMT-TWA/Maize/1/1	State of the Art of Molecular Techniques in Maize	David Zhang (FR)
BMT-TWA/Maize/1/2	Potential Use of Molecular Markers for Distinguishing Maize Inbred Lines and Hybrids in the UPOV Frame: A Draft Proposal	Jesus Moreno-González (ES)
BMT-TWA/Maize/1/3	Costs and Some Problems to Solve	[paper only: M. Echaide et al (AR)]
BMT-TWA/Maize/1/4	Current Test for Distinctness, Uniformity and Stability on Maize in the Federal Republic of Germany	Rudolf Becher (DE)
BMT-TWA/Maize/1/5	Observations on Maize Inbred Heterozygotes in SSR: Analysis from Two Laboratories	John Law (GB)
BMT-TWA/Maize/1/6	Possible Use of DNA Techniques for the Identification of Varieties	Anna Weitz (CPVO)
BMT-TWA/Oilseed Rape/1/6	Relationship between Phenotype and Genotype: Possible Use for Management of Reference Collection	Joël Guiard (FR)
Subgroup for Wheat		
BMT-TWA/Wheat/1/1 Rev.	Microsatellites for Wheat DUS testing	Paolo Donini (GB)
BMT-TWA/Wheat/1/2	Discovery and Implementation of Single Nucleotide Polymorphisms in Wheat Variety Identification	Daryl J. Somers (CA)
BMT-TWA/Wheat/1/3	Identification of Wheat by Molecular Markers	Jean-Marie Jacquemin (BE)
BMT-TWA/Wheat/1/4	Review of Biochemical and Molecular Methods	Robert J. Cooke (GB)
BMT-TWA/Wheat/1/5	Survey on Management of Reference Collection	Gerhard Deneken (DK)
BMT-TWA/Maize/1/6	Possible Use of DNA Techniques for the Identification of Varieties	José Elena (CPVO)
Subgroup for Oilseed Rape		
BMT-TWA/Oilseed Rape/1/1	Contribution paper	Adrian Roberts (GB)
BMT-TWA/Oilseed Rape/1/2	Uniformity issue: Oilseed Turnip Rape	Tome Christie (GB)
BMT-TWA/Oilseed Rape/1/3	Microsatellite Fingerprinting to Differentiate Brassica Varieties	[Paper only: Vincent Mulholland et al (GB)]
BMT-TWA/Oilseed Rape/1/4 Rev.	Development of Molecular Markers for DUS Testing in Oilseed Rape	Robert Cooke (GB)
BMT-TWA/Oilseed Rape/1/5	Identification of Genomic Regions Involved in DUS Traits in Oilseed Rape	Régine Delourme (FR)
BMT-TWA/Oilseed Rape/1/6	Relationship between Phenotype and Genotype: Possible Use for Management of Reference Collection	Claire Baril (FR)
BMT-TWA/Oilseed Rape/1/7	Development of Molecular Markers in Oilseed Rape	Vincent Lombard (FR)
BMT-TWA/Oilseed Rape/1/8	Assessment of Uniformity of Oilseed Rape Varieties with AFLP Markers	Vincent Lombard (FR)
BMT-TWA/Oilseed Rape/1/9	Problems in DUS tests of Winter Oilseed Rape Varieties	Beate Rücker (DE)
BMT-TWA/Oilseed Rape/1/10	Comparison between Roger Distances and GAIA Distances of Oilseed Rape Varieties	Françoise Blouet (FR)

Document number	Title	Expert presented in the Subgroup
Subgroup for Rose		
BMT-TWO/Rose/1/1	Microsatellite Markers for Identification and Registration of Rose Varieties	Ben Vosman (NL)
BMT-TWO/Rose/1/2	Evaluation of AFLPs for Variety Identification in Modern Rose	David Zhang (FR)
BMT-TWO/Rose/1/3	Genetic Diversity of a Collection of Rose Species and Cultivars Evaluated by Fluorescent AFLP	Jan De Riek (BE)
BMT-TWO/Rose/1/4	Analysis of Rose Data as Collected within MMEDV Project	Ben Vosman (NL)
BMT-TWO/Rose/1/5	Inventory of Molecular Techniques Used in Rose	Ton Kwakkenbos (CPVO)
Subgroup for Tomato		
BMT-TWV/Tomato/1/1	Construction of the STMs Database for Tomato	Ben Vosman (NL)
BMT-TWV/Tomato/1/2	Literature Review	Chrystelle Mondiere (FR)
BMT-TWV/Tomato/1/3	Brief Summary of Tomato DUS Practice: Existing Needs and Expectations for Molecular Techniques	Richard Brand (FR)
BMT-TWV/Tomato/1/4	Inventory of Molecular Techniques Used in Tomato	Sergio Semon (CPVO)
BMT-TWA/Maize/1/6	Possible Use of DNA Techniques for the Identification of Varieties	Sergio Semon (CPVO)

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