E

BMT/DUS/1 Draft 6
ORIGINAL: English
DATE: October 3, 2011

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

DRAFT

POSSIBLE USE OF MOLECULAR MARKERS IN THE EXAMINATION OF DISTINCTNESS, UNIFORMITY AND STABILITY (DUS)

Document prepared by the Office of the Union

to be considered by the Council at its forty-fifth ordinary session
to be held in Geneva on October 20, 2011

TABLE OF CONTENTS

1. INTRODUCTION

1.1 The purpose of this document is to provide guidance on the possible use of biochemical and molecular markers in the examination of Distinctness, Uniformity and Stability (DUS). The only binding obligations on members of the Union are those contained in the text of the UPOV Convention itself, and this document must not be interpreted in a way that is inconsistent with the relevant Act for the member of the Union concerned.

1.2 Possible application models for the use of biochemical and molecular markers in the examination of DUS are proposed to the *Ad hoc* Subgroup of Technical and Legal Experts of Biochemical and Molecular Techniques (BMT Review Group) (see *http://www.upov.int/en/about/structure.html*) by the Technical Committee, on the basis of the work of the Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular (BMT) and *Ad Hoc* Crop Subgroups on Molecular Techniques (Crop Subgroups) (see *http://www.upov.int/en/about/structure.html*).

1.3 The terms of reference of the BMT Review Group are as follows:

TERMS OF REFERENCE OF *AD HOC* SUBGROUP OF TECHNICAL AND LEGAL EXPERTS ON BIOCHEMICAL AND MOLECULAR TECHNIQUES ("BMT REVIEW GROUP")

1. The BMT Review Group should assess possible application models proposed by the Technical Committee, on the basis of the work of the BMT and crop subgroups, for the utilization of biochemical and molecular techniques in the examination of Distinctness, Uniformity and Stability in relation to the following:

(a) conformity with the UPOV Convention, and

(b) potential impact on the strength of protection compared to that provided by current examination methods and advise if this could undermine the effectiveness of protection offered under the UPOV system.

2. In conducting its assessment, the BMT Review Group may refer specific aspects to the Administrative and Legal Committee or the Technical Committee for clarification or further information as considered appropriate.

3. The BMT Review Group will report its assessment, as set out in paragraph 1 above, to the Administrative and Legal Committee, but this assessment will not be binding for the position of the Administrative and Legal Committee.

1.4 On the basis of the assessment of the BMT Review Group, the TC and CAJ propose guidance for inclusion in this document, which is adopted by the Council.

1.5 The following abbreviations are used in this document:

CAJ:	Administrative and Legal Committee
TC:	Technical Committee
TC-EDC:	Enlarged Editorial Committee
TWA:	Technical Working Party for Agricultural Crops
TWC:	Technical Working Party on Automation and Computer Programs
TWF:	Technical Working Party for Fruit Crops
TWO:	Technical Working Party for Ornamental Plants and Forest Trees
TWV:	Technical Working Party for Vegetables
TWP(s):	Technical Working Party(ies)
BMT:	Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular
BMT Review Group:	*Ad Hoc* Subgroup of Technical and Legal Experts on Biochemical and Molecular Techniques
Crop Subgroup:	*Ad Hoc* Crop Subgroup on Molecular Techniques

2. POSSIBLE APPLICATION MODELS

2.1 The following models were developed by the Crop Subgroups (see document BMT/7/2), BMT (see documents BMT/7/3 and BMT/7/19 "Report", paragraphs 42 to 52) and the TC (see document TC/38/14-CAJ/45/5) for consideration by the BMT Review Group at its meeting held on April 16, 2002:

Characteristic-specific molecular markers

(Title in document TC/38/14-CAJ/45/5)
Option 1: Molecular characteristics as a predictor of traditional characteristics

(a) Use of molecular characteristics which are directly linked to traditional characteristics (gene specific markers)

The Crop Subgroups noted that 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).

The BMT made a specific proposal to consider the acceptability of gene specific markers for predicting individual phenotypic characteristics. The characteristic of herbicide tolerance, introduced by genetic modification, is to be given as the example. The recommendation would need to be on the basis that there was reliable linkage between the marker and the expression of the characteristic. In considering this […] [example], the BMT Review Group would be requested to make a recommendation on the acceptability of differences arising from different markers developed for the same expression of a characteristic.

- *see Annex 1*

Calibrated molecular distances in the management of variety collections

(Title in document TC/38/14-CAJ/45/5)
Option 2: Calibration of threshold levels for molecular characteristics against the minimum distance in traditional characteristics

The Crop Subgroups developed this […] [model] with the aim to ensure that there would be no significant shift in the typical minimum distances as measured by traditional characteristics. However, they noted that the lack of a clear relationship between molecular marker distances and differences in traditional characteristics would 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 […] [markers] and the analysis of different decisions using molecular […] [markers] on the value of protection. The key is whether variety pairs, which are not distinct using traditional characteristics, would be judged as distinct using molecular […] [markers] and whether such decisions would be acceptable for maintaining the value of protection.

The BMT suggested that specific […] [examples] for this model should be presented on the basis of information from oilseed rape, maize and rose. These […] [examples] would be on the basis of a genetic distance assessment, rather than a characteristic by characteristic approach, and would be presented for use in the management of reference collections.

- *see Annex 2*

Use of molecular marker characteristics

(Title in document TC/38/14-CAJ/45/5)
Option 3: Development of a new system

The Crop Subgroups considered that this approach would mean that clearly distinguishable differences in molecular […] [markers] would be considered as threshold levels for judging distinctness. It noted that it would be necessary that the impact of the new system, compared to the existing system, should be analyzed, e.g. by a review of possible differences in decisions.

The BMT suggested that specific […] [examples] for this model should be presented on the basis of the […] [example] made in the Rose Crop Subgroup and on the basis of the information available from wheat. This […] [model] will be based on the use of molecular […] [markers] in the same way as existing non-molecular […] [markers].

- *see Annex 3*

2.2 The assessment of the BMT Review Group and the views of the TC and the CAJ on these models are presented in Section 3 of this document.

2.3 The following model was considered by the Crop Subgroups (see document BMT/7/2), BMT (see documents BMT/7/3 and BMT/7/19 “Report”, paragraphs 42 to 52) and the TC (see document TC/38/14–CAJ/45/5) but no example was put forward for consideration by the BMT Review Group at its meeting held on April 16, 2002:

> Molecular […] [markers] as a predictor of traditional characteristics: […] Use of a set of molecular […] [markers] which can be used reliably to estimate traditional characteristics; e.g. quantitative trait loci
>
> *The Crop Subgroups considered […] [an example] to predict the difference in traditional characteristics by a linear function of a set of molecular […] [markers].*
>
> *The BMT considered that […] [an example] based on this approach should not be presented at this time, but it was emphasized that work on this approach was ongoing.*

2.4 The following model, developed by experts from France, was agreed by the Crop Subgroup for Maize (see documents BMT-TWA/Maize/2/11 and BMT-TWA/Maize/2/12 "Report", paragraphs 8 to 10 and 19), BMT (see documents BMT/10/14, BMT/10/14 Add. and BMT/10/19 "Report", paragraphs 59 to 65), the Technical Working Party for Agricultural Crops (TWA) (see document TWA/37/14 "Report", paragraphs 36 to 40) and the TC (see document TC/45/15 "Report", paragraphs 51 and 52) for consideration by the BMT Review Group at its meeting held on April 1, 2009:

> Combining phenotypic and molecular distances in the management of variety collections
>
> - *see Annex 4*

2.5 The assessment of the BMT Review Group and the views of the TC and the CAJ on the models are presented in Section 3 of this document.

3. ASSESSMENT OF POSSIBLE APPLICATION MODELS

3.1 MODELS WITH A POSITIVE ASSESSMENT

Characteristic-specific molecular markers (see Annex 1)

3.1.1 The BMT Review Group met on April 16, 2002, to consider examples for the use of biochemical and molecular techniques contained in document TC/38/14–CAJ/45/5, Annex. It concluded as follows with regard to the example reproduced in Annex 1 to this document (Model: "Characteristic-specific molecular markers")[1]:

> "[…] [Example] 1 […] was, on the basis of the assumptions in the […] [example], acceptable within the terms of the UPOV Convention and would not undermine the effectiveness of protection offered under the UPOV system." (see document TC/38/14 Add.-CAJ/45/5 Add., paragraph 3)

[1] The Vice Secretary-General also made the following general remarks in relation to the BMT Review Group meeting on April 16, 2002. Firstly, concern had been raised regarding the accessibility of techniques covered by patents. Secondly, the group had emphasized the importance of considering if there were cost benefits arising from any new approaches. Thirdly, the importance of the relationship between phenotypic characteristics and molecular techniques had also been discussed. Finally, the importance of examining uniformity and stability on the same characteristics as used for distinctness had been emphasized (see document TC/38/14 Add.-CAJ/45/5 Add., paragraph 4).

3.1.2 The TC considered the conclusions of the BMT Review Group and agreed that example 1 could be pursued on the basis of the assumptions, whilst recognizing the need for further work to examine those assumptions (see document TC/38/14 Add.-CAJ/45/5 Add., paragraph 5).

3.1.3 The CAJ agreed with the conclusions of the BMT Review Group and endorsed the opinion of the TC (see document TC/38/14 Add.-CAJ/45/5 Add., paragraph 7).

3.1.4 In considering the model and example, as presented in Annex 1 of this document, the TC emphasized the importance of meeting the assumptions. In that regard, it clarified that it is a matter for the relevant authority to consider if the assumptions are met (see document TC/45/16 "Report", paragraph 152).

Combining phenotypic and molecular distances in the management of variety collections (see Annex 4)

3.1.5 At its meeting on April 1, 2009 (see document BMT-RG/Apr09/3 "Report", paragraphs 12 and 13), the BMT Review Group:

> (a) concluded that the "[…] [example] as presented in the Annex to document BMT-RG/Apr09/2 '[…] System for combining phenotypic and molecular distances in the management of variety collections', incorporating the clarifications set out in document BMT-RG/Apr09/3 'Report', paragraphs 7 and 8) [reproduced as Annex 4 to this document], where used for the management of variety collections, was acceptable within the terms of the UPOV Convention and would not undermine the effectiveness of protection offered under the UPOV system"; and
>
> (b) agreed that the example above "represented a model that might be applicable to other crops provided that the elements of the […] [example] were equally applicable. In that respect, it noted, for example, that the […] [example] above applied only to maize parental lines and did not extend to other types of maize. The BMT Review Group concluded that it was important to consider on a case by-case basis whether the model would be applicable."

3.1.6 The CAJ endorsed the recommendations of the BMT Review Group, as set out above (see document CAJ/60/11 "Report", paragraphs 53 and 54).

3.1.7 The TC noted that the CAJ had endorsed the recommendations of the BMT Review Group and endorsed the recommendations of the BMT Review Group, as set out above (see document TC/46/15 "Report on the Conclusions", paragraph 42).

Calibrated molecular distances in the management of variety collections (see Annex 2)

3.1.8 The BMT Review Group met on April 16, 2002, to consider examples for the use of biochemical and molecular techniques contained in document TC/38/14–CAJ/45/5, Annex. It concluded as follows with regard to the examples reproduced in Annex 2 to this document (Model: "Calibration of molecular distance")[1]:

> "[…] [Examples] 2, 3 and 4 ([…] Calibration of threshold levels for molecular […] [markers] against the minimum distance in traditional characteristics for Oilseed Rape, Maize and Rose, respectively), where used for the management of reference collections were, on the basis of the assumptions in the […] [examples], acceptable

> within the terms of the UPOV Convention and would not undermine the effectiveness of protection offered under the UPOV system."

3.1.9 The TC considered the conclusions of the BMT Review Group and agreed that examples 2, 3 and 4 could be pursued on the basis of the assumptions, whilst recognizing the need for further work to examine those assumptions and to improve the relationship between morphological and molecular distances.

3.1.10 The CAJ agreed with the conclusions of the BMT Review Group and endorsed the opinion of the TC (see document TC/38/14 Add.-CAJ/45/5 Add., paragraph 7).

3.1.11 In considering the model and example, as presented in Annex 2 of this document, the TC emphasized the importance of meeting the assumptions. In that regard, it clarified that it is a matter for the relevant authority to consider if the assumptions are met (see document TC/45/16 "Report", paragraph 152).

3.2 MODELS WITHOUT A POSITIVE ASSESSMENT

Use of molecular marker characteristics (see Annex 3)

3.2.1 The BMT Review Group met on April 16, 2002 to consider examples for the use of biochemical and molecular techniques contained in document TC/38/14–CAJ/45/5, Annex. It concluded as follows with regard to the examples reproduced in Annex 3 to this document (Model: "Use of molecular marker characteristics"):

> "Regarding […] [Example] 5 ([…] Rose) and […] [Example] 6 ([…] Wheat), it noted there was no consensus on the acceptability of these […] [examples] within the terms of the UPOV Convention and no consensus on whether they would undermine the effectiveness of protection offered under the UPOV system. Concerns were raised that, in these […] [examples], using this approach, it might be possible to use a limitless number of markers to find differences between varieties. The concern was also raised that differences would be found at the genetic level which were not reflected in morphological characteristics."

3.2.2 The TC considered the conclusions of the BMT Review Group and agreed with those conclusions. It noted the divergence of views which had been expressed regarding examples 5 and 6 (see document TC/38/14 Add.-CAJ/45/5 Add., paragraph 5).

3.2.3 The CAJ agreed with the conclusions of the BMT Review Group and endorsed the opinion of the TC (see document TC/38/14 Add.-CAJ/45/5 Add., paragraph 7).

[Annexes follow]

ANNEX 1

MODEL: CHARACTERISTIC-SPECIFIC MOLECULAR MARKERS

EXAMPLE 1: GENE SPECIFIC MARKER FOR HERBICIDE TOLERANCE

prepared by experts from France

Example

1. A variety is genetically modified by the insertion of a gene for tolerance to herbicide "Formula X." Varieties containing this gene are not harmed when sprayed with Formula X; however, varieties without this gene are always killed if sprayed with this particular herbicide. Tolerance of Formula X, examined in field trials by spraying of plots, is an accepted DUS characteristic, and it can be used to establish distinctness between varieties.

2. It is proposed that, rather than spraying varieties in the field (this is difficult to organize in the standard DUS trial), the characteristic "tolerance of Formula X" is examined by conducting a test for the presence of a molecular marker *linked* to the gene. This marker is located on a part of the gene "construct." The gene "construct" comprises all the elements which are inserted into the plant during the genetic modification and, in addition to the gene itself, contains additional elements for regulating the gene when in the plant. The marker may be located within the gene, partly on the gene or outside the gene itself.

Assumptions to be made in the example

3. The following assumptions are made:

(a) The DUS Examination

It is assumed that the test for the marker would be conducted to the same extent as for the field test, i.e. the same number of individual plants, over the same number of years and with the same criteria for distinctness, uniformity and stability.

(b) Reliability of the Linkage

It is assumed that the link between the marker and the gene would be checked to ensure that the marker is a reliable predictor of tolerance to Formula X. This check would be necessary to ensure, for example, that the marker does not become separated from the gene and that the presence of the gene is still resulting in tolerance to Formula X.

(c) Development of Different Molecular Markers for the Same Gene

It would be possible to develop different gene constructs containing Formula X tolerance and to identify separate molecular markers for these individual gene constructs, all of which would be linked to exactly the same gene for Formula X tolerance. If all the different markers for the same gene were accepted as different methods for examining the *same existing phenotypic characteristic*, the consideration of the approach would be the same. For the use of "Molecular […] [markers] as a predictor of traditional characteristics," it is

necessary to work on the basis that the markers correspond to a traditional, i.e. existing, approved characteristic. Therefore, it is assumed that different markers for the same gene would be treated as different methods for examining the same characteristic, i.e. tolerance to Formula X.

(d) Different Genes Producing Tolerance to the Same Herbicide

It might be possible to develop different genes which confer tolerance to Formula X. In the simplest case, this could be considered in the same way as different markers for the same gene, i.e. the different genes, with their respective markers, would be considered as different methods for examining the same characteristic, i.e. tolerance to Formula X. However, the different genes are likely to have a different chemical mechanism to produce the tolerance to Formula X. Thus, the chemicals produced from the different genes will be different and, these different chemicals might be a basis for establishing distinctness in some circumstances. Nevertheless, under this model, it would first be necessary to approve the chemical components as UPOV characteristics, before accepting molecular markers linked to these potential characteristics. This in turn would be a separate example. Therefore, it is assumed that different genes would be treated as different methods for examining the same characteristic, i.e. tolerance to Formula X.

(e) Different Gene Constructs Producing the Same Herbicide Tolerance but With Different Control of the Expression

It is also possible that different gene constructs could be developed which contain the same gene for tolerance to Formula X, but which had different regulatory control. For example, the regulatory elements may result in the Formula X tolerance only being switched on at certain stages of development. For simplicity, in considering this example, it is assumed that the different markers linked to different regulatory elements for the same gene would all be treated as different methods for examining the same characteristic of tolerance to Formula X. However, it is also assumed that further consideration would be given to this matter at a later stage.

[Annex 2 follows]

ANNEX 2

MODEL: CALIBRATED MOLECULAR DISTANCE

EXAMPLE 2: OILSEED RAPE

prepared by experts from France

Example

1. This model is based on a calibration of threshold levels for molecular markers against threshold levels in traditional characteristics, principally based on information obtained in France on Maize, Oilseed Rape and Rose. In this particular example, the threshold levels in the traditional characteristics are based on an overall distance assessment, rather than a characteristic-by-characteristic approach and the application of the example is in the "management of reference collections." In this context, the term "management of reference collections" encompasses, in particular, the selection of varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness, on the basis of comparing harmonized descriptions. A key feature of the process of eliminating varieties of common knowledge prior to the growing trial is that the threshold for deciding which varieties can be safely excluded (i.e. are distinct on the basis of descriptions), can be set with a suitable margin of safety, because those varieties which are not eliminated, but which are actually distinct, will be discovered in the growing trial. This threshold, with a safety margin, is termed the "Distinctness plus" threshold in this paper. In this example, the aim is to develop a Distinctness plus threshold for molecular markers.

Measuring distance in traditional characteristics

2. The first step is to consider how to measure the distance between varieties using traditional characteristics. This example is based on the use of an approach, using the GAÏA computer software, developed by France (see document TWA/30/15). This approach works by estimating the phenotypical difference between two varieties, based on the addition of the differences observed for the different characteristics. Each difference observed is weighted by the crop expert according to the value of the difference and to the reliability of each characteristic.

Measuring differences in molecular markers

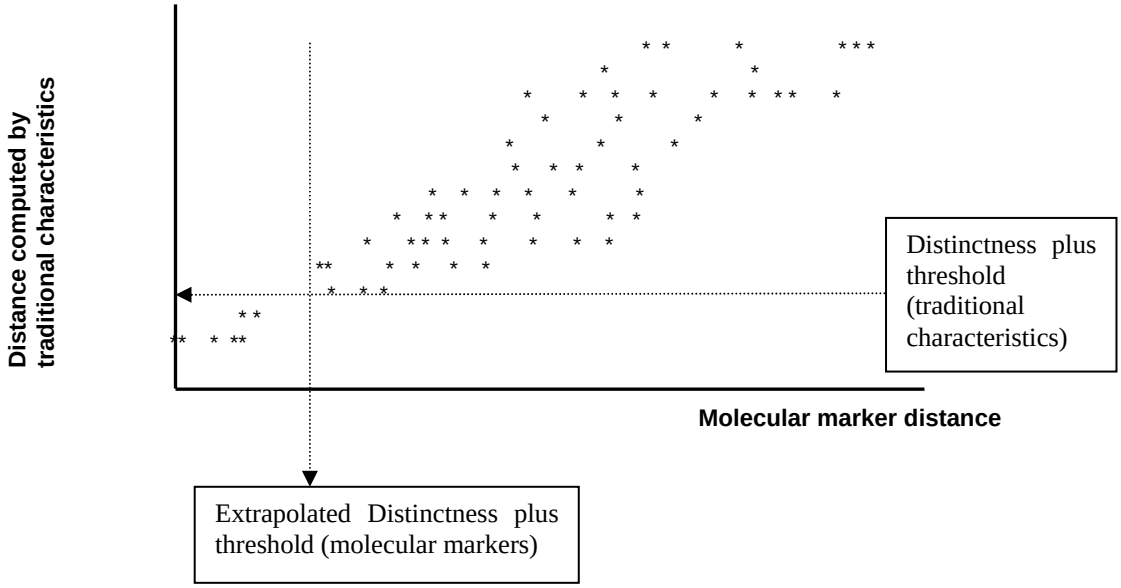
3. The difference between varieties on the basis of information from molecular markers is calculated, in this example, by the use of Rogers' distances.

Calibrating threshold levels for molecular markers against the minimum distance in traditional characteristics

4. The calibration of threshold levels for differences in molecular markers against differences in traditional characteristics would be straightforward if there was a strong correlation between these two ways of measuring the differences between varieties. In such a

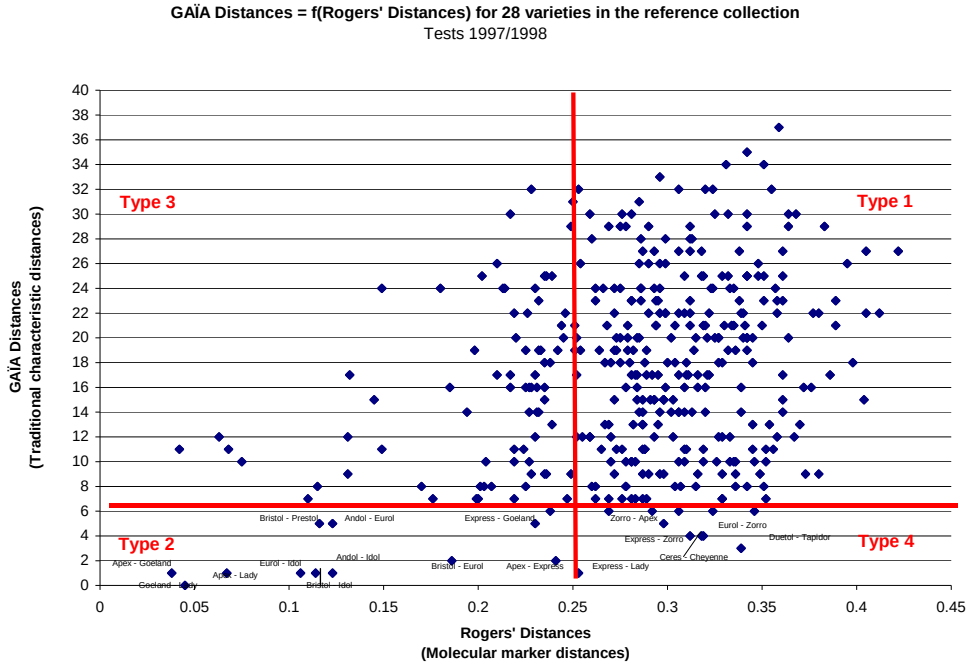
situation, a graph of the different methods would look like figure 1. The threshold for Distinctness plus in molecular markers could be extrapolated from the Distinctness plus threshold in traditional characteristics in such a way that the same decisions would be made, regardless of which method of assessing variety differences was used.

Figure 1



5. However, in the case of Oilseed Rape, the correlation is less good, as illustrated in figure 2. It can be seen that, wherever the Distinctness plus threshold is set for the molecular markers, there would be some varieties with different decisions according to the method used for calculating the differences. The implications of this situation are explored in the section "Potential Impact."

Figure 2



Assumptions to be made in the example

6. The following assumptions are made:

(a) Uniformity and Stability

The uniformity and stability requirements for the molecular markers have not been developed in this example. However, the available information suggests that variability for molecular markers within varieties seems to be higher than that observed in traditional characteristics. It is assumed that the differences calculated between varieties on the basis of molecular markers fully take into account the variation within varieties. Furthermore, it is assumed that suitable uniformity standards could be developed for molecular markers without requiring varieties, in general, to be more uniform. This assumption is on the basis that molecular markers would be used for the establishment of a "Distinctness plus" threshold, based on genetic distance, in the management of reference collections and not for the judgement of distinctness on a characteristic by characteristic approach.

(b) Application of the example

As explained in the Introduction, this example is made on the basis that it would only be used for the establishment of a "Distinctness plus" threshold in the management of reference collections.

(c) Reliability of the techniques

It is assumed that the techniques would meet all the normal requirements for any characteristic to be used in the DUS examination and, in particular, would be checked to ensure they are sufficiently consistent and repeatable.

Potential Impact

8. The graph provided in figure 2 highlights the possible ways in which this example could have an impact on the strength of protection. In summary, the situation can be represented as follows:

	Distinctness plus (Traditional characteristics)	Distinctness plus (Molecular markers)
Type 1	Yes	Yes
Type 2	No	No
Type 3	Yes	No
Type 4	No	Yes

9. Types 1 and 2 outcomes would have no impact on the strength of protection because the result is the same for both methods used.

10. Type 3 outcomes would also have no impact on the strength of protection because the varieties would be discovered to be distinct using traditional characteristics in the growing trial.

11. Type 4 outcomes could have an impact on the strength of protection because they could result in varieties being considered to be distinct which would not have previously been considered to be distinct. Determining whether type 4 outcomes could undermine the

effectiveness of protection offered under the UPOV system would require an analysis of such cases.

12. At present, type 4 cases are known in oilseed rape (examples can be provided). However, these cases only relate to pairs of varieties which were found to be distinct in a growing trial. The situation in which different decisions on distinctness would result can only be investigated where varieties are rejected for lack of distinctness in the growing trial. This would require analysis of pairs of varieties rejected for lack of distinctness in the past or, if such material is unavailable, a system of "parallel running" of the two systems in real time on candidate varieties. It would then be possible to discover if any such cases would occur and if these would undermine the effectiveness of protection. If it was considered that these cases would undermine the effectiveness of protection it could then be decided if a sufficiently high threshold could be set to eliminate these cases without losing the benefit of the approach for the management of reference collections.

13. It should be recognized that the case studies, envisaged in paragraphs 10 and 11, may not provide a complete assessment of the potential impact, since breeders would be operating under the existing system of DUS examination. Consideration should also be given, for example, to whether it would be easier under the proposed new system, if accepted, for new varieties to be selected from entirely within existing protected varieties. If this was the case, it could encourage "breeders" to try to select new varieties in this way, whereas, under the existing system there would be no incentive to do so because the varieties would not be considered distinct. This situation might be more likely to occur if the uniformity criteria for molecular markers was lower than for traditional characteristics.

EXAMPLE 3: MAIZE

prepared by experts from France

This example for Maize was on the same basis as the example for Oilseed Rape.

EXAMPLE 4: ROSE

prepared by experts from France

This example for Rose was on the same basis as the example for Oilseed Rape.

[Annex 3 follows]

ANNEX 3

MODEL: USE OF MOLECULAR MARKER CHARACTERISTICS

EXAMPLE 5: ROSE

prepared by experts from the Netherlands

Example

1. The basis of this example is that a set of molecular markers would be used in the same way as existing non-molecular characteristics.

2. A study of 76 rose varieties has shown that all these varieties, except for mutant variety pairs, could be distinguished using a limited number of molecular markers. Furthermore, when the individual plants of a number of varieties were examined they were all found to be uniform. The STMS ("sequence tagged micro-satellite") markers concerned seek certain repeat sequences in the plant DNA. At these marker sites, the plant DNA is amplified and the resultant fragments are run on a gel, which produces a set of bands or peaks corresponding to each fragment. Different banding or peak patterns resulting from the same markers indicate differences in the marker sites. It should be noted that it is unlikely that these sequences are linked with any existing Test Guidelines' characteristics and should be thought of as indicators of structural differences in the plant DNA.

3. The uniformity of the banding pattern for all the plants within a variety means that it would be possible to distinguish varieties on the basis of a single band difference. However, such a difference could result from a single mutation**,** i.e. by chance. For this reason, it is proposed that varieties would be considered to be clearly distinguishable only if there were three band/peak differences between varieties.

4. The following scheme is proposed:

Step 1: Use a fixed set of seven STMS markers (Set 1) to examine two plants of the candidate variety to see if it is clearly distinguishable from all other varieties.

If the candidate variety has at least 3 band/peak differences from all other varieties, using this first set of markers, it would be considered to be distinct. It would then be grown in a field trial to examine uniformity and stability for the relevant non molecular characteristics. In other cases, or where there are missing values, it would proceed to step 2.

Step 2: If the candidate variety is not considered distinct using the Set 1 markers, it is tested with a second, different set of seven STMS markers (Set 2).

If the candidate variety has at least 3 band/peak differences from all other varieties, using both sets of markers combined, it would be considered to be distinct. It would then be grown in a field trial to examine uniformity and stability for the relevant non-molecular characteristics. In other cases, or where there are missing values for more than one marker set, it would proceed to step 3.

Step 3: If the candidate variety was not considered to be distinct using both sets of markers, it is likely that it would be an existing variety or genetically very similar to an existing variety, e.g. resulting from a mutation. Such candidate varieties would be included in the growing trial to examine distinctness, as well as uniformity and stability, using non-molecular characteristics.

Assumptions to be made in the example

5. The following assumptions are made:

(a) The DUS Examination

It is assumed that the field examination would be conducted on the same number of plants as now. Only two plants would be necessary for the STMS marker examination because any variant plants would be seen in the subsequent field examination. This can be assumed because the chance of a mutation occurring in a marker site and not being seen in the non molecular characteristics is extremely small.

(b) Reliability of the Techniques

It is assumed that the STMS markers would meet all the normal requirements for any characteristic to be used in the DUS examination and, in particular, would be checked to ensure they are sufficiently consistent and repeatable.

(c) Uniformity

It is assumed that the situation found in the initial study, regarding the uniformity of the existing varieties, would be consistent when examined throughout the entire variety collection, or that there would be only very occasional single band differences within the varieties.

Potential Impact

7. The way in which this example could have a potential impact on the strength of protection is if varieties, which would not have been considered distinct using existing Test Guidelines' characteristics, would be considered distinct by this approach. The initial study suggests that this is unlikely, because the most similar varieties considered distinct under the existing system (i.e. mutant variety pairs) are *not* considered distinct using the two sets of STMS markers.

8. It is noted above that the risk of mutation exists and that this could produce a "distinct" variety from an existing variety, if the mutation occurred at an STMS marker site. However, this risk is reduced within the example by the requirement for differences in three bands to be able to consider a variety distinct using STMS marker sets. This would require three separate mutations to occur, all within marker sites. If the rate of mutation is assumed to be 1 in 10,000, then the chance of finding a plant with three mutations is 1 in $10,000^3$ i.e. 1 in 1,000,000,000,000 and the need for these three mutations to occur in marker sites would make the possibility of screening for such variants uneconomic.

EXAMPLE 6: WHEAT

prepared by experts from the United Kingdom

Example

1. The basis of this example is that a set of molecular markers would be used in wheat (i) to expand and organize the reference collection, and (ii) to screen candidates prior to field testing.

2. Currently there is considerable discrepancy in the constitution of reference collections in different countries, and it is considered that the existence of a database of DNA profiles of varieties, used as in this example, would improve this situation and strengthen the value of PBR.

3. Final decisions on distinctness of candidates could be made on the basis of the screening using molecular markers or, if this is not conclusive, on the basis of a reduced set of existing non-molecular characteristics recorded in field trials.

4. A study of 40 wheat varieties has shown that all of these varieties, except for one pair of sister lines, could be distinguished using 8 microsatellite (simple sequence repeat, SSR) markers. Microsatellites are highly polymorphic, tandemly repeated DNA sequences with a basic repeat unit (or core sequence) of 2-8 base pairs (e.g. GA, CTT and GATA). The polymorphism found in microsatellites is due to variations in the copy number of the basic repeat unit. In various crop species, multiple such variations ("alleles") have been shown to exist for many microsatellites in different varieties, arising from these differences in copy number. Microsatellites can be analyzed as sequence-tagged sites (STMS), which require the use of pairs of DNA primers (short sequences) that flank the microsatellite. The use of these primer pairs in a polymerase chain reaction (PCR) amplifies the microsatellite region. Different alleles of the microsatellite site ("locus") can then be separated and visualized by electrophoresis or other analytical techniques.

5. It should be noted that it is unlikely (but not impossible) that these microsatellite sequences are linked to existing UPOV characteristics. However, they can be mapped and their inheritance can be followed in crosses. The expression of the alleles, for instance as bands on a gel, is not affected by the environment or by the developmental stage of the plant.

6. The 8 SSRs are all known to map to different chromosomal locations in the wheat genome and can be reliably and repeatably examined.

7. The uniformity of the 40 varieties with respect to the 8 SSR loci has been studied. Preliminary analysis showed that the uniformity of the banding pattern for all the plants within a variety depended on the variety and the molecular marker. In 15 out of the 40 varieties, no variant banding patterns were found out of 48 plants, for any of the 8 SSRs. A further 8 varieties had only one variant in 48 plants, whilst 2 varieties had an individual plant with different alleles at 2 loci. This analysis has yet to be completed, but will ultimately provide an indication of the uniformity of existing, protected varieties at these loci, i.e. what is achieved by wheat breeders currently with no specific effort to purify varieties for these characteristics.

8. The following scheme is proposed:

Step 1: A candidate variety is received by the testing office. It is then profiled using an agreed and fixed set of 8 SSR markers.

Step 2: The initial DNA profile information is used to determine if the candidate is clearly distinguishable from the varieties of common knowledge, and/or to determine from which varieties it is not clearly distinguishable (according to the agreed basis below).

Step 3: If the candidate variety can be clearly distinguished using this set of markers, it is considered distinct. One basis for distinctness might be the occurrence of a different allele at one marker locus for which the candidate and the reference variety are sufficiently uniform. However, it is possible that a more strict requirement (e.g. different alleles at more than one locus, i.e. differences in more than one marker) could be used ("Distinctness Plus"), although this would, of course, reduce the discriminating power of the markers.

Step 4: The uniformity standard will be based on that currently found in protected varieties (see 7 above), which, in turn, will determine the number of individuals to be analyzed. If a "Distinctness Plus" approach is taken, then the Uniformity criteria will have to be similarly adjusted. Plants for which the difference was less than that used to establish distinctness would not be regarded as variants for the purposes of assessing uniformity.

Step 5: Candidates which are not sufficiently uniform for any of the 8 markers will not undergo further testing and will not be protected.

Step 6: If the candidate cannot be clearly distinguished from all varieties of common knowledge, then the varieties from which it is not distinct (according to an agreed criterion) are selected for inclusion in the field trial.

Step 7: The process is repeated for all candidates, and the field trial is then planned so that similar varieties are grown close together, i.e. comparisons can be readily made between most similar groups of candidates/reference varieties. The planning could also utilize information supplied by the breeder on the TQ.

Step 8: All candidates are sown in field trials, to check uniformity and stability of the relevant, non molecular characteristics.

Step 9: The characteristics recorded in the field trials would comprise a reduced set of those currently recorded, based e.g., on an analysis of their discriminating power, or on their lack of environmental interaction, or on their usefulness for descriptive purposes (including certification).

Step 10: If the establishment of distinctness is still difficult, additional characters could be used, in a special test. Such characteristics would have to meet the same criteria as existing characteristics.

Step 11: The variety description would consist of both the DNA profile and the recorded field trial characteristics.

Assumptions to be made in the example

9. The following assumptions are made:

(a) The DUS Examination

It is assumed that the standards for the use of the SSR markers would be agreed (see 7 above, plus 8, steps 2-4). The uniformity and stability standards for the marker data would be determined as in 7 above, based on what is achievable currently. There is no need to examine marker data in more than one year. The same standards as now would apply to the field trials, with the currently used criteria for uniformity and stability.

(b) Reliability of the Techniques

It is assumed that the SSR markers would meet all the normal requirements for any characteristic to be used in the DUS examination (see "General Introduction"), including the need to be sufficiently consistent and repeatable.

(c) The Set of Markers

The set of 8 SSR markers used for creating the database and assessing candidates would be 'fixed.' However, should improved and/or additional markers become available over time, the original marker set might be augmented, or alternatively less useful markers replaced. Any such additional markers would have to be tested in the same way as the original set of eight.

(d) Uniformity

It is assumed that the situation found in the initial study on 40 varieties, particularly regarding the uniformity of existing varieties, would be broadly indicative of all existing, protected varieties.

(e) Database of DNA Profiles

It is assumed that a suitable database can be created and maintained, incorporating the DNA profiles of varieties of common knowledge, probably also partitioned, for example, according to the origin of the variety and/or agri-climatic regions.

Potential Impact

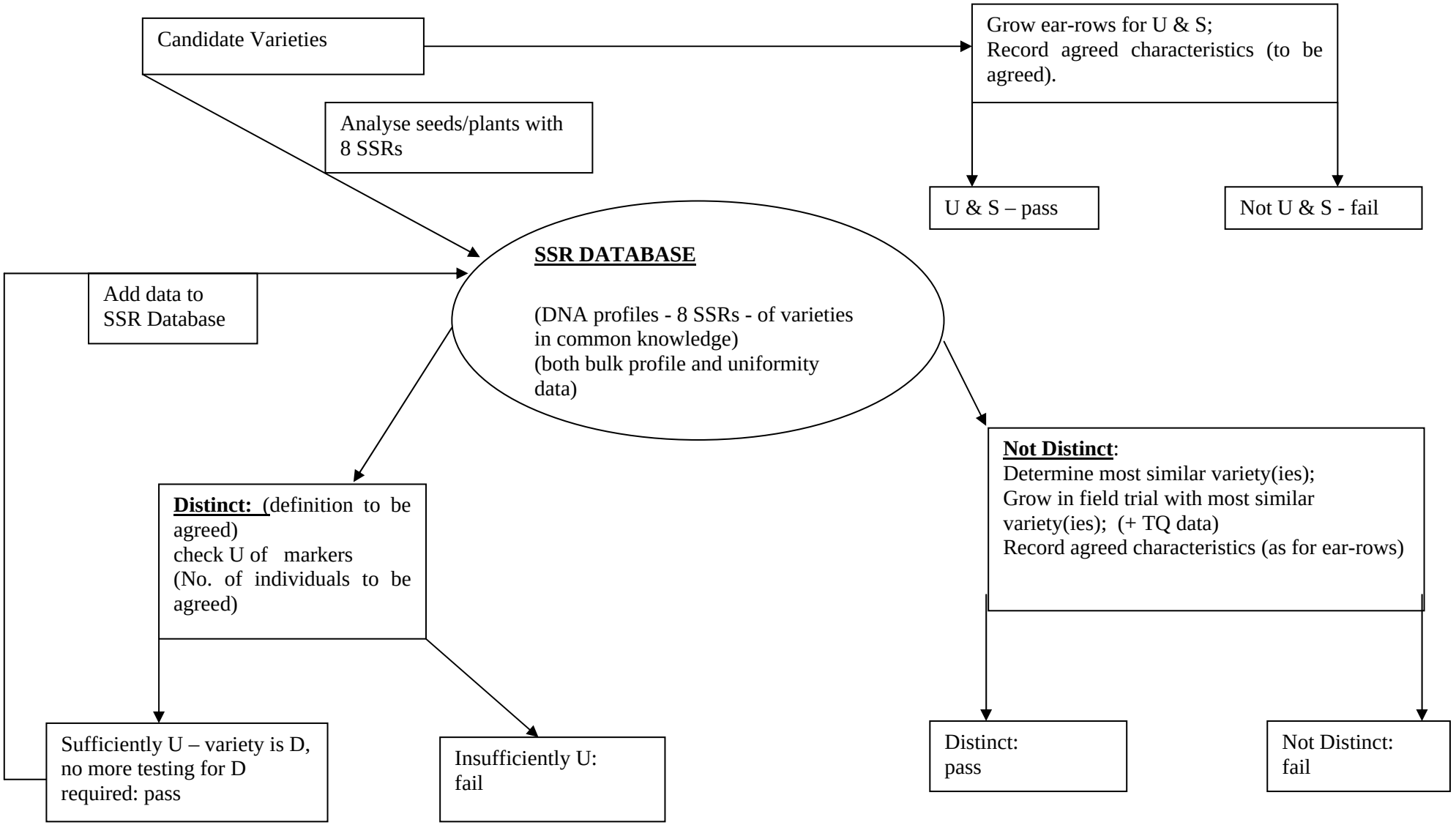
11. A significant positive impact on the strength and quality of protection would be the potential to screen a much more comprehensive reference collection. It is now well established that reference collections vary widely in their coverage of varieties of common knowledge, and that environmental interactions with many morphological characters compromise the effectiveness of published descriptions (see document TWA/30/16). This example offers an opportunity to address both of these problems.

12. It is possible that the proposed system could allow varieties to be declared D, U and S in a single year of testing.

13. One way in which this example could have a potential negative impact on the strength of protection is if varieties, which would not have been considered distinct using traditional characteristics, would be considered distinct using this approach. This could be assessed by a parallel running exercise over an agreed number of years (or, where possible, could be done retrospectively).

14. If a breeder sought to produce a new variety by changing only the molecular marker profile, this could become apparent from the description of the variety (and could then presumably trigger an investigation of possible EDV status).

15. The risk of a new variety being produced by selection from an existing variety could be minimized by requiring differences at more than one SSR locus to be able to consider a variety distinct (see 8, steps 3 and 4 above). In any case, this risk is no greater with this example than that which currently exists. This example preserves the link between the size of differences required to establish clear distinctness and uniformity standards. Therefore, it would be futile to select and purify parts of a sufficiently uniform variety because such a collection of plants would not be clearly distinct from the original variety.



[Annex 4 follows]

ANNEX 4

MODEL: COMBINING PHENOTYPIC AND MOLECULAR DISTANCES IN THE MANAGEMENT OF VARIETY COLLECTIONS

EXAMPLE: PARENT LINES IN MAIZE

prepared by experts from France

1. Description

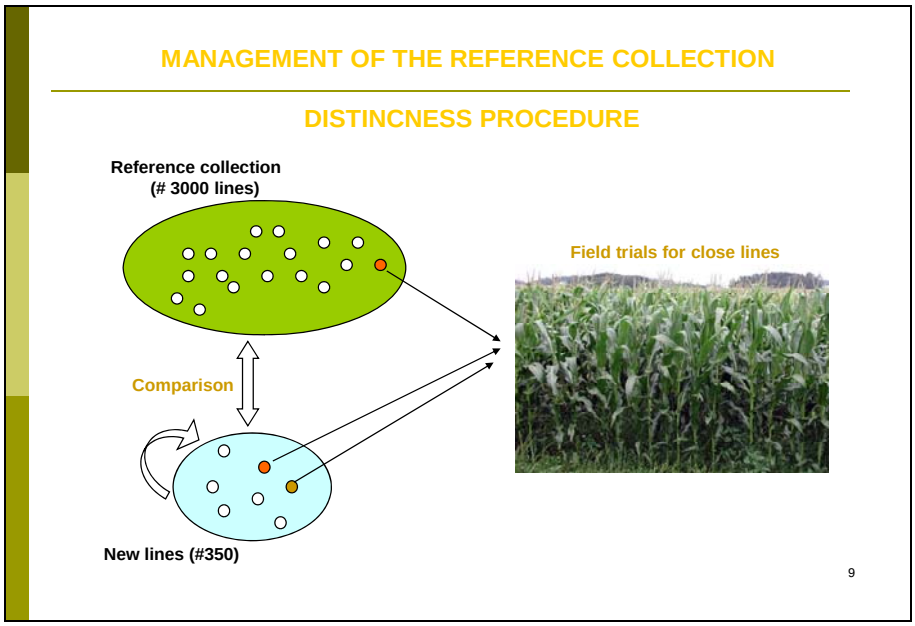
1.1 A key feature of the process of eliminating varieties of common knowledge prior to the DUS growing trial is that the threshold for deciding which varieties can be safely excluded (i.e. are distinct on the basis of descriptions), can be set with a suitable margin of safety, because those varieties which are eliminated, will not be included in the growing trial. This threshold, with a safety margin, is termed the "Distinctness plus" threshold which means that the distances between a candidate variety and "distinct plus" varieties are robust enough to take a decision without direct comparison in the growing trial.

1.2 The objective of this example is to develop an efficient tool, based on a combination of phenotypic and molecular distances, to identify within the variety collection, those varieties which need to be compared with candidate varieties (see Figure 1) in order to improve the selection of "distinct plus" varieties and so to limit the workload without decreasing the quality of the test. The challenge is to develop a secure system that:

(a) only selects varieties which are similar to the candidate varieties; and

(b) limits the risk of not selecting a variety in the variety collection which needs to be compared in the field, especially when there is a large or expensive variety collection.

Figure 1



1.3 The new system has been elaborated on the following background:

(a) Studies done on molecular distances in maize for DUS testing and essential derivation, which showed the link with the parentage between varieties (see documents BMT/3/6 “The Estimation of Molecular Genetic Distances in Maize or DUS and ED Protocols: Optimization of the Information and new Approaches of Kinship” and document BMT/3/6 Add.)

(b) An experiment done by GEVES on a set of maize parental lines, which showed that there is a link between the evaluation of distinctness by experts (global assessment) and a molecular distance computed on Simple Sequence Repeat (SSR) molecular data (see Figure 2).

1.4 Components of the system

1.4.1 GAIA distance

The GAIA distance component is computed with the GAIA software developed by GEVES. The GAIA distance is a combination of differences observed on phenotypic characteristics, where each difference contributes to the distance according to the reliability of the characteristics, especially regarding its variability and its susceptibility to environment. The larger the size of the difference and the greater the reliability of the characteristic, the more the difference contributes to the GAIA distance. Only differences that are equal or larger than the minimum distance required for each individual characteristic are included.

1.4.2 Molecular distance

The molecular distance component is computed on the differences observed on a set of markers. Different types of molecular markers and distances can be used. In the case of the study done in France on maize, 60 SSR markers and Roger’s distance have been used. It is important that sufficient markers, with a good distribution on the chromosomes, are used. The type of markers, the effect of the number of markers and the distribution of the markers need to be considered according to the species concerned.

1.4.3 Before combining these two components, an evaluation of the link between molecular distance and a global assessment of distinctness by a panel of experts needs to be done on a set of pairs of varieties. In the case of maize, that evaluation was made on the following basis:

Material : 504 pairs of varieties tested in parallel with molecular markers

Field design : pairs of varieties grown side by side
(1 plot = 2 rows of 15 plants)

Visual assessment by maize crop experts:

Scale of similarity:

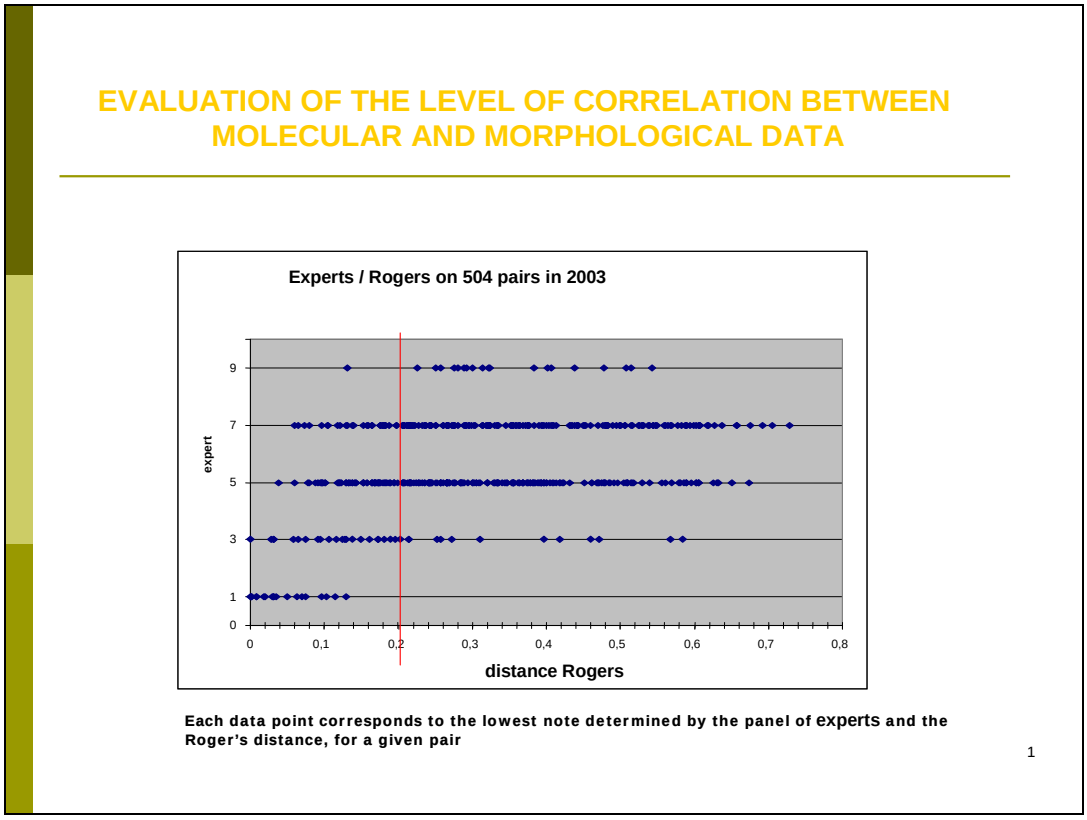
1. the two varieties are similar or very close
3. the two varieties are distinct but close
5. the comparison was useful, but the varieties are clearly distinct

7. the comparison should have been avoided because the varieties are very different
9. the comparison should have been avoided because the varieties are totally different

("even" notes are not used in the scale)

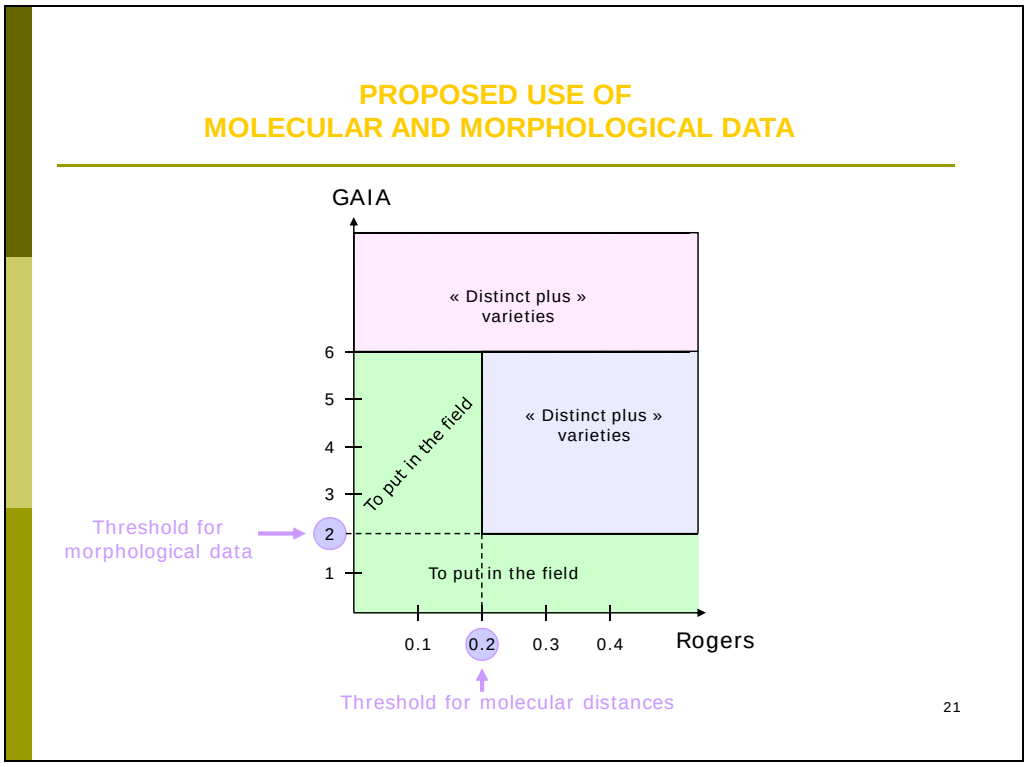
In the case of maize, this evaluation showed that no parental lines with a molecular distance greater than 0.15 were considered as similar or very close by a DUS expert evaluation (see Figure 2).

Figure 2



1.4.4 On the basis of that result, the combination of morphological and molecular distances offers the possibility to establish a decision scheme as follows (see Figure 3):

Figure 3



1.4.5 All pairs of varieties with a GAIA distance equal to, or larger than, 6 and all varieties with a GAIA distance between 2 and 6, plus a molecular distance equal to, or larger than, 0.2 are declared “Distinct plus”.

1.4.6 This scheme shows that less parental lines need to be observed in the field compared to the situation where only a GAIA distance of 6 is used on its own.

1.4.7 The robustness of this system has been studied with different GAIA and molecular distances.

2. Advantages and constraints

2.1 Advantages

(a) Improvement of the management of variety collections with less varieties needing to be compared in the field;

(b) Use of morphological and molecular distances with thresholds defined by DUS experts. GAIA was also calibrated against DUS experts’ evaluations when developed by GEVES;

(c) Use of molecular data that are not susceptible to the environment; the set of markers and the laboratory protocol are well defined;

(d) Use of only phenotypic characteristics with a good robustness and possibility to use descriptions coming from different origins under close cooperation (The maize database that has been developed in cooperation between Germany, France, Spain and the Community Plant Variety Office of the European Union (CPVO) is a good example to illustrate the value of this approach with a variety collection shared between different offices);

(e) Electrophoresis characteristics can also be replaced; and

(f) There is no influence of lack of uniformity in molecular profiles provided enough markers are used and the number of variants is low. In the case of maize parental lines, the level of molecular uniformity is high but could be a problem in some other crops.

2.2 Constraints

(a) Not efficient, or less efficient, for species with synthetic varieties or populations;
(b) Necessity to have enough good DNA markers and enough phenotypic characteristics with low susceptibility to environment; and
(c) Preliminary work with calibration in comparison with DUS expert evaluation of distinctness.

[End of Annex 4 and of document]