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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL COMMITTEE

Thirty-Eighth Session
Geneva, April 15 to 17, 2002

ADMINISTRATIVE AND LEGAL COMMITTEE

Forty-Fifth Session
Geneva, April 18, 2002

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

Document prepared by the Office of the Union

Background

1. This document explains the developments which have taken place in response to a proposal from the Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular (hereinafter referred to as "the BMT"), for a meeting of a group of technical and legal experts to consider certain important issues regarding biochemical and molecular techniques.

2. At the sixth session of the BMT, held in Angers, France, from March 1 to 3, 2000, the BMT considered that there were a number of legal or policy type problems, concerning these techniques, which should be discussed by a special working group.

3. The Technical Committee (hereinafter referred to as "the TC") considered this proposal from the BMT and decided to ask the Office of the Union (hereinafter referred to as "the Office") to take action and to contact the Chairmen of the Administrative and Legal Committee (hereinafter referred to as "the CAJ") and of the BMT concerning the possibility of creating a subgroup formed by legal and technical experts (see document TC/36/11, paragraph 123).

4. At its forty-second session, on October 23 and 24, 2000, the Chairman of the CAJ noted there was a consensus for the setting up of an *ad hoc* subgroup of technical and legal experts on biochemical and molecular techniques (hereinafter referred to as "the BMT Review Group") as suggested by the BMT. The terms of reference, drafted by the Office in document CAJ/43/3, were agreed by the CAJ at its forty-third session, on April 5, 2001, and are presented in Box I.

BOX 1

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.

5. At its seventh session, held in Hanover, Germany, from November 21 to 23, 2001, the BMT considered that it was important for the BMT Review Group to consider models for the use of biochemical and molecular techniques in DUS testing, and make recommendations on the acceptability of these models, before further consideration of the technical aspects. The BMT proposed that recommendations be sought on the basis of selected proposals developed in the *ad hoc* crop subgroups (hereinafter referred to as "Crop Subgroups") and discussed at its seventh session.

6. Aware of the importance of the issue and need for the UPOV Committees to be able to provide guidance in a timely way, for those working on these techniques, the Chairmen of the TC, CAJ and BMT considered it appropriate to convene a meeting of the BMT Review Group prior to the next sessions of the TC and CAJ.

7. The composition of the BMT Review Group proposed by the Chairmen of the CAJ, the TC and the BMT, and modified in accordance with the responses received to Circular U 3178, is as follows:

Chairman:	Mr. Rolf Jördens (Office)
Members:	Ms. Nicole Bustin (FR) Mr. Michael Camlin (GB) Mr. Doug Waterhouse (AU) Ms. Julia Borys (PL) Mr. Joël Guiard (FR) Ms. Adelaida Harries (AR) Mr. Michael Köller (DE) Mr. Mike Wray (GB)
Experts:	Ms. Françoise Blouet (FR) Mr. Robert Cooke (GB) Mr. Ben Vosman (NL)
Observers:	Community Plant Variety Office (CPVO) International Association of Plant Breeders for the Protection of Plant Varieties (ASSINSEL) International Association of Breeders of Ornamental and Fruit Plants (CIOPORA)
Office:	Mr. Peter Button Ms. Yolanda Huerta Mr. Makoto Tabata

8. The BMT Review Group, as set out above, will meet during the week of the TC and CAJ sessions in April 2002.

Models to be considered by the BMT Review Group

9. As explained above, the BMT considers that it is important for the BMT Review Group to consider models for the use of biochemical and molecular techniques in DUS testing and make recommendations on the acceptability of these models before further consideration of the technical aspects. It considers that the recommendations should be sought on the basis of models developed in the Crop Subgroups and specific proposals discussed by the BMT at its seventh session.

10. The following section summarizes the models developed by the Crop Subgroups and the specific proposals suggested by the BMT for consideration by the BMT Review Group. These suggestions have been developed into full proposals by the relevant member of the Union and are presented in the Annex.

Model for the Possible Introduction of Molecular Techniques:

Distinctness including "pre-screening"

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 proposal, 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: Proposal 1.

(b): Use of a set of molecular characteristics which can be used reliably to estimate traditional characteristics; e.g. quantitative trait loci

The Crop Subgroups considered a proposal to predict the difference in traditional characteristics by a linear function of a set of molecular characteristics.

The BMT considered that a proposal based on this approach should not be presented at this time, but it was emphasized that work on this approach was ongoing.

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

The Crop Subgroups developed this option 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 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, would be judged as distinct using molecular characteristics and whether such decisions would be acceptable for maintaining the value of protection.

The BMT suggested that specific proposals for this model should be presented on the basis of information from oilseed rape, maize and rose. These proposals 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: Proposals 2 to 4.

Option 3: Development of a new system

The Crop Subgroups considered that this approach would mean that clearly distinguishable differences in molecular characteristics 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 proposals for this model should be presented on the basis of the proposal made in the Rose Crop Subgroup and on the basis of the information available from wheat. This option will be based on the use of molecular characteristics in the same way as existing non-molecular characteristics.

See Annex: Proposals 5 and 6.

11. The BMT Review Group will consider and make recommendations on the six individual proposals presented in the Annex to this document on the basis of certain assumptions, which need to be made regarding information which is not yet available for the crops used in the illustrations. The Vice Secretary-General will make a verbal report to the TC and the CAJ, at their April 2002 sessions, on these recommendations.

12. The TC is invited to consider the recommendations made by the BMT Review Group and pass its opinion to the CAJ.

13. The CAJ is invited to consider the recommendations made by the BMT Review Group and the opinion of the TC.

14. The TC and CAJ are invited to request the Office to produce a document containing the recommendations of the BMT Review Group, together with the opinions of the TC and CAJ, for circulation to the members of the Technical Working Parties.

[Annex follows]

ANNEX

PROPOSAL 1

Prepared by experts from France

OPTION 1(a) for a Gene Specific Marker for Herbicide Tolerance Introduced by Genetic Modification

Note
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)

Proposal

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 Proposal

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. Under Option 1, "Molecular characteristics 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 Option 1, 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 proposal. 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 proposal, 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.

Conformity with the UPOV Convention

4. The BMT Review Group is invited to consider if this proposal would conflict with the UPOV Convention.

Potential Impact

5. In the basic proposal and on the basis of the assumptions made in section 2(a) to (e), it would appear that the potential impact on the strength of protection compared to that provided by the "current" examination method (i.e. the field test for tolerance to Formula X) should be nil, because the results of the DUS examination would always be the same regardless of whether the field test or test for the marker was used.

6. On the basis of the assumptions specified in paragraph 3, the BMT Review Group is invited to consider the potential impact, of this proposal, on the strength of protection compared to that provided by the current examination method envisaged within the proposal and advise if this could undermine the effectiveness of protection offered under the UPOV system.

PROPOSAL 2

Prepared by experts from France

("OPTION 2" for Oilseed Rape)

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

Proposal

1. Option 2 is based on a calibration of threshold levels for molecular characteristics against threshold levels in traditional characteristics, principally based on information obtained in France on Maize, Oilseed Rape and Rose. In this particular proposal, 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 proposal 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 proposal, the aim is to develop a Distinctness plus threshold for molecular characteristics.

Measuring distance in traditional characteristics

2. The first step is to consider how to measure the distance between varieties using traditional characteristics. This proposal 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 characteristics

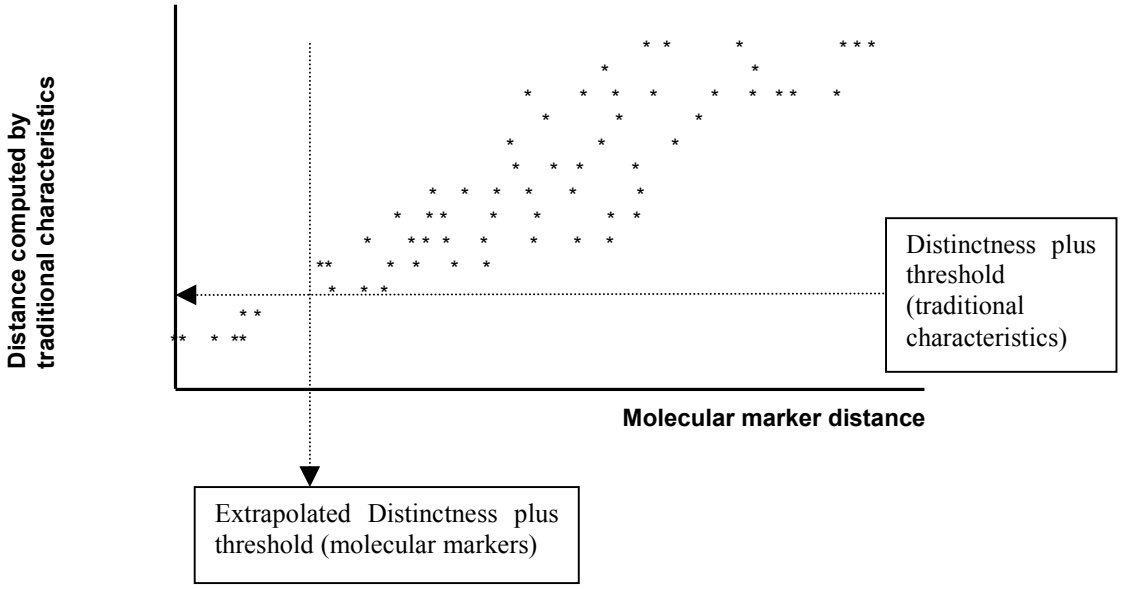
3. The difference between varieties on the basis of information from molecular markers is calculated, in this option, by the use of Rogers' distances.

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

4. The calibration of threshold levels for differences in molecular characteristics 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

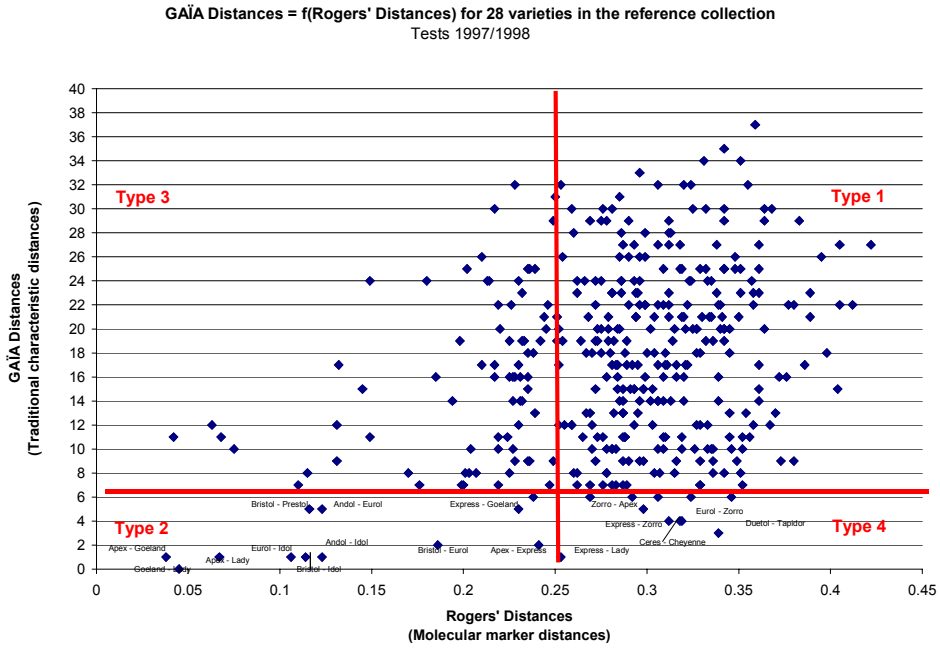
threshold in traditional characteristics in such a way that the same decisions would be made, regardless of which method of assessing variety differences was used.

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 Proposal

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 proposal. However, the available information suggests that variability for molecular characteristics 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 Proposal

As explained in the Introduction, this proposal 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.

Conformity with the UPOV Convention

7. The BMT Review Group is invited to consider if this proposal would conflict with the UPOV Convention.

Potential Impact

8. The graph provided in figure 2 highlights the possible ways in which this proposal 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 distinctness in the growing trial. This would require analysis of pairs of varieties rejected for 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.

14. On the basis of the assumptions specified in paragraph 6, the BMT Review Group is invited to consider the potential impact, of this proposal, on the strength of protection compared to that provided by the current examination methods and advise if this could undermine the effectiveness of protection offered under the UPOV system.

PROPOSAL 3

Prepared by experts from France

("OPTION 2" for Maize)

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

This proposal for Maize will be on the same basis as Proposal 2 (Option 2 for Oilseed Rape). The experts from France will present information obtained on Maize at the meeting.

PROPOSAL 4

Prepared by experts from France

("OPTION 2" for Rose)

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

This proposal for Rose will be on the same basis as Proposal 2 (Option 2 for Oilseed Rape). The experts from France will present information obtained on Rose at the meeting.

PROPOSAL 5

Prepared by experts from the Netherlands

("OPTION 3" for Rose)

Option 3: Development of a New System

Proposal

1. The basis of this proposal is that a set of molecular characteristics 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 Proposal

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.

Conformity with the UPOV Convention

6. The BMT Review Group is invited to consider if this proposal would conflict with the UPOV Convention.

Potential Impact

7. The way in which this proposal 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 proposal 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.

9. On the basis of the assumptions specified in paragraph 5, the BMT Review Group is invited to consider the potential impact, of this proposal, on the strength of protection compared to that provided by the current examination methods and advise if this could undermine the effectiveness of protection offered under the UPOV system.

PROPOSAL 6

Prepared by experts from the United Kingdom

("OPTION 3" for Wheat)

Option 3: Development of a New System

Proposal

1. The basis of this proposal 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 proposal, 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 Proposal

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.

Conformity with the UPOV Convention

10. The BMT Review Group is invited to consider if this proposal would conflict with the UPOV Convention.

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 proposal offers an opportunity to address both of these problems.

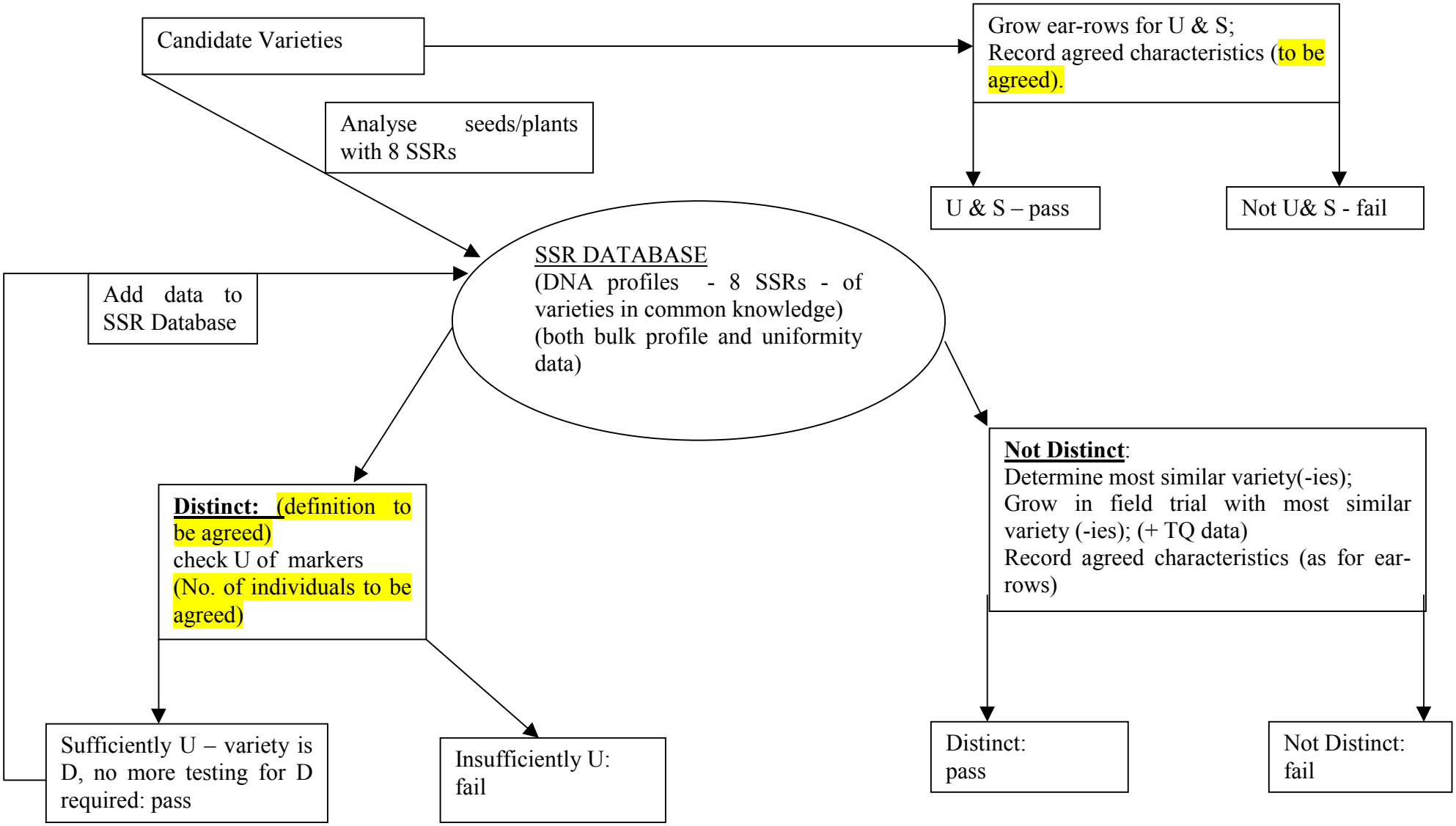
12. It is possible that the proposed system could allow varieties to be declared D, U and S in a single year of testing.

13. One way in which this proposal 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 proposal than that which currently exists. This proposal 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.

16. On the basis of the assumptions specified in paragraph 9, the BMT Review Group is invited to consider the potential impact, of this proposal, on the strength of protection compared to that provided by the current examination methods and advise if this could undermine the effectiveness of protection offered under the UPOV system.



[End of Annex and of document]