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Associated Document to the
General Introduction to the Examination of Distinctness, Uniformity and Stability
and the Development of Harmonized Descriptions of New Varieties of Plants (document TG/1/3)

DOCUMENT TGP/15

GUIDANCE ON THE USE OF BIOCHEMICAL AND MOLECULAR MARKERS IN THE EXAMINATION OF DISTINCTNESS, UNIFORMITY AND STABILITY (DUS)

Document prepared by the Office of the Union

to be considered by

the Technical Committee at its fifty-fifth session
to be held in Geneva on October 28 and 29, 2019,

the Administrative and Legal Committee at its seventy-sixth session
to be held in Geneva on October 30, 2019

and

the Council at its fifty-third ordinary session
to be held in Geneva on November 1, 2019

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1. INTRODUCTION

1.1 Document UPOV/INF/18 "Possible Use of Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)" considers possible application models for the use of biochemical and molecular markers in the examination of DUS that were proposed to the *Ad hoc* Subgroup of Technical and Legal Experts of Biochemical and Molecular Techniques (BMT Review Group) by the Technical Committee (TC), 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/about/en/organigram.html). The assessment of the BMT Review Group and the views of the Technical Committee, the Administrative and Legal Committee (CAJ) on those models are presented in document UPOV/INF/18.

1.2 The purpose of this document is to provide guidance on the use of biochemical and molecular markers in the examination of Distinctness, Uniformity and Stability (DUS) on the basis of the models that have received a positive assessment and for which accepted examples have been provided.

1.3 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.

1.4 The following abbreviations are used in this document:

CAJ:	Administrative and Legal Committee
TC:	Technical Committee
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. APPLICATION MODELS

2.1 Characteristic-Specific Molecular Markers (see Annex I)

2.1.1 Molecular markers can be used as a method of examining DUS characteristics that satisfy the criteria for characteristics set out in the General Introduction, Chapter 4, section 4.2, on the following basis:

(a) the test for the marker is conducted on the same number of individual plants, with the same criteria for distinctness, uniformity and stability as for the examination of the characteristic by a bioassay;

(b) there is verification of the reliability of the link between the marker and the characteristic;

(c) different markers for the same characteristic are different methods for examining the same characteristic;

(d) markers linked to different genes conferring expression of the same characteristic are different methods for examining the same characteristic; and

(e) markers linked to different regulatory elements for the same gene conferring expression of the same characteristic are different methods for examining the same characteristic: 2.1.2 Annex I to this document "Gene Specific Marker for Herbicide Tolerance" provides an example of the use of characteristic-specific molecular markers.

2.1.2 It is a matter for the relevant authority to consider if the assumptions are met when applying the model and example, as presented in Annex I of this document.

2.1.3. In order to include a method based on the model in Annex I of this document in Test Guidelines the relevant Technical Working Party and the TC would need to agree that the requirement for reliability of the link between the gene and the expression of the characteristic was satisfied.

2.2 Combining Phenotypic and Molecular Distances in the Management of Variety Collections (see Annex II)

2.2.1 A key feature of the process of eliminating varieties of common knowledge prior to the DUS growing trial is that the threshold is set with a suitable margin of safety. This threshold 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.

2.2.2 A combination of phenotypic differences and molecular distances can be used to identify within the variety collection, those varieties which need to be compared with candidate varieties in order to improve the selection of “Distinct plus” varieties, on the following basis:

(a) there is reliable information that the molecular distances are sufficiently related to phenotypic differences, such that

(b) the method selects varieties in the variety collection which are similar to the candidate varieties; and

(c) the method does not create an increased risk of not selecting a variety in the variety collection which needs to be compared to the candidate varieties in the field.

2.2.3 Annex II to this document “Combining Phenotypic and Molecular Distances in the Management of Variety Collections” provides an example of the use of combining phenotypic differences and molecular distances in the management of variety collections.

2.3 Genetic Selection of Similar Varieties for the First Growing Cycle (see Annex III)

2.3.1 This approach involves a step to check for genetic similarity before the first growing cycle.

2.3.2 In cases where the minimum duration of tests is normally two growing cycles, a selection of similar varieties in the variety collection for comparison with candidate varieties in the first growing cycle is made according to genetic similarity. As a next step, the information provided by the applicant in the Technical Questionnaire (TQ) is used to see if some of the genetically similar varieties do not have to be compared in a growing trial because of differences in DUS characteristics.

2.3.3 On the basis of the variety description of DUS characteristics produced in the first growing cycle, a further search is made of varieties in the variety collection to identify any similar varieties that were not compared in the first growing cycle and which should be compared with the candidate variety in the second growing cycle.

2.3.4 Annex III to this document “Genetic Selection of Similar Varieties for the First Growing Cycle” provides an example of the genetic selection of similar varieties for the first growing cycle.

[Annexes follow]

ANNEX I

MODEL: CHARACTERISTIC-SPECIFIC MOLECULAR MARKERS

EXAMPLE: 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 II follows]

ANNEX II

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

EXAMPLE: PARENT LINES IN MAIZE

prepared by experts from France

1. Description

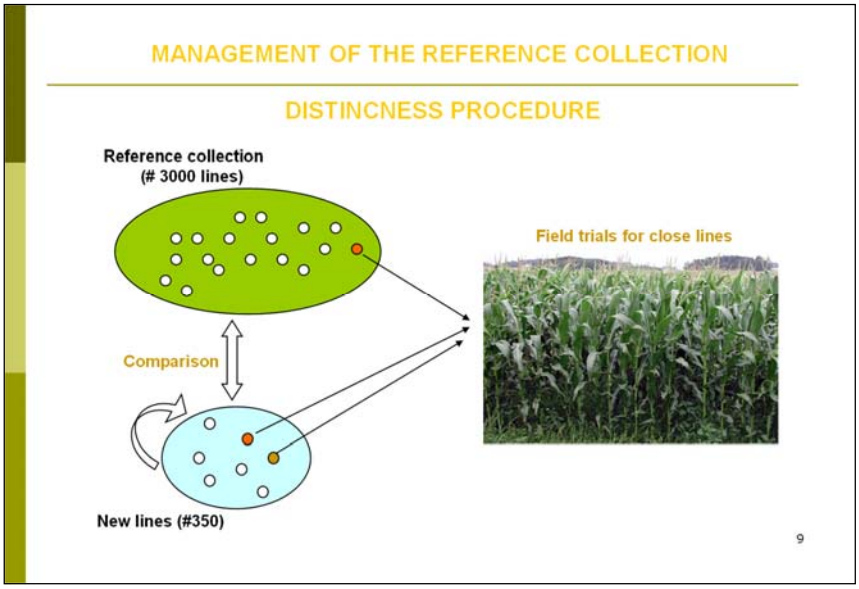
1.1 A key feature of the process of eliminating varieties of common knowledge prior to the DUS growing trial is that the threshold for deciding which varieties can be safely excluded (i.e. are distinct on the basis of descriptions), can be set with a suitable margin of safety, because those varieties 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:

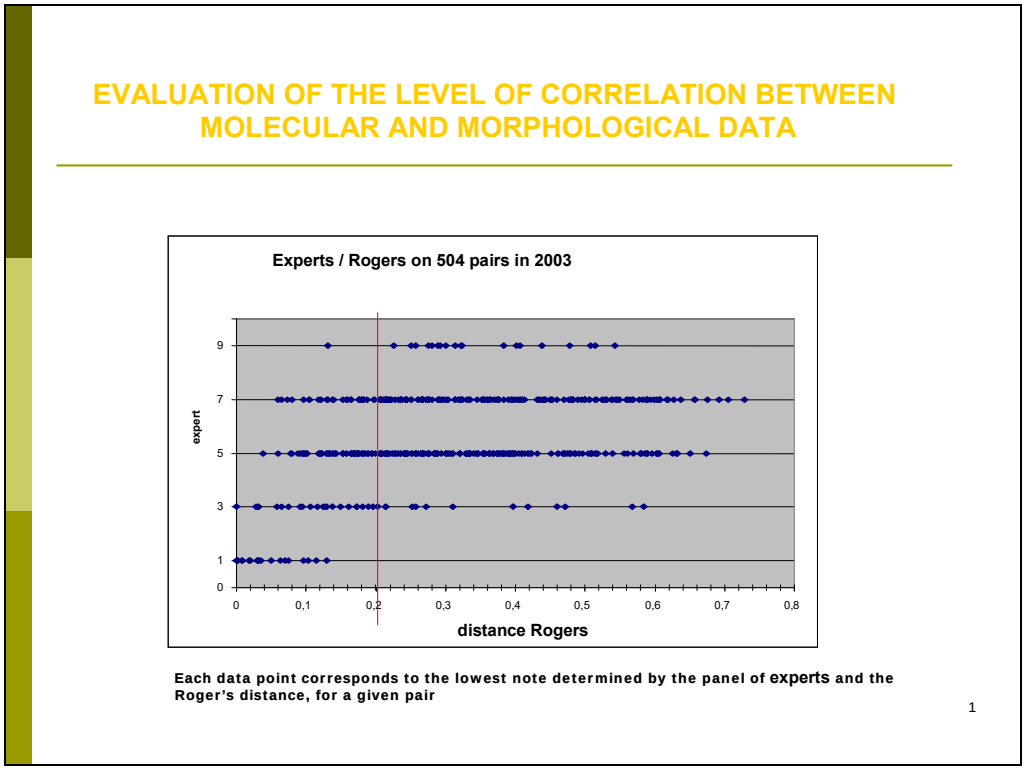
Scale of similarity:

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)

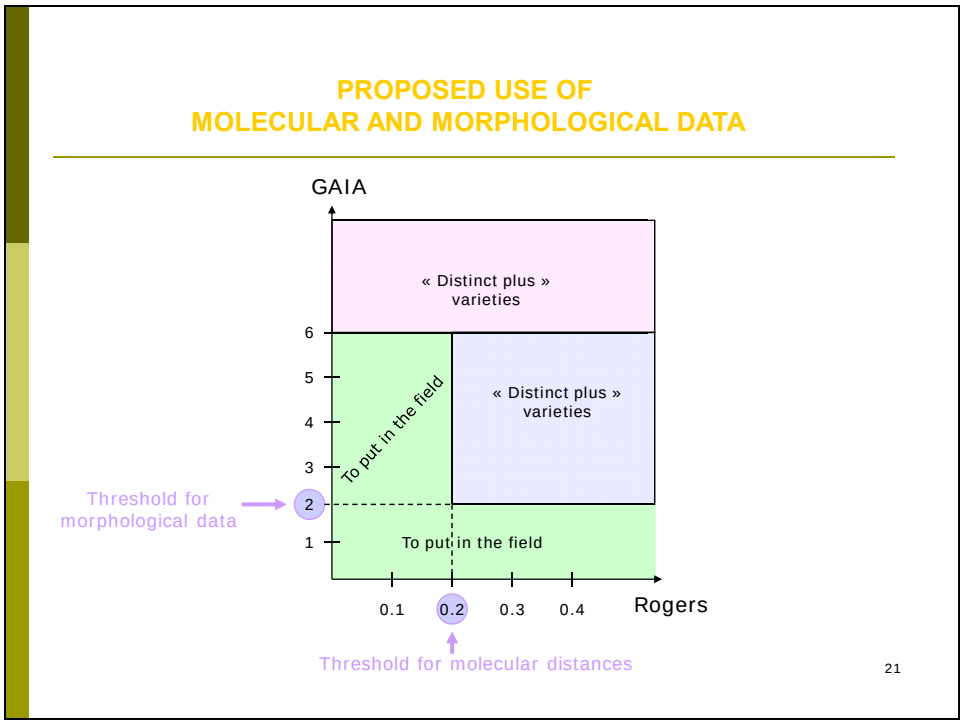
In the case of maize, this evaluation showed that no parental lines with a molecular distance greater than 0.15 were considered as similar or very close by a DUS expert evaluation (see Figure 2).

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 France, Germany, 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.

[Annex III follows]

ANNEX III

MODEL: GENETIC SELECTION OF SIMILAR VARIETIES FOR THE FIRST GROWING CYCLE

EXAMPLE: FRENCH BEAN

prepared by experts from the Netherlands

1. Introduction

1.1 This approach involves a step to check for genetic similarity before the first growing cycle.

1.2 In cases where the minimum duration of tests is normally two growing cycles, a selection of similar varieties in the variety collection for comparison with candidate varieties in the first growing cycle is made according to genetic similarity. As a next step, the information provided by the applicant in the Technical Questionnaire (TQ) is used to see if some of the genetically similar varieties do not have to be compared in a growing trial because of differences in DUS characteristics.

1.3 On the basis of the variety description of DUS characteristics produced in the first growing cycle, a further search is made of varieties in the variety collection to identify any similar varieties that were not compared in the first growing cycle and which should be compared with the candidate variety in the second growing cycle.

2. Procedure

Determine genetic similarity

2.1 The DNA-profile of the candidate variety is produced as soon as plant material is received.

2.2 The DNA-profile is compared with the profiles of all varieties in the variety collection and genetically similar varieties are identified.

Technical Questionnaire information

2.3 The information provided by the applicant in the Technical Questionnaire (TQ) is then used to see if there are clear differences in DUS characteristics from some of the genetically similar varieties so that they do not need to be compared with candidate varieties in a growing trial.

Field trial

First growing cycle:

2.4 The candidate and the genetically similar varieties selected by the procedure above are grown in the same field trial. A complete description of the DUS characteristics of the candidate variety is produced and is compared to the descriptions of all varieties in the variety collection using a database containing descriptions produced at the same location in previous years.

Possible outcomes:

2.5 If the candidate variety is not distinct from the genetically similar varieties on the basis of DUS characteristics, the test will be continued for another growing cycle.

2.6 In any case, the description of the candidate variety produced in the first growing cycle is compared to the descriptions of the varieties in the variety collection using a database containing descriptions produced at the same location:

(a) If the candidate variety is found to be distinct from all varieties grown in the first growing cycle and to all other varieties in the variety collection at the end of the first growing cycle and it fulfills the uniformity and stability requirements the DUS test may be concluded after the first growing cycle.

(b) In all other cases a second growing cycle is performed.

Second growing cycle

2.7 In the second growing cycle, the candidate variety is grown with all varieties in the variety collection from which it was not found to be distinct at the end of the first growing cycle.

2.8 At the end of the second growing cycle, an assessment of DUS is made. If it is not possible to reach a decision on DUS at the end of the second growing cycle, a further growing cycle may be conducted.

[End of Annex III and of document]