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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

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

Meeting
Geneva, April 1, 2009

PROPOSAL FOR USE OF MOLECULAR TECHNIQUES IN DUS TESTING OF MAIZE

document prepared by experts from France

Introduction

1. On the basis of studies described under Option 2 "Calibration of threshold levels for molecular characteristics against the minimum distance in traditional characteristics" in document TC/38/14-CAJ/45/5, new investigations have been undertaken in France to explore the possibility to use molecular data in DUS testing.

2. The initial approach with a calibration of a threshold for molecular characteristics against the minimum distance in traditional characteristics has not been studied further due to the absence of correlation between distances computed on molecular data and phenotypic data respectively. This situation has been observed on different species (maize, oilseed rape, rose, etc.) when non-coding markers, such as Simple Sequence Repeats (SSRs), are used. It could be reconsidered in the future with coding markers.

3. Instead of developing a system in which molecular data would replace phenotypic data, a new approach has been considered with the combination of the both types of data. That approach has been developed into the proposal set out in the Annex to this document, for consideration by the *Ad hoc* Subgroup of Technical and Legal Experts of Biochemical and

Molecular Techniques (BMT Review Group) at its meeting to be held in Geneva on April 1, 2009.

Future work

4. This new approach has been largely tested in France on maize.

5. It has also been developed on oilseed rape but must still be improved mainly due to lack of quality of the markers.

6. On barley, the first results are positive and need to be extended to a larger collection.

7. Applications are also under consideration with a new set of molecular markers on maize and on other species (peach, flax, sunflower, lettuce).

Conclusion

8. The new system could be considered as an option derived from Option 2, as described in the document TC/38/14-CAJ/45/5, because it uses a calibration of molecular distances against a DUS expert's evaluation of distinctness.

9. All candidate varieties are examined in a DUS trial in the normal way. Thereafter, with regard to the varieties of common knowledge that were excluded from comparison in the DUS growing trial, they would only be excluded if they had a reliable and meaningful difference in a phenotypic characteristic.

10. The use of distances as used in this approach is a new concept in the framework of UPOV methods. It is used to define a threshold based on a DUS expert's evaluation and with which only "distinct plus" varieties are excluded from the comparisons in the field. For the varieties compared in the field, the decision rules do not change and are still based on phenotypic characteristics.

11. This approach is a way to use DNA polymorphism for the management of variety collections with all advantages of these descriptors (large variability, robustness, possibility to store descriptions from different origins, usefulness to enforce the right) but still with decisions on distinctness between close varieties strictly based on phenotype.

9. The BMT Review Group is invited to consider the proposal in the Annex to this document, 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.

[Annex follows]

ANNEX

Proposal: "System for combining phenotypic and molecular distances in the management of variety collections"

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 proposal 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 Appendix, slide 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.

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 explanations in Appendix, slides 2 and 3).

1.4 Components of the system

1.4.1 GAIA distance

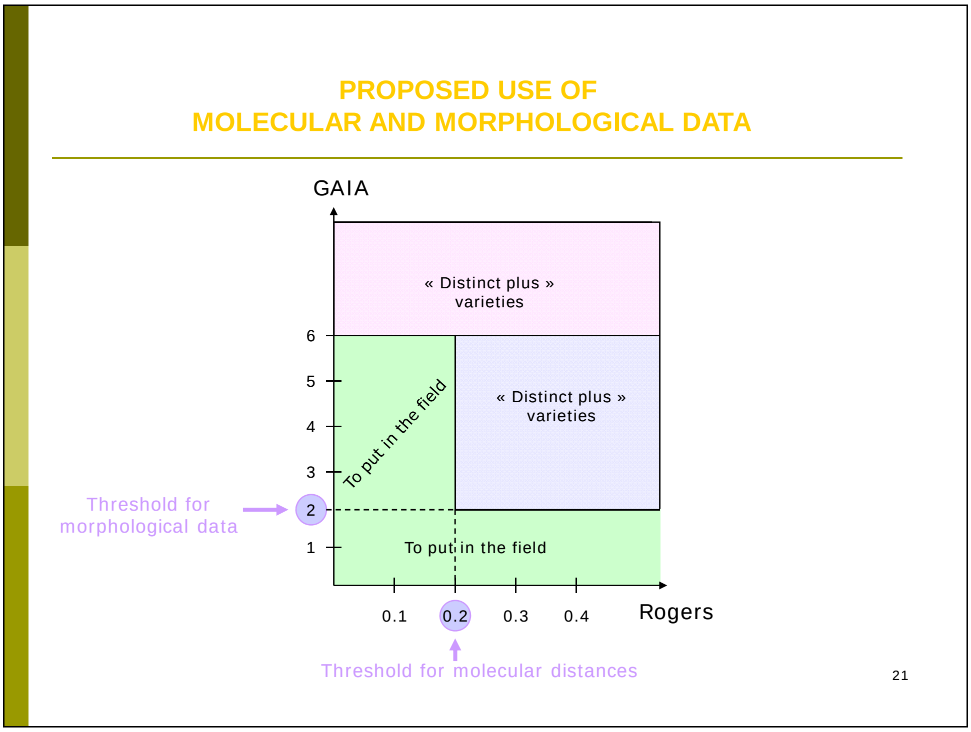
The GAIA distance component is computed with the GAIA software developed by GEVES. The GAIA distance is a combination of differences observed on phenotypic characteristics, where each difference contributes to the distance according to the reliability of the characteristics, especially regarding its 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, 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 Appendix).

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:



PROPOSED USE OF
MOLECULAR AND MORPHOLOGICAL DATA

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 Community (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.

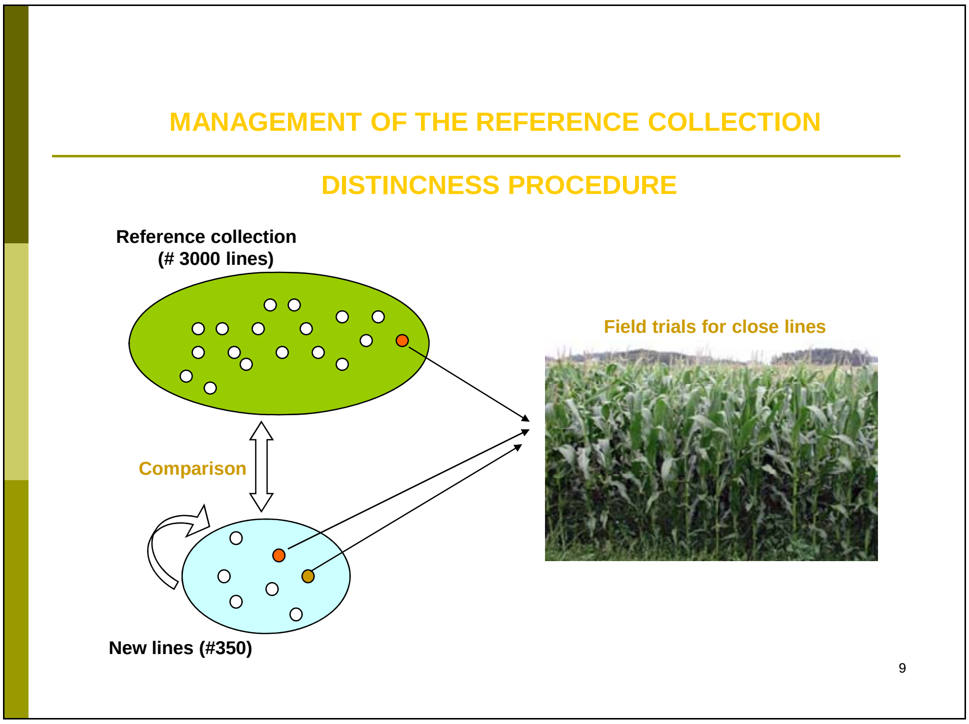
2.2 Constraints

(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.

[Appendix follows]

APPENDIX

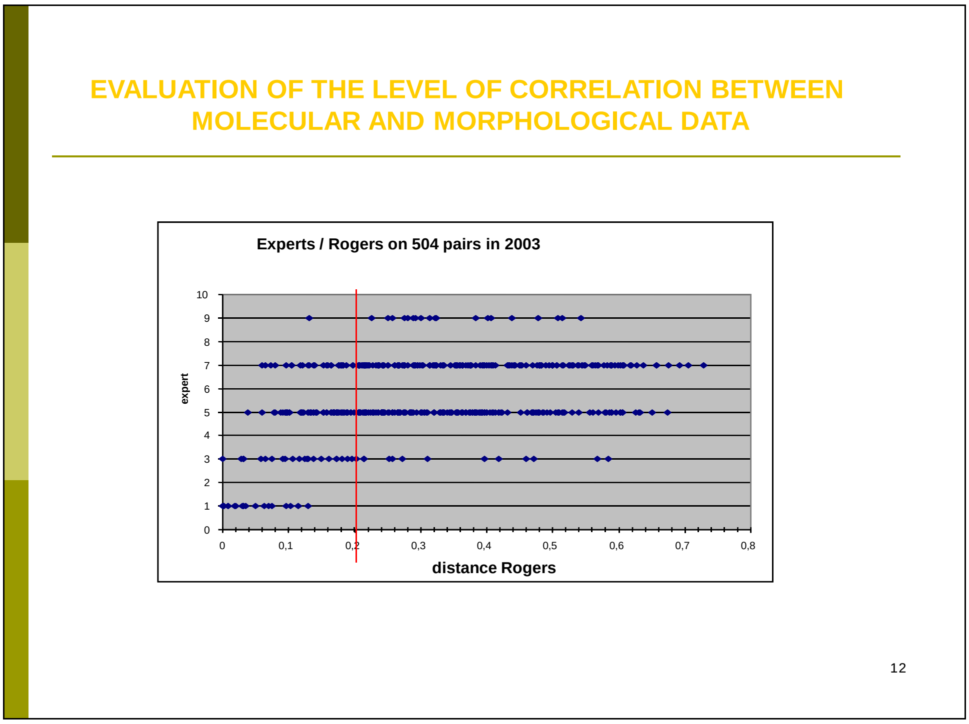
Slide 1



MANAGEMENT OF THE REFERENCE COLLECTION

DISTINCNESS PROCEDURE

Slide 2



EVALUATION OF THE LEVEL OF CORRELATION BETWEEN MOLECULAR AND MORPHOLOGICAL DATA

Experts / Rogers on 504 pairs in 2003

[End of Appendix and of document]