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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

ADDENDUM TO
PROPOSAL FOR USE OF MOLECULAR TECHNIQUES IN DUS TESTING OF MAIZE

document prepared by experts from France

1. At the meeting of the *Ad hoc* Subgroup of Technical and Legal Experts of Biochemical and Molecular Techniques (BMT Review Group) on April 1, 2009, Mr. Joël Guiard (France) made a presentation on the proposal for the use of molecular techniques in DUS testing of Maize, as set out in document BMT-RG/Apr09/2. A copy of that presentation follows:

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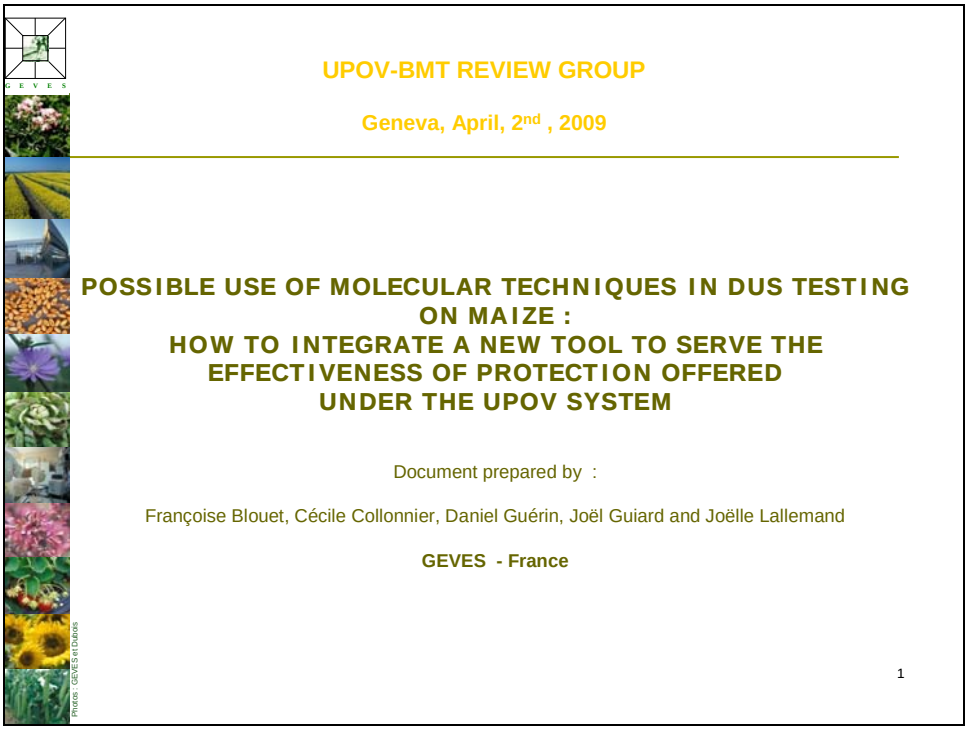

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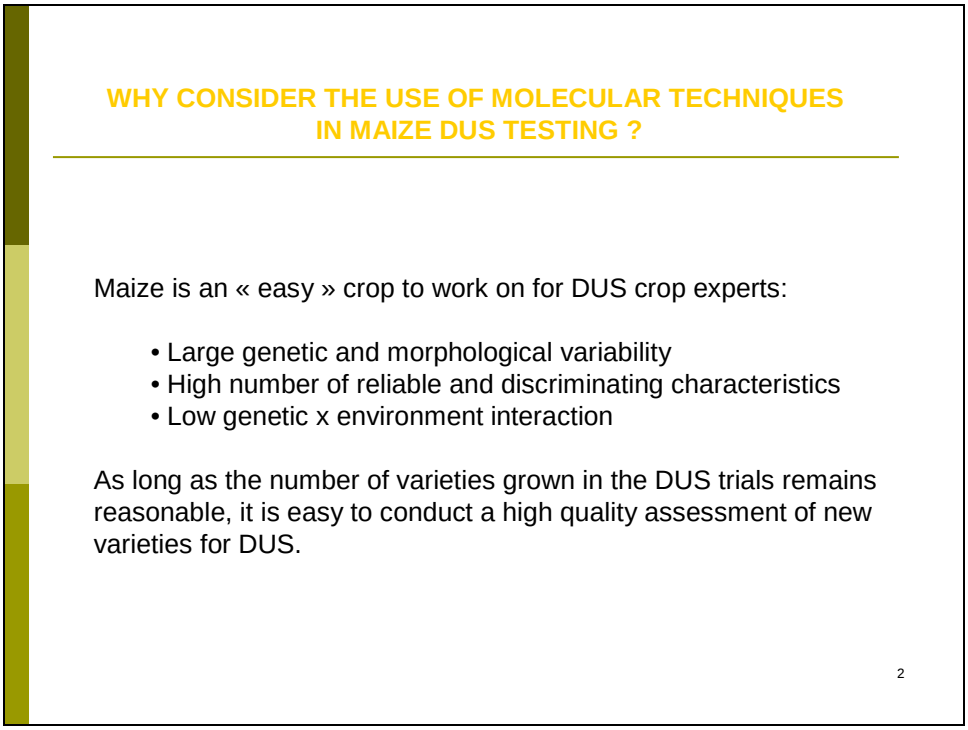

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WHY CONSIDER THE USE OF MOLECULAR TECHNIQUES IN MAIZE DUS TESTING?

We do not need to find new characteristics to establish the distinctness of the new candidates.

What we need is to find tools and procedures to handle a huge number of varieties.

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WHY CONSIDER THE USE OF MOLECULAR TECHNIQUES IN MAIZE DUS TESTING?

Maize is a « huge » crop to work on for DUS crop experts:

As in example in France, in 2005, we had:

- 279 new lines applied in first year
- 2,673 lines in our reference collection

The number of comparisons to establish the distinctness of the new lines was 823,329.

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WHY CONSIDER THE USE OF MOLECULAR TECHNIQUES IN MAIZE DUS TESTING ?

The challenge we face is to maintain the high level of quality of the distinctness assessment,

- considering several thousands varieties of common knowledge and candidates,
- avoiding prohibitive costs ; and
- avoiding lengthening the duration of the tests.

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WHY CONSIDER THE USE OF MOLECULAR TECHNIQUES IN MAIZE DUS TESTING?

Main changes over the recent past:

- integration of characteristics derived from electrophoresis in combination with field characteristics
- development of the concept of combination of differences observed on the different characteristics
- development of the GAIA software to select the varieties which need to be grown in the field trials
- development of a technical cooperation with Spain and Germany; construction of a common database for phenotypic data

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WHY CONSIDER THE USE OF MOLECULAR TECHNIQUES IN MAIZE DUS TESTING ?

Next steps under study:

- integration of genetic distances in combination with phenotypic characteristics to assess distinctness
- integration of molecular techniques as tools to check the identity of lines and hybrids during the test and for the maintenance of the reference collection

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Management of the reference collection

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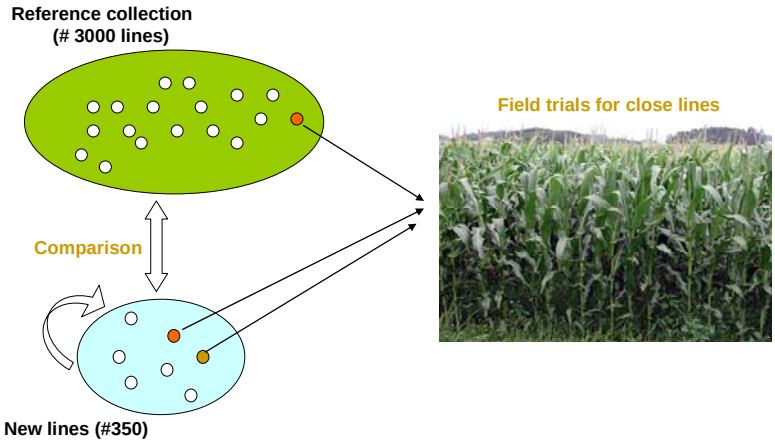
MANAGEMENT OF THE REFERENCE COLLECTION

DISTINCNESS PROCEDURE



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MANAGEMENT OF THE REFERENCE COLLECTION

CORRELATION BETWEEN MOLECULAR AND MORPHOLOGICAL DATA ?

- Previous studies showed that the relation between genetic distances and morphological distances is not linear

  ⇒ how then define an appropriate way of integrating molecular data into the decision ?

- We decided to use “the expert’s appreciation of degree of similarities/differences” between varieties and to compare it with the molecular distances (preliminary study in maize in 1994-95)

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THE EXPERT'S APPRECIATION OF DEGREE OF SIMILARITY/DIFFERENCE BETWEEN 2 VARIETIES

- **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

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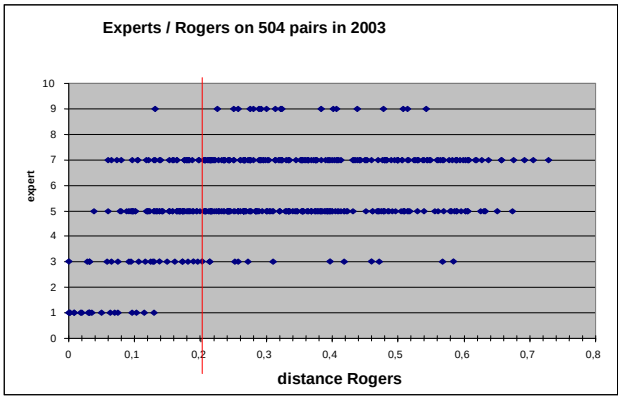
EVALUATION OF THE LEVEL OF CORRELATION BETWEEN MOLECULAR AND MORPHOLOGICAL DATA



Each data point corresponds to the lowest note determined by the panel of experts on a given couple

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METHODS **Data analysis : Roger's distance**

- LCDMV software (Calculation Software of Molecular Distances between Varieties) for fingerprinting and Genetic Diversity Studies (DUBREUIL P. et al., 2004).

					Confidence interval at 95 %	
Var_A	Var_B	Nb_Loci	Rogers distance	StdDev	Lower boundary	Upper boundary
1	10	51	0.544	0.069	0.407	0.681
1	103	51	0.382	0.068	0.249	0.516
1	104	48	0.609	0.070	0.471	0.747
321	204	47	0.021	0.021	-.020	0.063
321	347	50	0.020	0.019	-.019	0.059
83	207	50	0.820	0.054	0.714	0.926

$$D_R^{ij} = \frac{1}{2L}\sum_{l=1}^{L}\sum_{a=1}^{A_l}\left(P_{al}^{i} - P_{al}^{j}\right)^2$$

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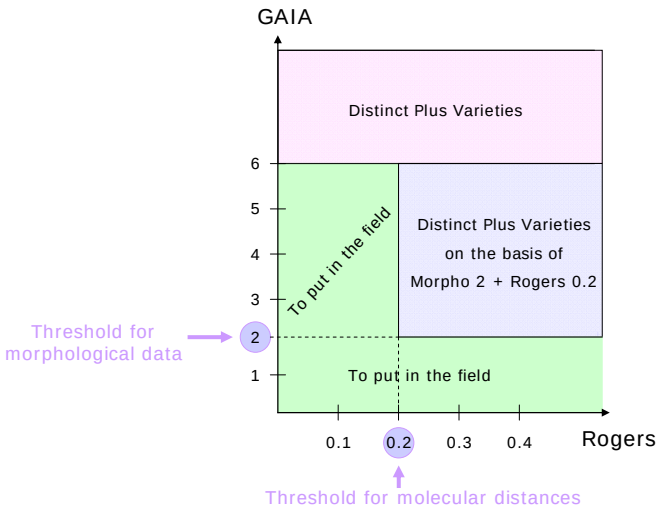
PROPOSED USE OF MOLECULAR AND MORPHOLOGICAL DATA



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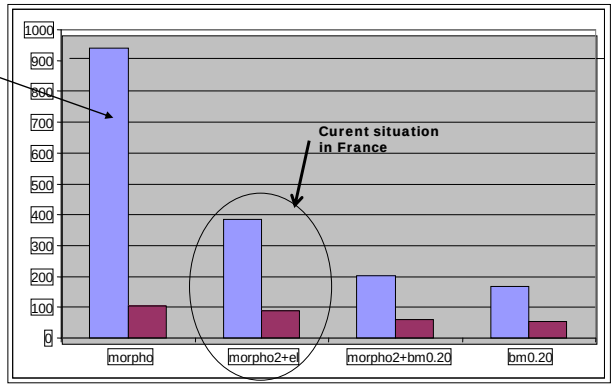
COMPARISON WITH OTHER EXISTING SYSTEMS

Candidate cv. vs Ref collec.
+
Candidate cv. vs Candidate cv.



Number of pairs of varieties to grow in the field trials

Number of reference varieties to grow in the field trials
(morpho = 'GAIA index<6'; morpho2 = 'GAIA index at 2' ; el = 'isoenzymes'; bm0.2 = 'Rogers distance at 0.2')

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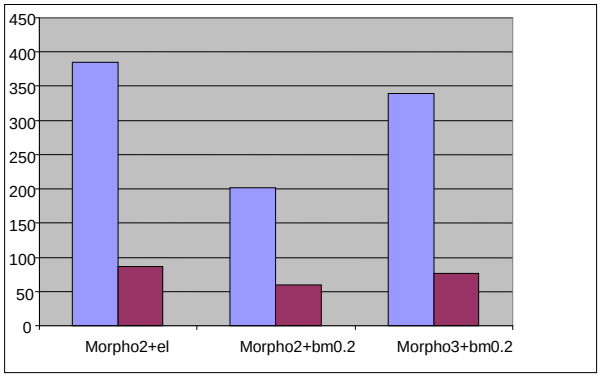
COMPARISON WITH OTHER EXISTING SYSTEMS



Impact of different levels of contributions of morphological data for a fixed molecular distance.

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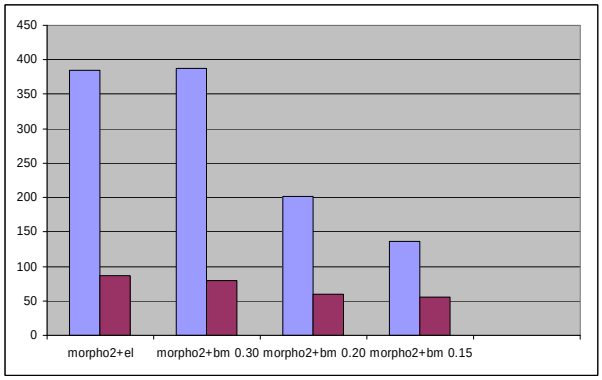
COMPARISON WITH OTHER EXISTING SYSTEMS



Impact of three different thresholds for molecular distances used in combination with a fixed contribution of morphological data

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CONCLUSIONS AND PERSPECTIVES

1. The work we are conducting is derived from option 2 approach

Molecular markers are used as a help for structuring the reference collection and not for the judgement of distinctness on a characteristic by characteristic approach. Close varieties and all candidates are still compared on the basis of phenotype

- the information from molecular markers is calculated by use of a genetic distance and calibrate on the basis of an expert evaluation. It's the main innovation in this system.

- the genetic distance is combined with morphological characteristics. Under the GAÏA system, at least one significant difference on phenotype is requested

- the use of molecular markers in combination with the more robust phenotypical characteristics improve the management of the reference collection

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CONCLUSIONS AND PERSPECTIVES

2 Future Work

- confirmation of the efficiency on the full reference collection (~ 3,000 lines) in progress
- confirmation of the threshold for the genetic distance and the minimum requirement for the morphological difference
  GAIA index 2 + Rogers 0.2
- evaluation of the cost of the new system in relation with the abandonment of electrophoresis
- check the security of the new system and the quality of the protection by running in parallel the new system and the current system in progress

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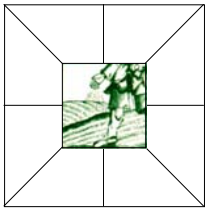
THANK YOU FOR YOUR ATTENTION !



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