UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR
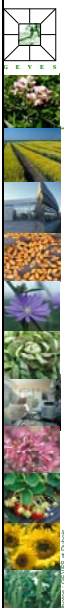
Eleventh Session
Madrid, September 16 to 18, 2008

ADDENDUM

REPORT ON DEVELOPMENTS IN UPOV CONCERNING BIOCHEMICAL AND
MOLECULAR TECHNIQUES

Document prepared by experts from the Office of the Union

At the eleventh session of the Working Group on Biochemical and Molecular
Techniques and DNA Profiling in Particular (BMT), Mr. Joël Guiard made a presentation on
the approach presented in documents BMT/10/14 and BMT-TWA/Maize/2/11 to be put
forward for consideration at the BMT Review Group as a potential option for the use of
molecular markers in DUS examination (see document BMT/11/2, paragraph 26(d)). A copy
of that presentation follows:

UPOV-BMT TWA
Ad Hoc Crop Subgroup on Molecular Techniques
for Maize

Chicago, United States of America, December 3, 2007

POSSIBLE USE OF MOLECULAR TECHNIQUES IN DUS TESTING ON MAIZE :
HOW TO INTEGRATE A NEW TOOL TO SERVE THE EFFECTIVENESS OF PROTECTION OFFERED UNDER THE UPOV SYSTEM

(Presented at the WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES
AND DNA PROFILING IN PARTICULAR
Tenth Session, Seoul, November 21 to 23, 2006)

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GEVES - France

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

Maize is an « easy » crop to work on for DUS crop experts:

- Large genetic and morphological variability
- High number of reliable and discriminating characteristics
- Low genetic x environment interaction

As long as the number of varieties grown in the DUS trials remains reasonable, it is easy to conduct a high quality assessment of new varieties for DUS.

2

**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
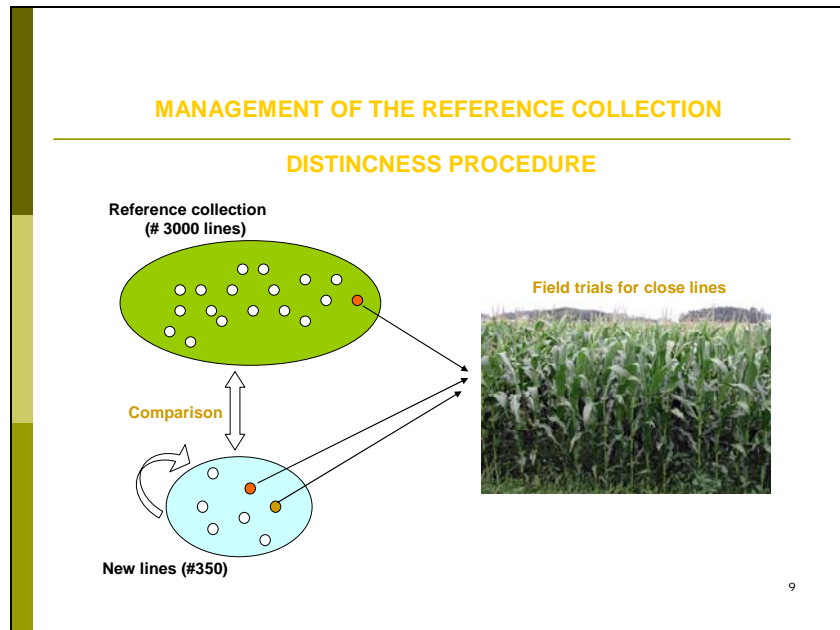IN MAIZE DUS TESTING ?**

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

Reference collection
(# 3000 lines)

Field trials for close lines

Comparison

New lines (#350)

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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

$\Rightarrow$ 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**

Experts / Rogers on 504 pairs in 2003
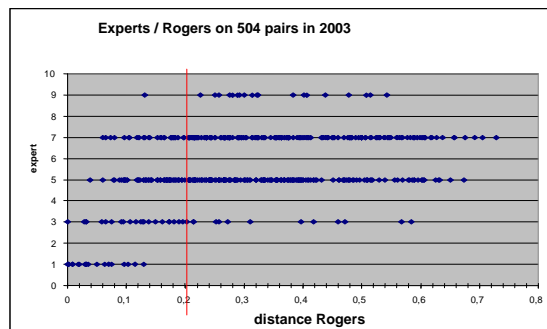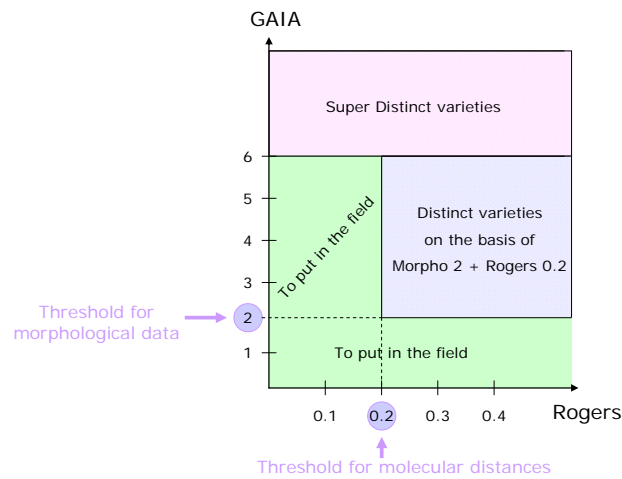
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METHODS   **Data analysis : Roger's distance**

☐ LCDMV software (Calculation Software of Molecular Distances between Varieties) for fingerprinting and Genetic Diversity Studies (DUBREUIL P. et al., 2004).
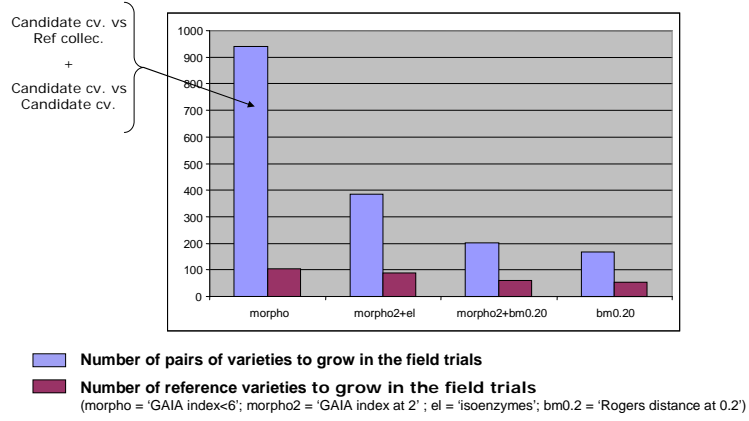
					Confidence interval at 95 %	
Var_A	Var_B	Nb_Loci	Rogers distance	StdDev	Lower boundary	Upper boundary
1	10	51	0.544	0.069	0.407	0.681
1	103	51	0.382	0.068	0.249	0.516
1	104	48	0.609	0.070	0.471	0.747
321	204	47	0.021	0.021	-.020	0.063
321	347	50	0.020	0.019	-.019	0.059
83	207	50	0.820	0.054	0.714	0.926

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

**PROPOSED USE OF
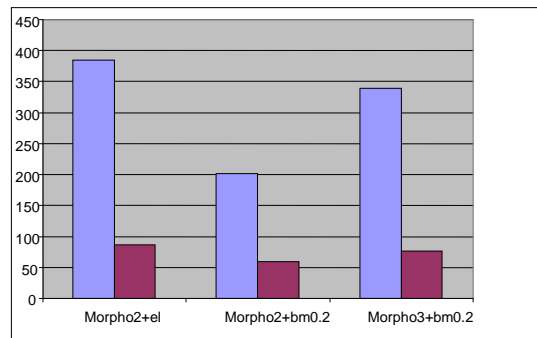MOLECULAR AND MORPHOLOGICAL DATA**

GAIA

Super Distinct varieties

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To put in the field

4

Distinct varieties
on the basis of
Morpho 2 + Rogers 0.2

3

Threshold for
morphological data → 2

1   To put in the field

0.1   0.2   0.3   0.4   Rogers

Threshold for molecular distances

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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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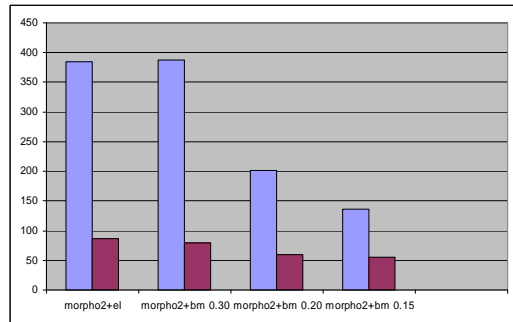**COMPARISON WITH OTHER EXISTING SYSTEMS**

**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 under 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.

• the information from molecular markers is calculated by use of a genetic distance

• the genetic distance is combined with morphological characteristics

• the calibration of the new system against the existing one is a crucial point, requiring a "parallel running" of the two systems.
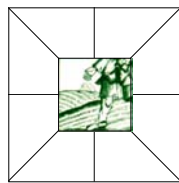
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CONCLUSIONS AND PERSPECTIVES

2. Genetic distances are promising tools for the management of the reference collection in maize in combination with morphological characteristics

We need now to:

- confirm their efficiency on the real reference collection (~ 3,000 lines)
- specify a threshold for the genetic distance and the minimum requirement for the morphological difference
    → GAIA index 2 + Rogers 0.2
- estimate the cost of the new system in relation with the abandonment of electrophoresis → to be done
- check the security of the new system and the quality of the protection by running in parallel the new system and the current system → to be done

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THANK YOU FOR YOUR ATTENTION !

G E V E S

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