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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

TECHNICAL WORKING PARTY ON AUTOMATION AND COMPUTER PROGRAMS

Twenty-Sixth Session
Jeju, Republic of Korea, September 2 to 5, 2008

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

Document prepared by experts from France

Summary

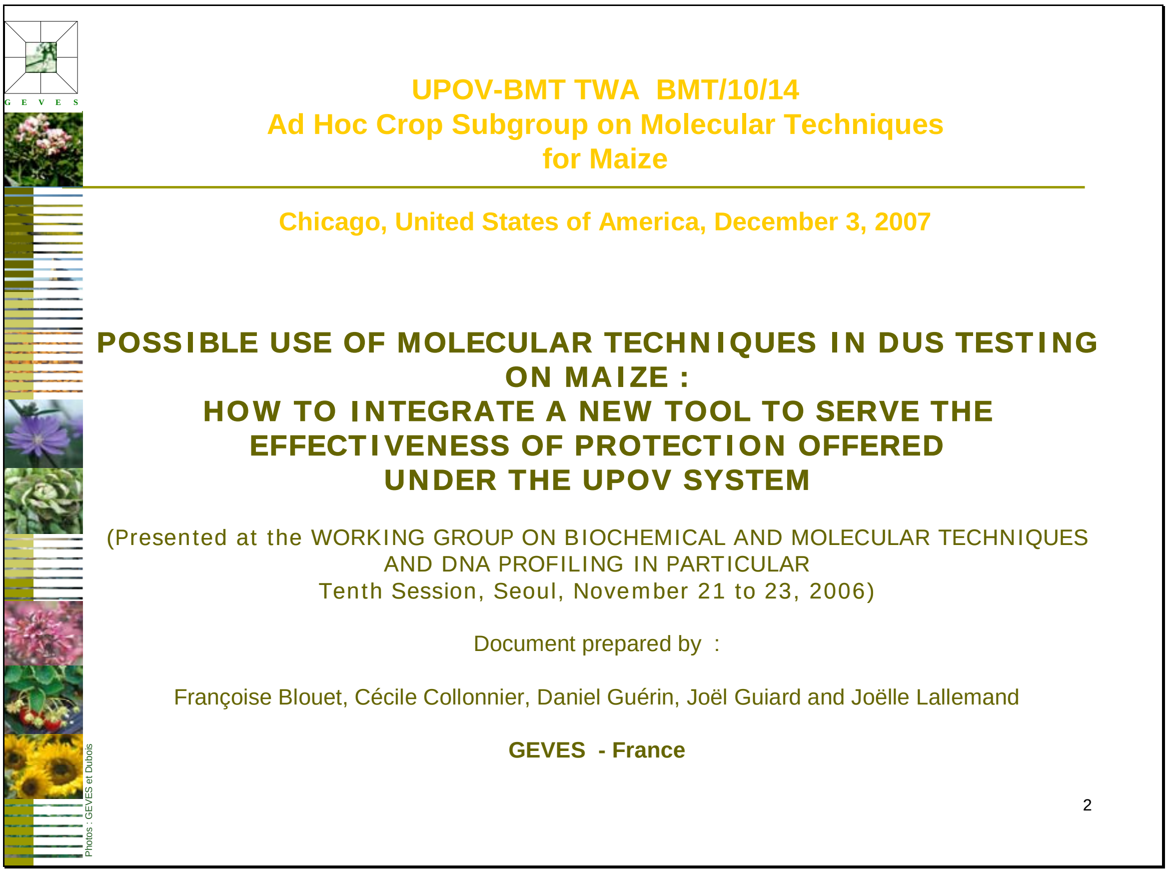
BMT/10/14
POSSIBLE USE OF MOLECULAR TECHNIQUES IN DUS TESTING ON MAIZE :
HOW TO INTEGRATE A NEW TOOL TO SERVE THE EFFECTIVENESS OF PROTECTION OFFERED UNDER THE UPOV SYSTEM

TWC/26/18 last 2 slides
follow up of yesterday presentation on oil seed rape

Context
- **too many reference varieties to grow all of them**
- **data are available on reference and candidate varieties**

Concept
- **1 use present UPOV characteristics**
- **2 if needed combine with electrophoresis or biomelcular data**

Graphical view of possibilities
- **quantify on basis of past data, the possible reduction of reference varieties to grow with candidates**



G E V E S

UPOV-BMT TWA BMT/10/14
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

Photos : GEVES et Dubois

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

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

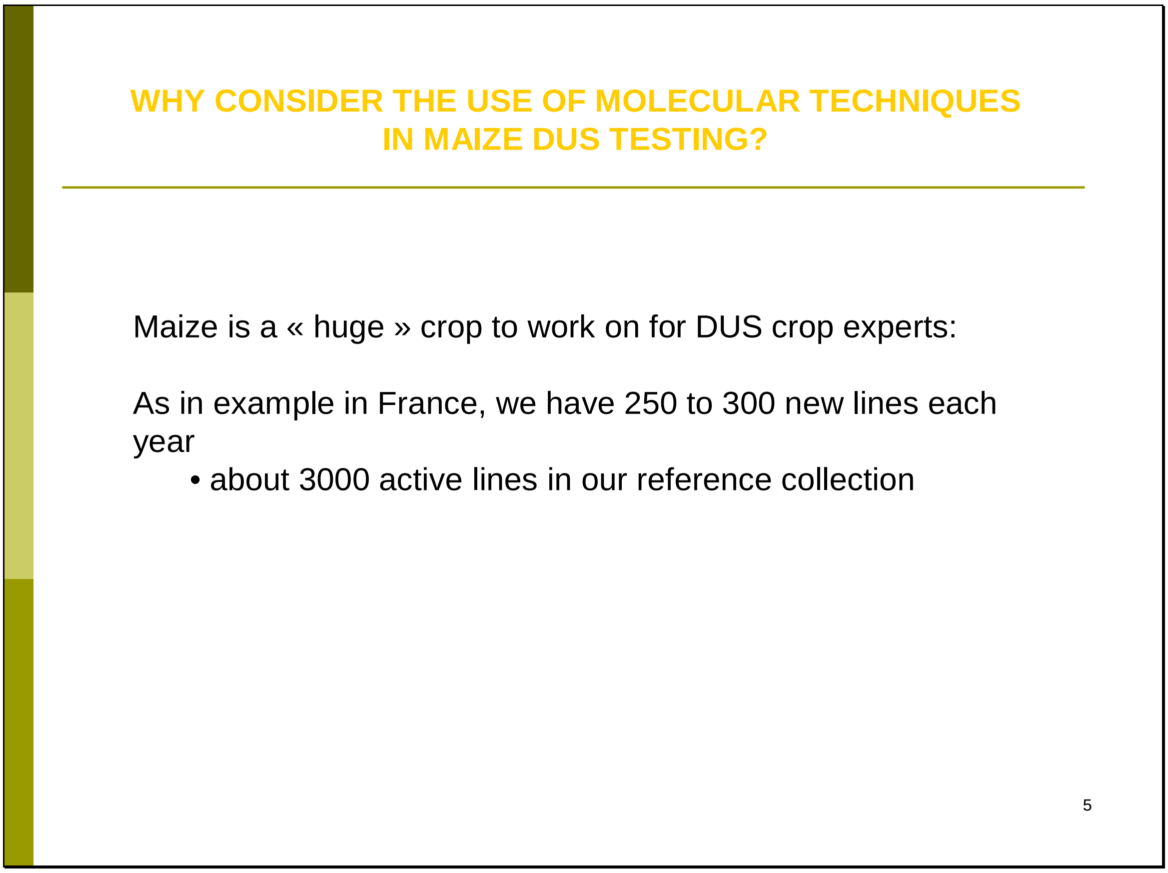
- Large genetic and morphological variability
- High number of reliable and discriminating characteristics
- Low genetic x environment interaction

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.

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.

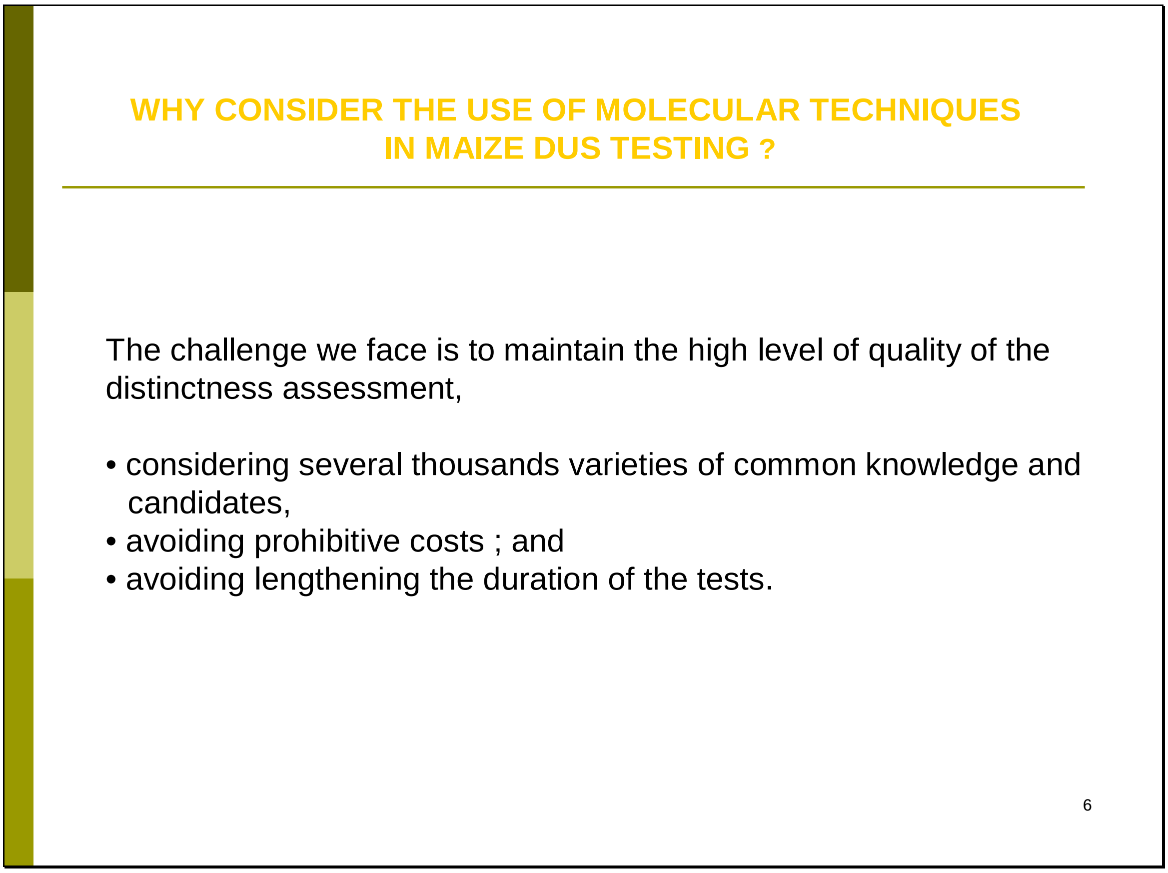


WHY CONSIDER THE USE OF MOLECULAR TECHNIQUES IN MAIZE DUS TESTING?

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

As in example in France, we have 250 to 300 new lines each year

- about 3000 active lines in our reference collection



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.

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:

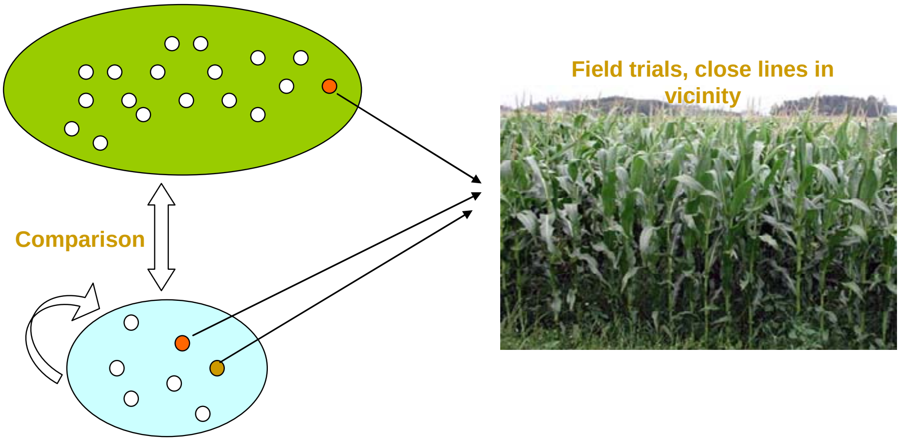
- integration of genetic distances in combination with phenotypic characteristics to assess distinctness

- 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

Management of the reference collection

MANAGEMENT OF THE REFERENCE COLLECTION

DISTINCTNESS PROCEDURE

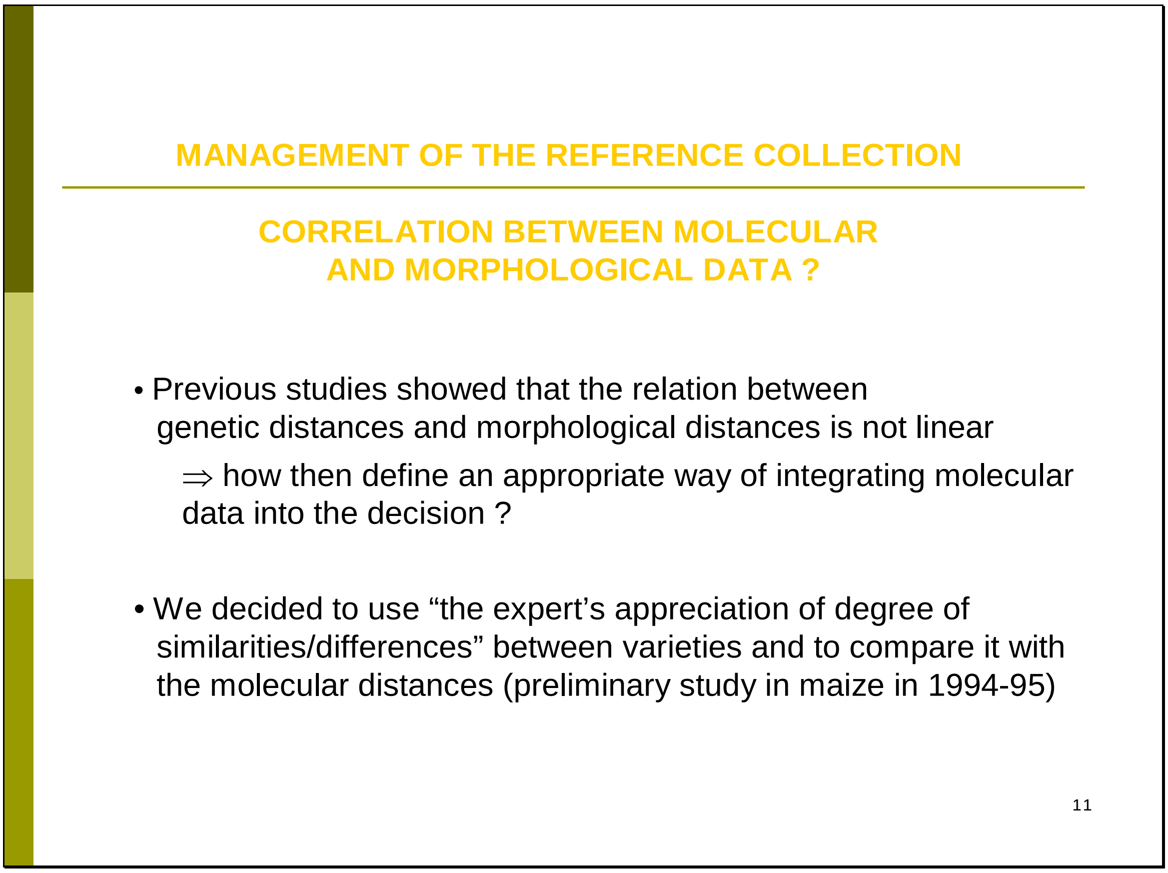


Reference collection
(# 3000 lines)

Field trials, close lines in vicinity

Comparison

New lines (#350)



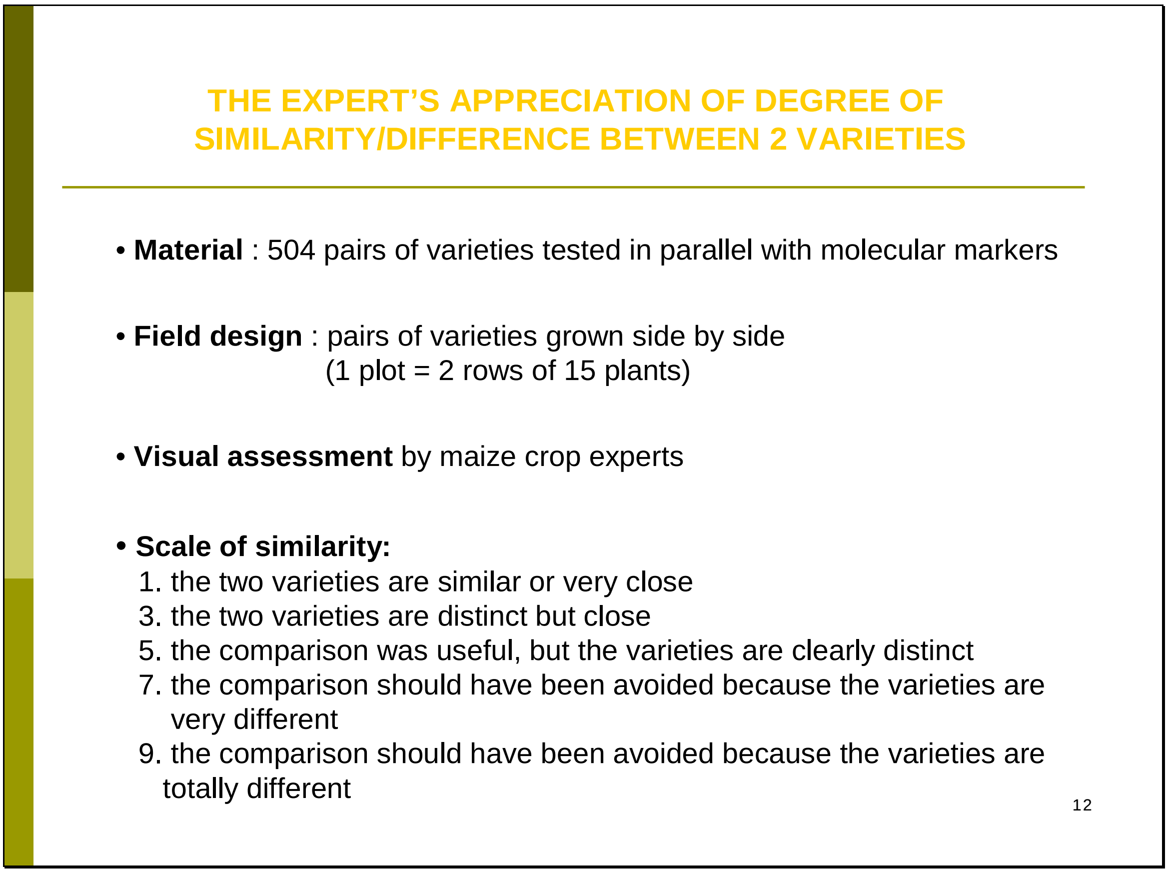
MANAGEMENT OF THE REFERENCE COLLECTION

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)



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

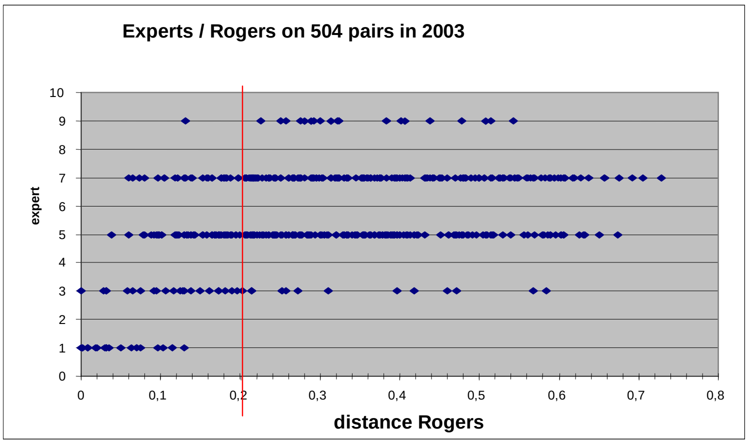
- **Material** : 504 pairs of varieties tested in parallel with molecular markers

- **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

EVALUATION OF THE LEVEL OF CORRELATION BETWEEN MOLECULAR AND MORPHOLOGICAL DATA


Experts / Rogers on 504 pairs in 2003

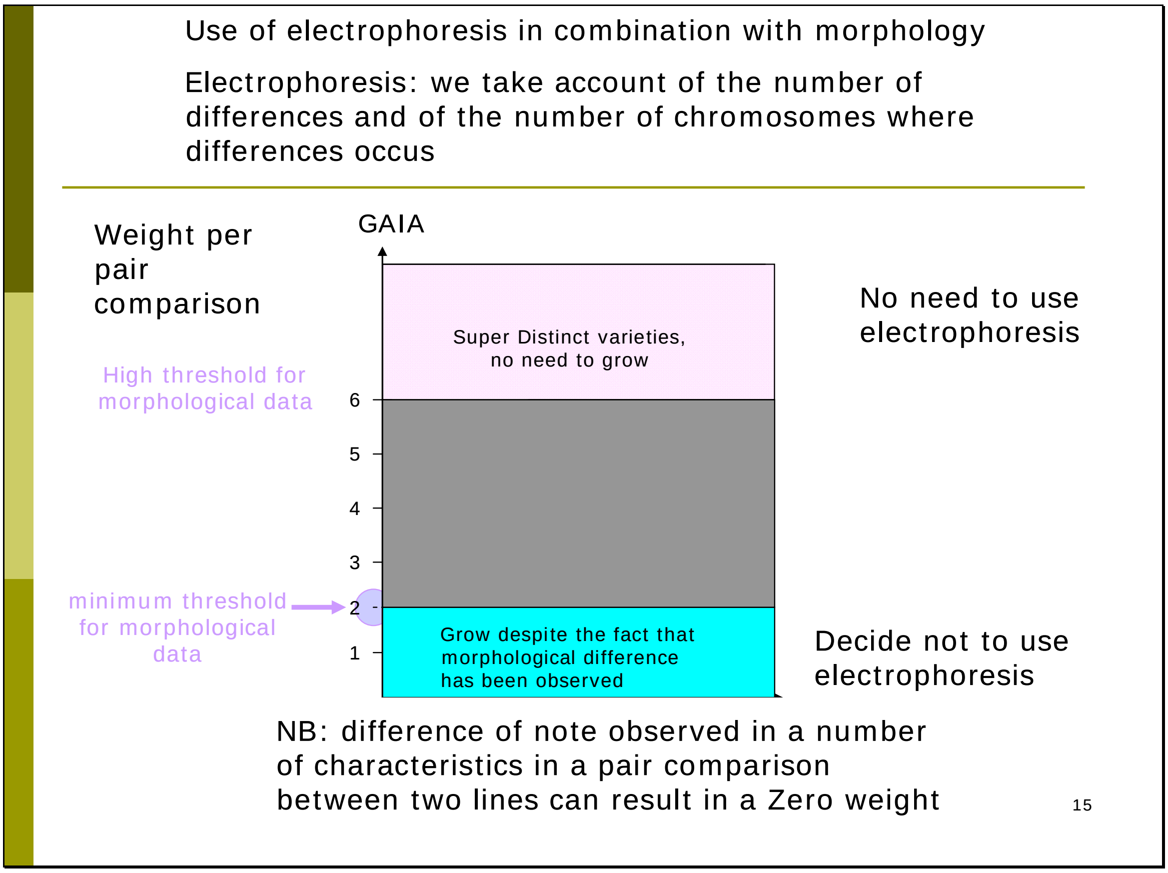
METHODS

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

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



Use of electrophoresis in combination with morphology

Electrophoresis: we take account of the number of differences and of the number of chromosomes where differences occus

Weight per pair comparison

High threshold for morphological data

minimum threshold for morphological data

GAIA

Super Distinct varieties, no need to grow

Grow despite the fact that morphological difference has been observed

No need to use electrophoresis

Decide not to use electrophoresis

NB: difference of note observed in a number of characteristics in a pair comparison between two lines can result in a Zero weight

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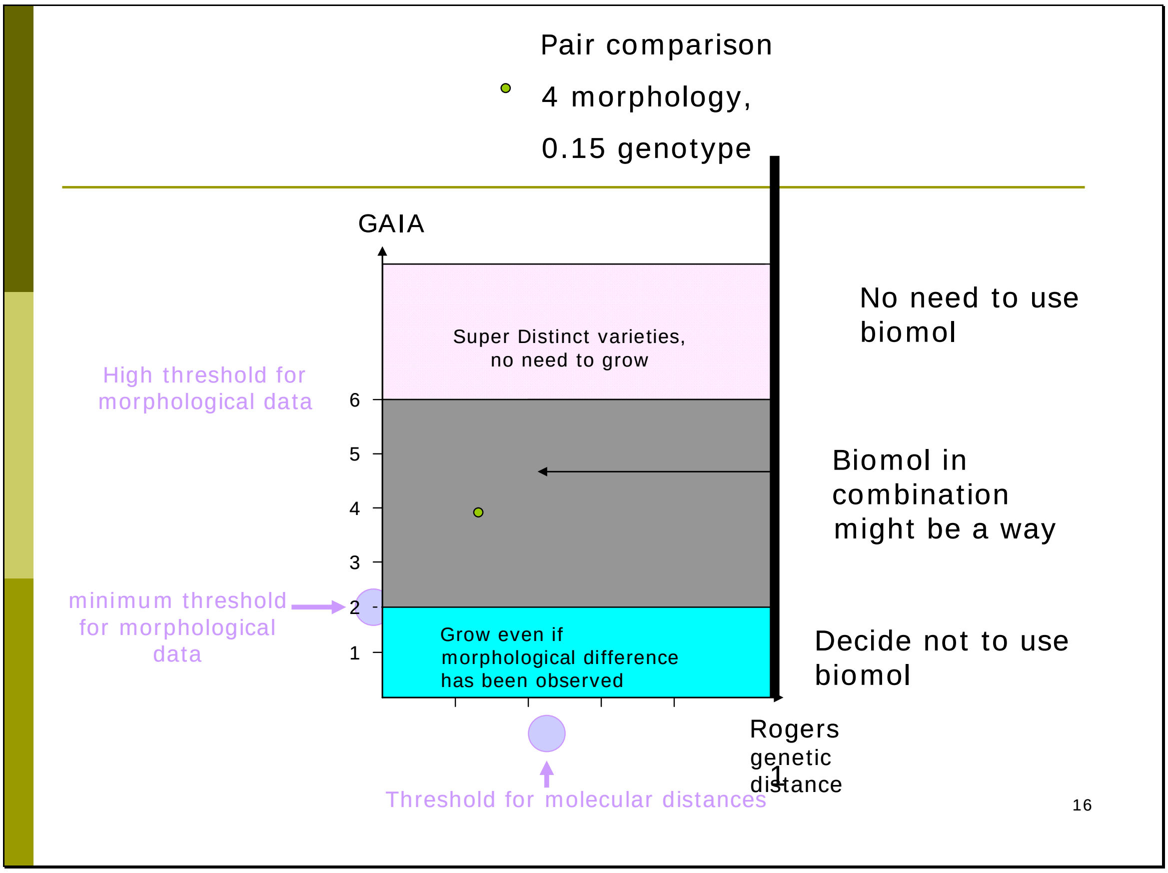

Pair comparison

- 4 morphology,

0.15 genotype

GAIA

High threshold for morphological data

minimum threshold for morphological data

Super Distinct varieties, no need to grow

Grow even if morphological difference has been observed

No need to use biomol

Biomol in combination might be a way

Decide not to use biomol

Rogers genetic distance

Threshold for molecular distances

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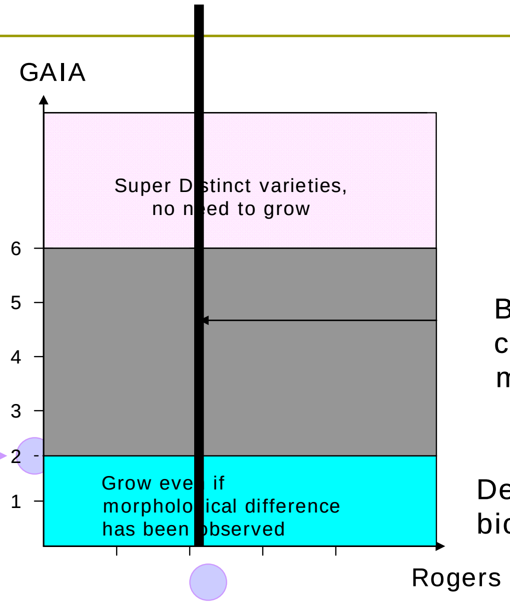


High threshold for morphological data

minimum threshold for morphological data

No need to use biomol

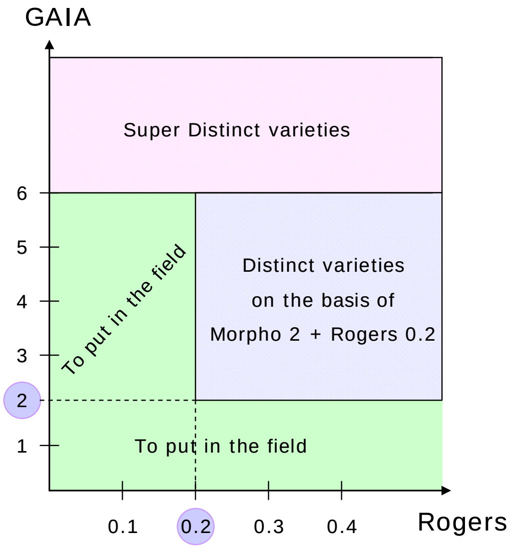
Biomol in combination might be a way

Decide not to use biomol

1

Threshold for molecular distances

PROPOSED USE OF MOLECULAR AND MORPHOLOGICAL DATA

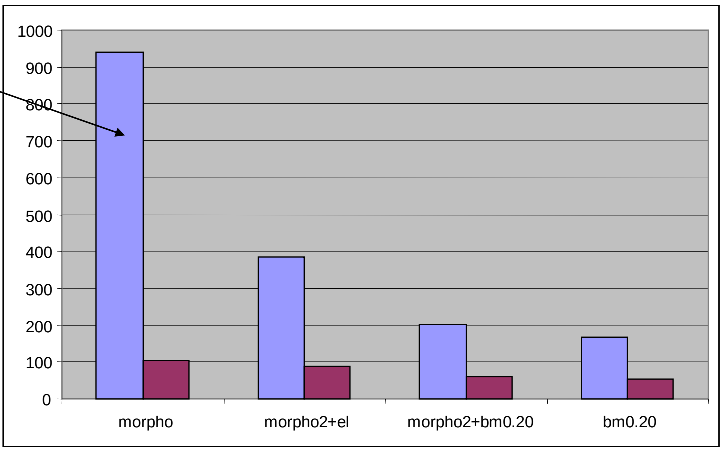


Threshold for morphological data

Threshold for molecular distances

COMPARISON WITH OTHER EXISTING SYSTEMS

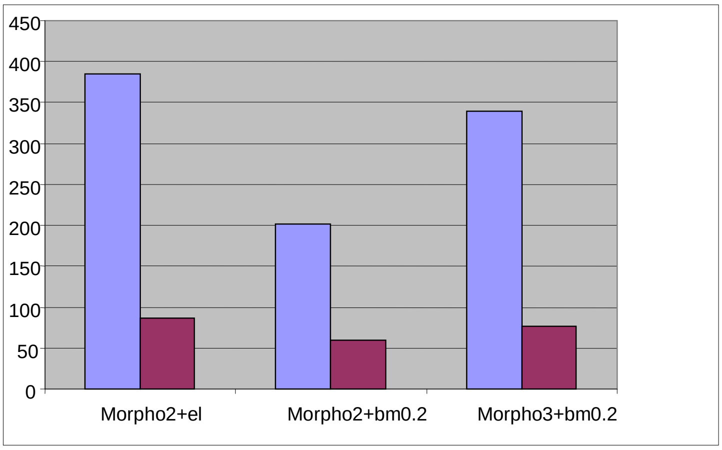
Candidate cv. vs
Ref collec.
+
Candidate cv. vs
Candidate cv.



🟦 **Number of pairs of varieties to grow in the field trials**

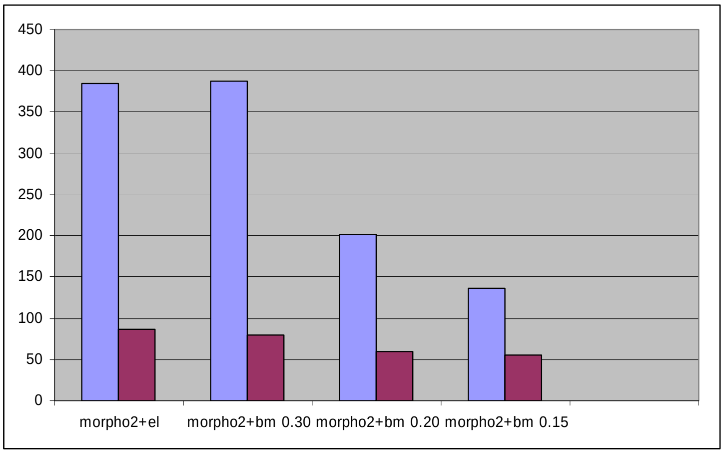
🟥 **Number of reference varieties to grow in the field trials**
(morpho = ‘GAIA index<6’; morpho2 = ‘GAIA index at 2’ ; el = ‘isoenzymes’; bm0.2 = ‘Rogers distance at 0.2’)

COMPARISON WITH OTHER EXISTING SYSTEMS



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

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.

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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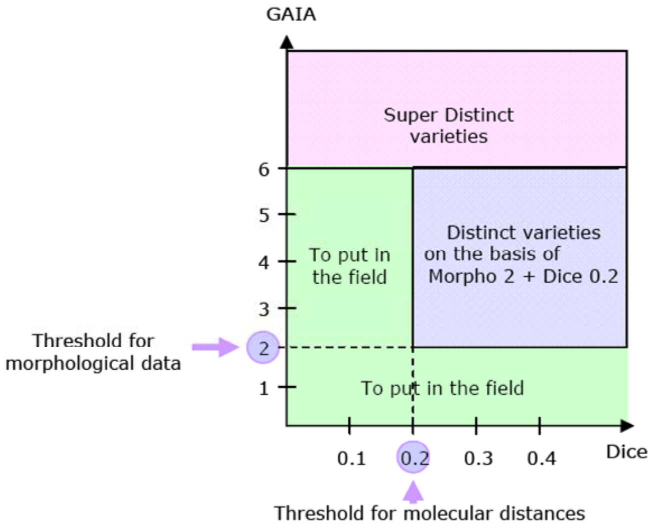
THANK YOU FOR YOUR ATTENTION !

G E V E S

TWC/26/18 oil seed rape



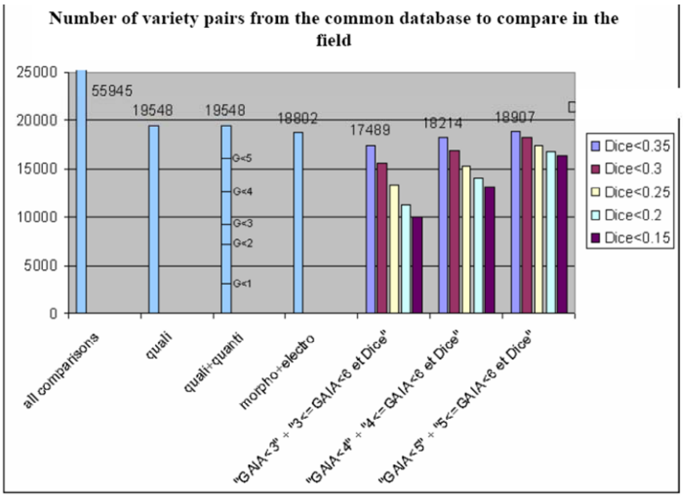


Figure 3: Number of variety pairs from the common database to compare in the field, selected according to the following criteria:

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