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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR ROSE

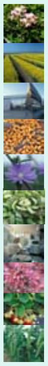
Second Session

Angers, France, April 18, 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

Document prepared by experts from France

This document contains a copy of the presentation made by experts from France, in relation to document 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”.



UPOV WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

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

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

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.

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, in 2005, we had:
- 279 new lines applied in first year
- 2,673 lines in our reference collection

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

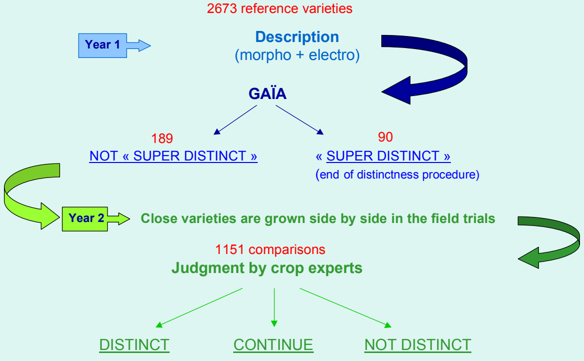
WHY CONSIDER THE USE OF MOLECULAR TECHNIQUES IN MAIZE DUS TESTING ?

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.

Ex : 2005

DISTINCTNESS PROCEDURE



279 new lines in 1st year
2673 reference varieties

Year 1 → Description (morpho + electro)

GAÏA

189 NOT « SUPER DISTINCT »

90 « SUPER DISTINCT » (end of distinctness procedure)

Year 2 → Close varieties are grown side by side in the field trials

1151 comparisons

Judgment by crop experts

DISTINCT | CONTINUE | NOT DISTINCT

Notion of super distinctness

- This notion relies on the fact that pairs of varieties are more or less different.
- Some pairs of varieties are so much different that they can easily be declared « super distinct » just after the first comparison. It is useless (and costly) to repeat the comparison and to continue the distinctness test because the risk that these varieties would not finally be declared « distinct » is null.
- GAIA is a software created in order to select the « super distinct » pairs of varieties and to avoid to make unnecessary comparisons in the field trials.
- The requirements for « super distinctness » are higher than for distinctness.

CRITERIA FOR BEING « SUPER DISTINCT »

Present System

- **Only with morphology**
  - Morphological distance = 6
    (according to the weightings
    of the differences observed
    in GAIA)

or

- **Combination of morphology and electrophoresis**
  - Morphological distance = 2
    and
    Electrophoresis distance = 4

Maize example of morphological distance

	Type of grain	Attitude of blade	Attitude of lateral branches	Anthocyanin coloration of glumes	Length of husks	Number of rows of grain	Diameter of the ear	Time of anthesis	……
Variety A	4	3	7	2	5	3	3	6	……
Variety B	4	3	5	1	7	3	5	7	……
									……
Difference	0	0	2	1	2	0	2	1	
Weight	0	0	2	0	0	0	2	0	

Σ Weights = 4

Estimation of the distance between A and B
$D_{morpho} = 4$

Maize example of electrophoresis distance

	Chromosome 8		Chromosome 6	
	Idh1 4	Idh1 6	Idh2 4	Idh2 6
Variety A	**0**	**1**	**1**	**0**
Variety B	**0**	**1**	**0**	**1**
Difference	**0**	**0**	**1**	**1**

Number of differences

Number of chromosomes on which differences are observed

Distance electro = 2 x 0,25 + 1 x 1 = 1,5

Weight associated to the number of differences

Weight associated to chromosomes

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Possible new System

- **Only with morphology**
  - Morphological distance = 6
    (according to the weightings
    of the differences observed
    in GAIA)

or

- **Combination of morphology and molecular information**

•Morphological distance = 2
and
Genetic distance ?

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

THREE STEPS

- Development and evaluation of suitable molecular markers
- Evaluation of the level of correlation between molecular and morphological data

3. Combination of molecular data with phenotypical data and comparison with existing systems

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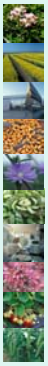
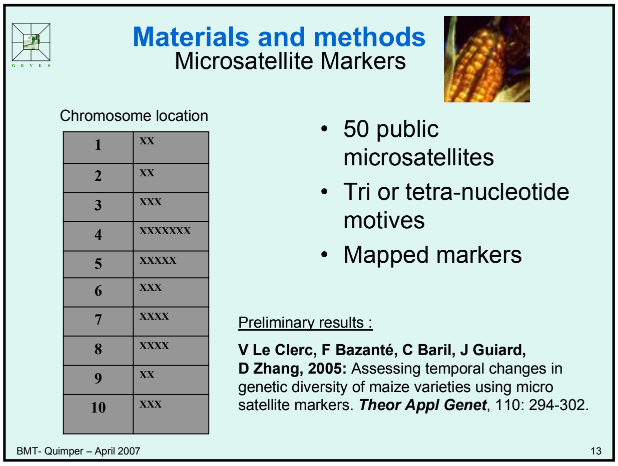


- **DEVELOPMENT AND EVALUATION OF SUITABLE MOLECULAR MARKERS**



Materials and methods
Microsatellite Markers

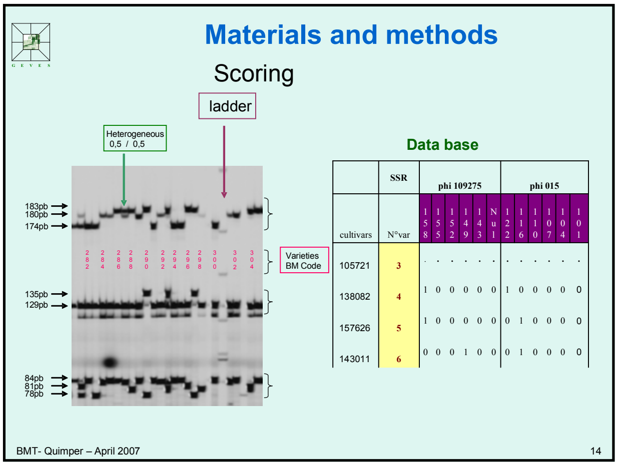
Chromosome location

1	XX
2	XX
3	XXX
4	XXXXXXX
5	XXXXX
6	XXX
7	XXXX
8	XXXX
9	XX
10	XXX

- 50 public microsatellites
- Tri or tetra-nucleotide motives
- Mapped markers

Preliminary results :

V Le Clerc, F Bazanté, C Baril, J Guiard, D Zhang, 2005: Assessing temporal changes in genetic diversity of maize varieties using micro satellite markers. ***Theor Appl Genet***, 110: 294-302.



Materials and methods
Scoring

Data base

	SSR	phi 109275						phi 015					
cultivars	N°var	158	155	152	149	143	Nul	122	116	110	107	104	101
105721	3	.	.	.	.	.	.	.	.	.	.	.	.
138082	4	1	0	0	0	0	0	1	0	0	0	0	0
157626	5	1	0	0	0	0	0	0	1	0	0	0	0
143011	6	0	0	0	1	0	0	0	1	0	0	0	0

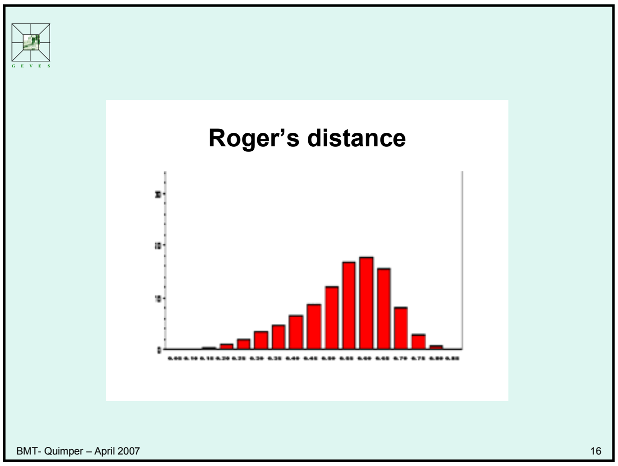
Data analysis

Roger's distance

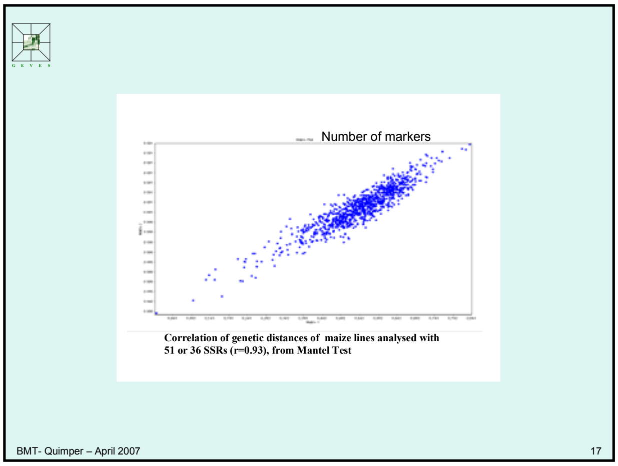
- LCDMV software (Calculation Software of Molecular Distances between Varieties) for fingerprinting and Genetic Diversity Studies (DUBREUIL P. et al., 2004).

					CI at 95 %	
Var_A	Var_B	Nb_Loc	Rogers distance	E_type	B_inf	B_sup
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

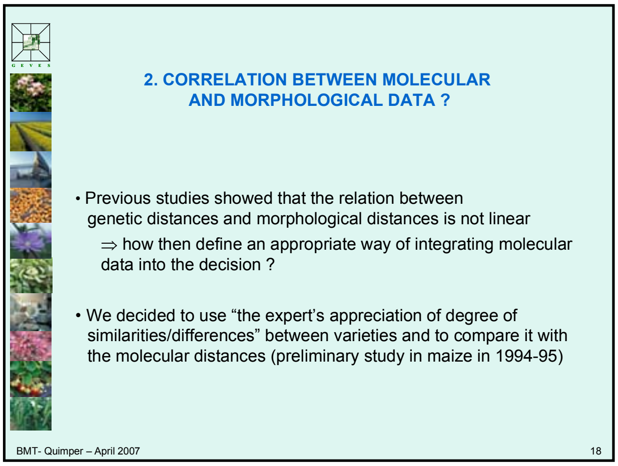
$$D_R^{ij} = \frac{1}{2L}\sum_{l=1}^{L}\sum_{a=1}^{A_l}\left(P_{al}^{i} - P_{al}^{j}\right)^2$$



Roger's distance



Correlation of genetic distances of maize lines analysed with 51 or 36 SSRs (r=0.93), from Mantel Test



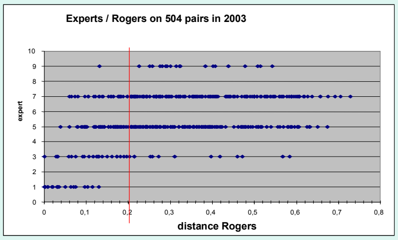
2. CORRELATION BETWEEN MOLECULAR AND MORPHOLOGICAL DATA ?

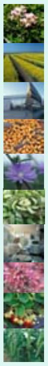
- Previous studies showed that the relation between genetic distances and morphological distances is not linear
  - ⇒ how then define an appropriate way of integrating molecular data into the decision ?
- We decided to use "the expert's appreciation of degree of similarities/differences" between varieties 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





- **COMBINATION OF MOLECULAR DATA AND COMPARISON WITH EXISTING SYSTEMS**

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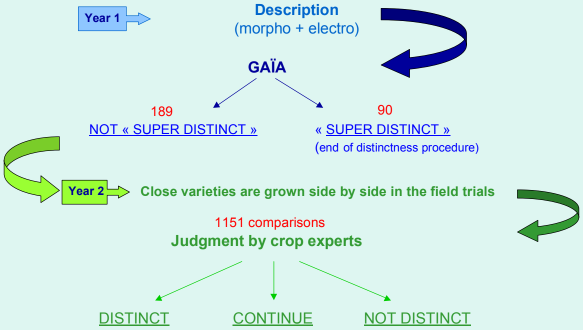

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CONDITION FOR BEING « SUPER DISTINCT »

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    and
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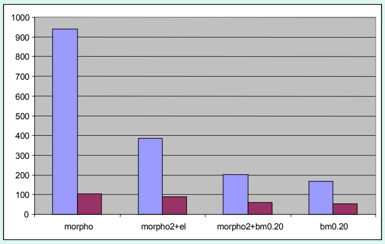
Possible new System

- **Only with morphology**
  - Morphological distance = 6
    (according to the weightings of the differences observed in GAIA)

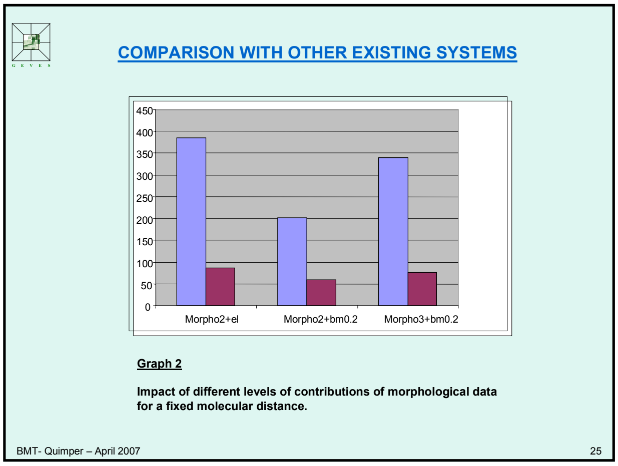
or

- **Combination of morphology and genetic distance**
- Morphological distance = 2
  and
  Genetic distance = 20%

COMPARISON WITH OTHER EXISTING SYSTEMS



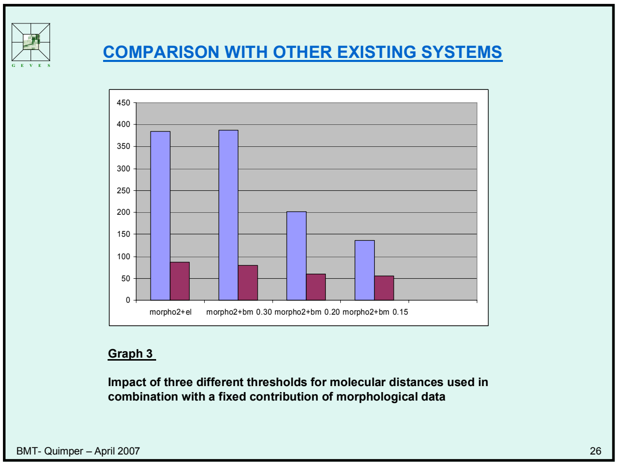
▨ **Number of pairs of varieties to grow in the field trials**
▨ **Number of varieties "non super distinct" (index<6)**



COMPARISON WITH OTHER EXISTING SYSTEMS

Graph 2

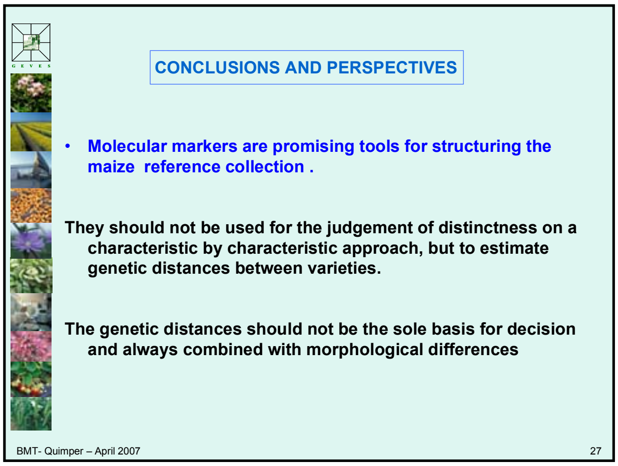
Impact of different levels of contributions of morphological data for a fixed molecular distance.



COMPARISON WITH OTHER EXISTING SYSTEMS

Graph 3

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

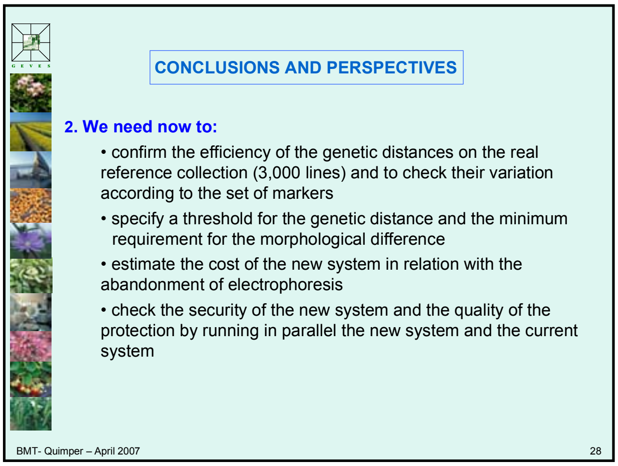


CONCLUSIONS AND PERSPECTIVES

- **Molecular markers are promising tools for structuring the maize reference collection .**

They should not be used for the judgement of distinctness on a characteristic by characteristic approach, but to estimate genetic distances between varieties.

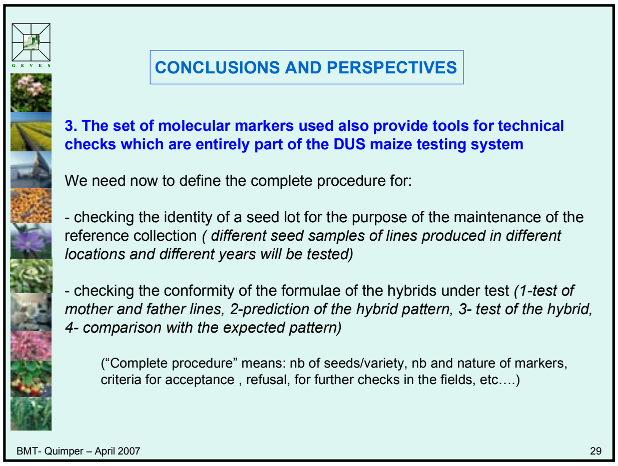
The genetic distances should not be the sole basis for decision and always combined with morphological differences



CONCLUSIONS AND PERSPECTIVES

2. We need now to:

- confirm the efficiency of the genetic distances on the real reference collection (3,000 lines) and to check their variation according to the set of markers
- specify a threshold for the genetic distance and the minimum requirement for the morphological difference
- estimate 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



CONCLUSIONS AND PERSPECTIVES

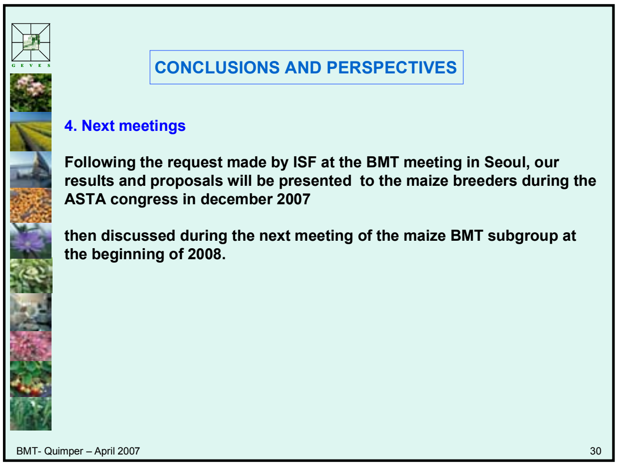
3. The set of molecular markers used also provide tools for technical checks which are entirely part of the DUS maize testing system

We need now to define the complete procedure for:

- checking the identity of a seed lot for the purpose of the maintenance of the reference collection *(different seed samples of lines produced in different locations and different years will be tested)*

- checking the conformity of the formulae of the hybrids under test *(1-test of mother and father lines, 2-prediction of the hybrid pattern, 3- test of the hybrid, 4- comparison with the expected pattern)*

(“Complete procedure” means: nb of seeds/variety, nb and nature of markers, criteria for acceptance , refusal, for further checks in the fields, etc….)



CONCLUSIONS AND PERSPECTIVES

4. Next meetings

Following the request made by ISF at the BMT meeting in Seoul, our results and proposals will be presented to the maize breeders during the ASTA congress in december 2007

then discussed during the next meeting of the maize BMT subgroup at the beginning of 2008.