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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR MAIZE

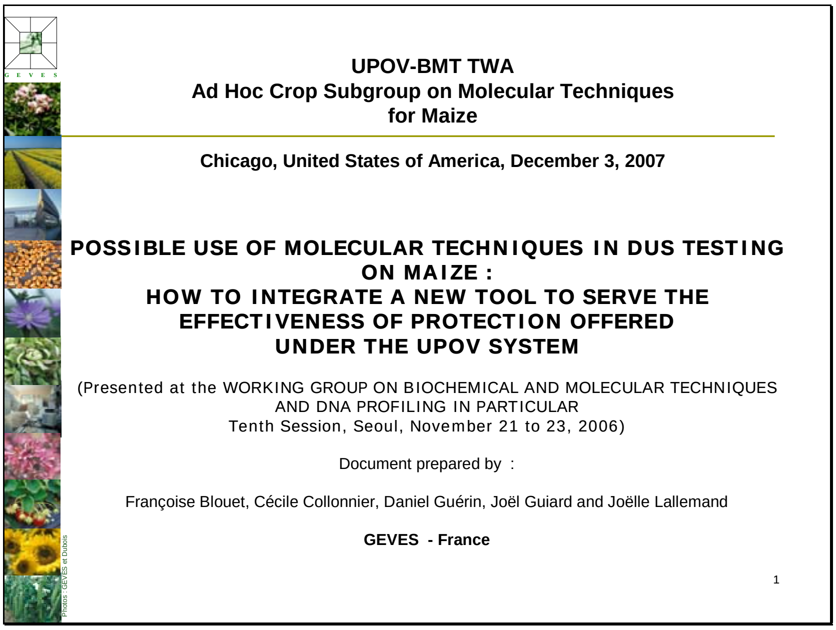
Second Session
Chicago, United States of America, December 3, 2007

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

Document prepared by experts from France

Slide 1



GEVES

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

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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)

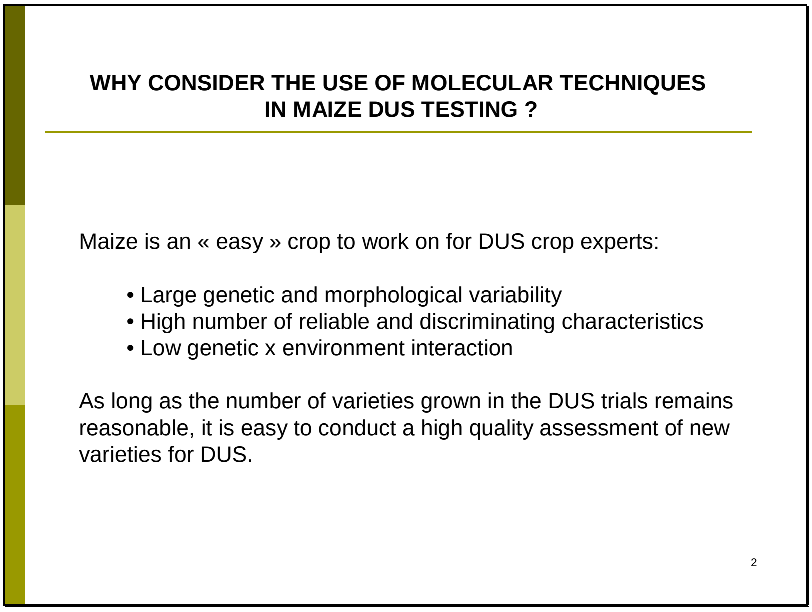
Document prepared by :

Françoise Blouet, Cécile Collonnier, Daniel Guérin, Joël Guiard and Joëlle Lallemand

GEVES - France

Photos : GEVES et Dubois

Slide 2



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.

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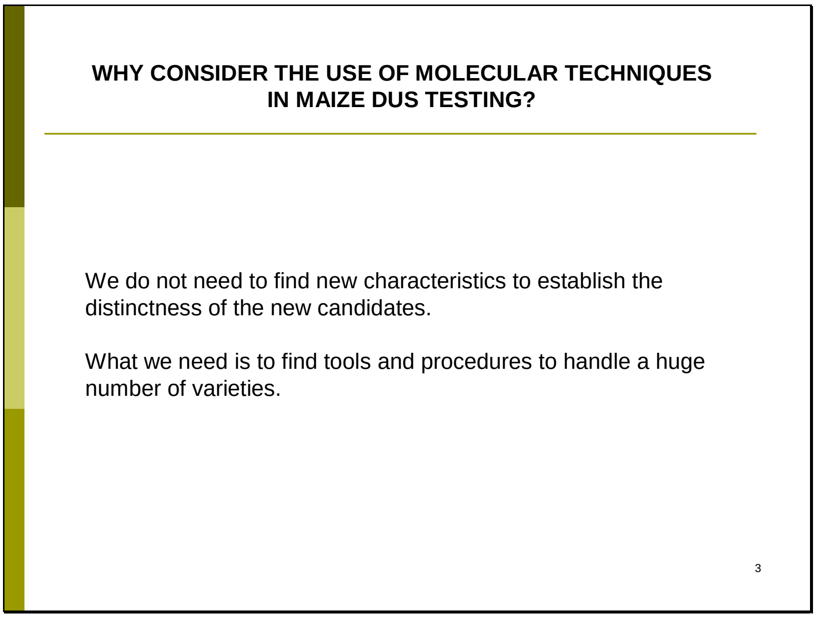
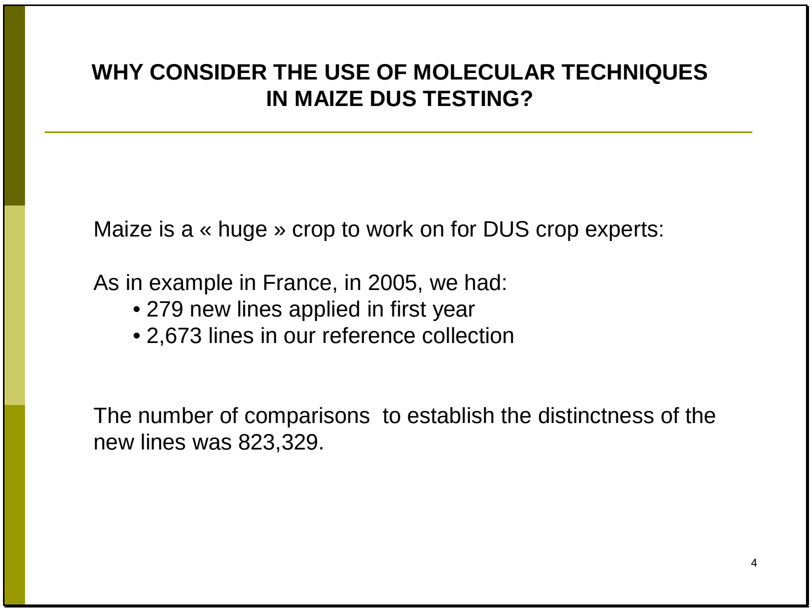


WHY CONSIDER THE USE OF MOLECULAR TECHNIQUES IN MAIZE DUS TESTING?

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.

Slide 4



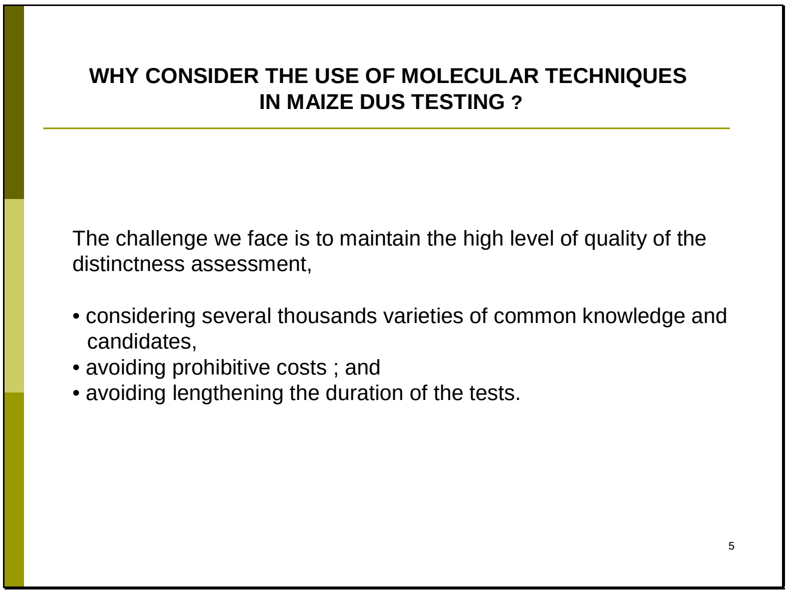
WHY CONSIDER THE USE OF MOLECULAR TECHNIQUES IN MAIZE DUS TESTING?

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.

Slide 5

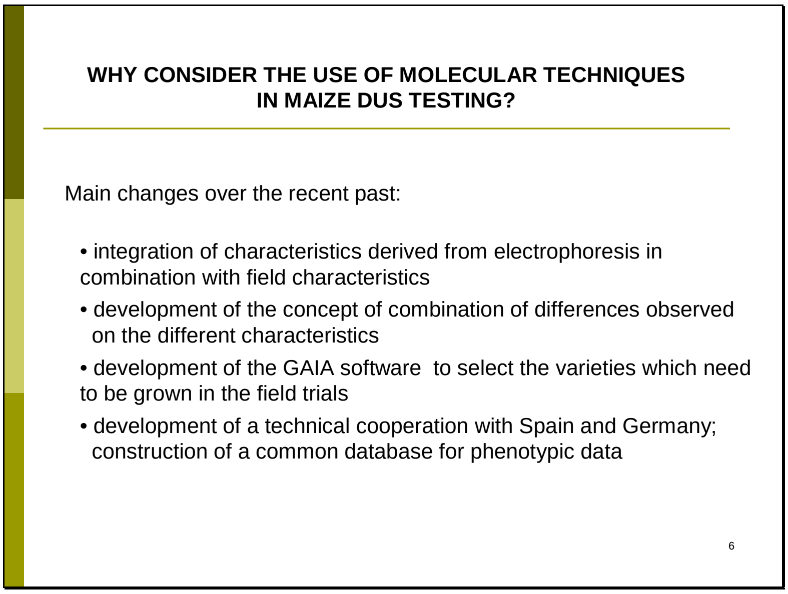


WHY CONSIDER THE USE OF MOLECULAR TECHNIQUES IN MAIZE DUS TESTING ?

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.

Slide 6

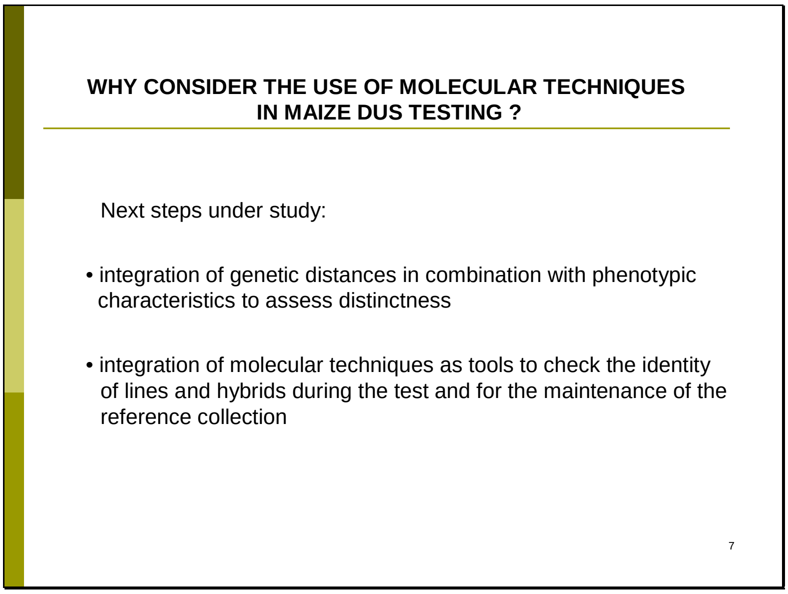


WHY CONSIDER THE USE OF MOLECULAR TECHNIQUES IN MAIZE DUS TESTING?

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

Slide 7



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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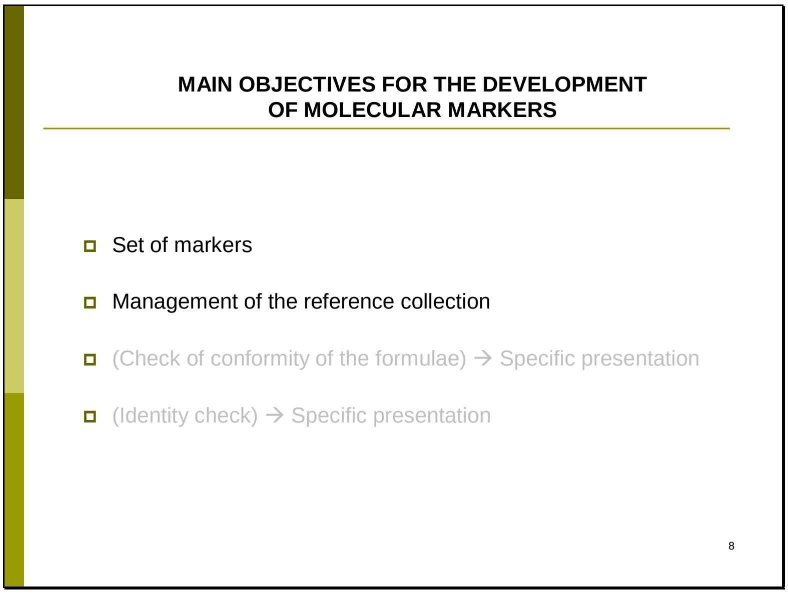


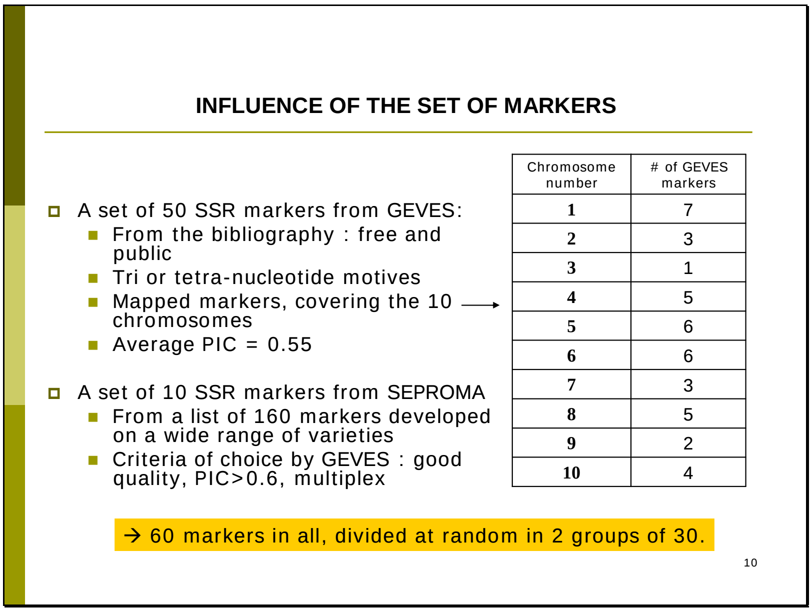
MAIN OBJECTIVES FOR THE DEVELOPMENT OF MOLECULAR MARKERS

- Set of markers
- Management of the reference collection
- (Check of conformity of the formulae) → Specific presentation
- (Identity check) → Specific presentation

Slide 9

Set of markers

Slide 10



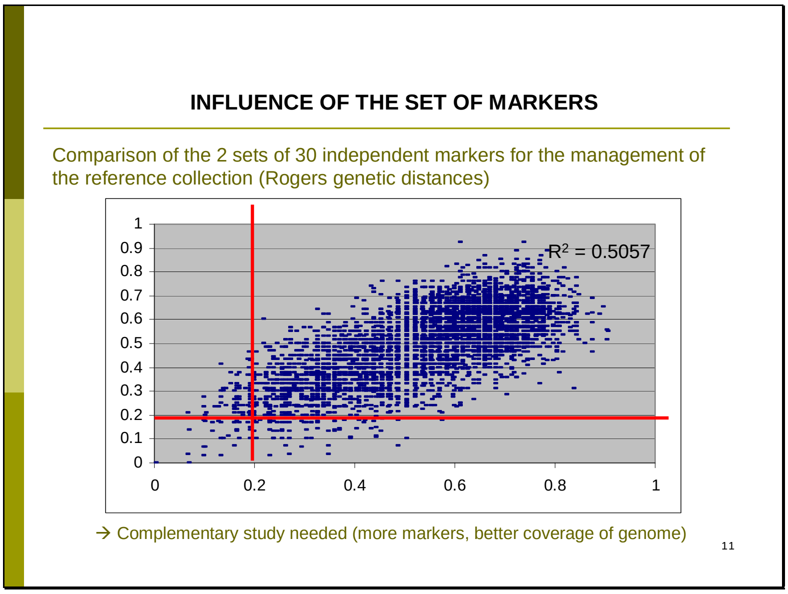
INFLUENCE OF THE SET OF MARKERS

- A set of 50 SSR markers from GEVES:
  - From the bibliography : free and public
  - Tri or tetra-nucleotide motives
  - Mapped markers, covering the 10 chromosomes →
  - Average PIC = 0.55
- A set of 10 SSR markers from SEPROMA
  - From a list of 160 markers developed on a wide range of varieties
  - Criteria of choice by GEVES : good quality, PIC>0.6, multiplex

Chromosome number	# of GEVES markers
1	7
2	3
3	1
4	5
5	6
6	6
7	3
8	5
9	2
10	4

→ 60 markers in all, divided at random in 2 groups of 30.

Slide 11

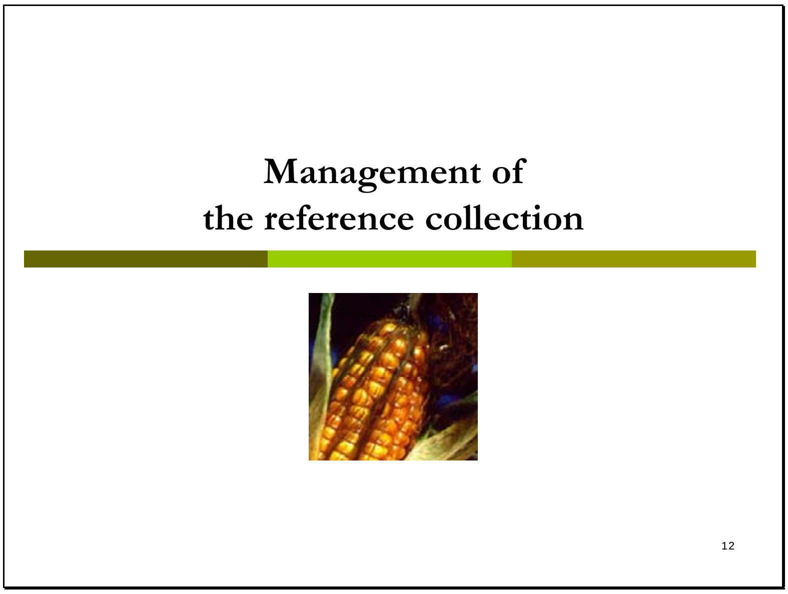


INFLUENCE OF THE SET OF MARKERS

Comparison of the 2 sets of 30 independent markers for the management of the reference collection (Rogers genetic distances)

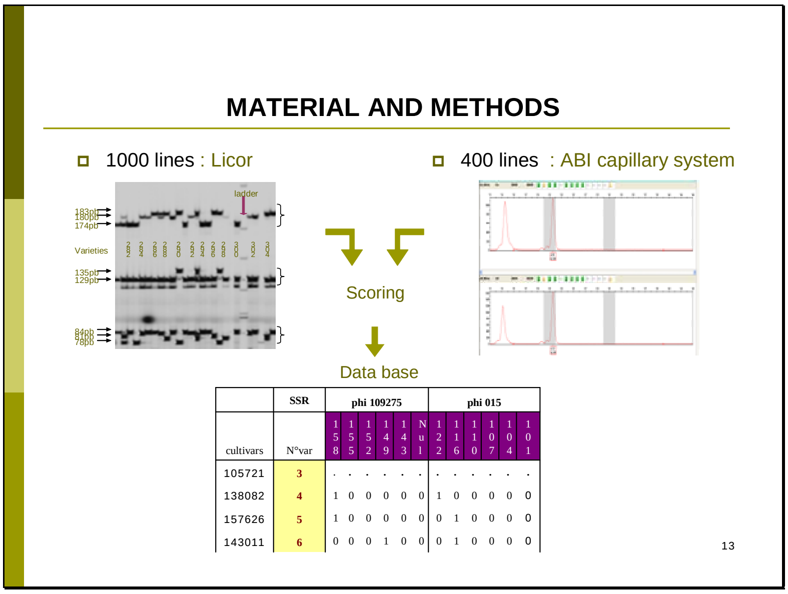
→ Complementary study needed (more markers, better coverage of genome)

Slide 12



Management of the reference collection

Slide 13



MATERIAL AND METHODS

- 1000 lines : Licor

- 400 lines : ABI capillary system

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

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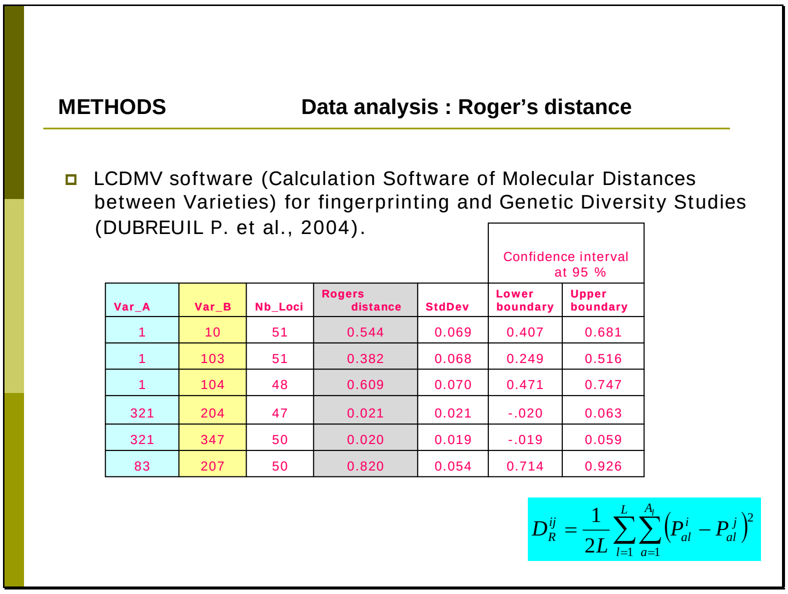


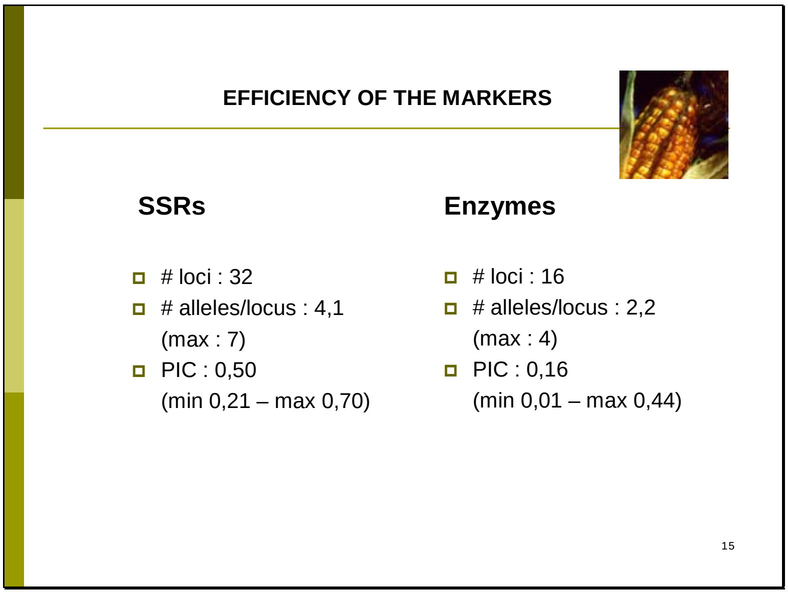
METHODS Data analysis : Roger's distance

- 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

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

Slide 15



EFFICIENCY OF THE MARKERS

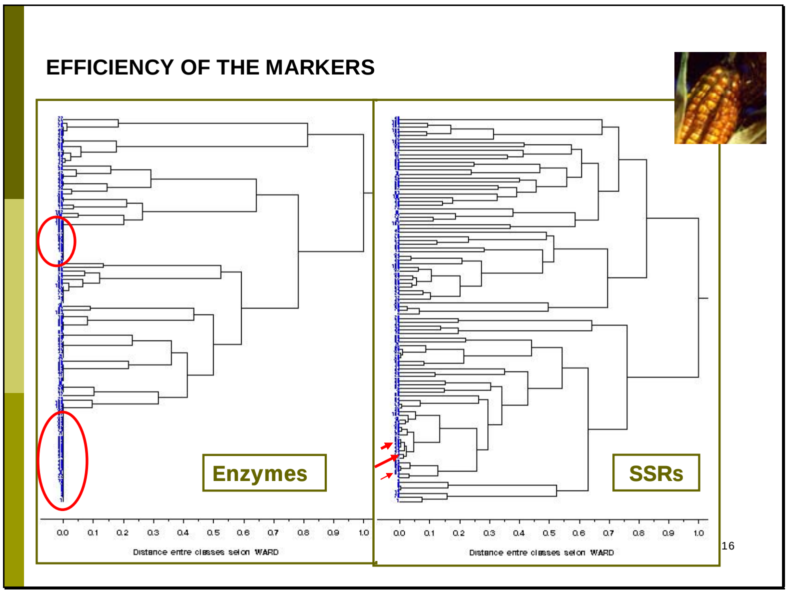
SSRs

- # loci : 32
- # alleles/locus : 4,1 (max : 7)
- PIC : 0,50 (min 0,21 – max 0,70)

Enzymes

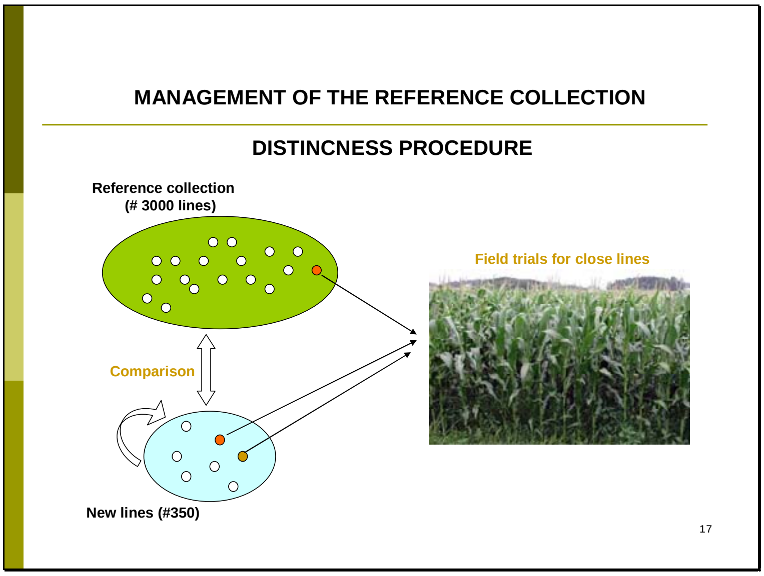
- # loci : 16
- # alleles/locus : 2,2 (max : 4)
- PIC : 0,16 (min 0,01 – max 0,44)

Slide 16



EFFICIENCY OF THE MARKERS

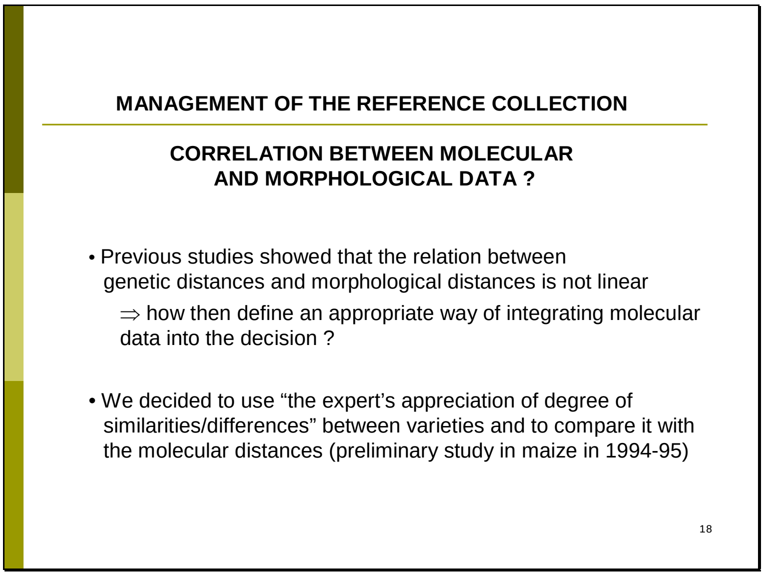
Slide 17



MANAGEMENT OF THE REFERENCE COLLECTION

DISTINCNESS PROCEDURE

Slide 18



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)

Slide 19

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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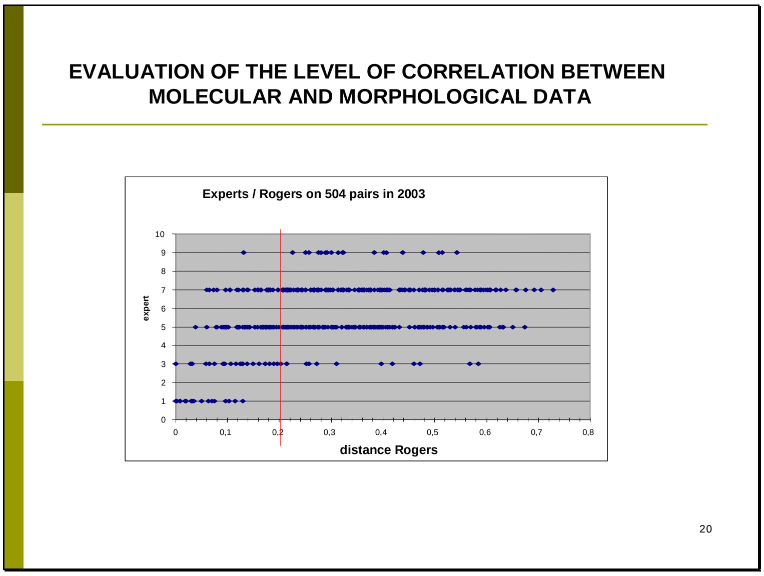
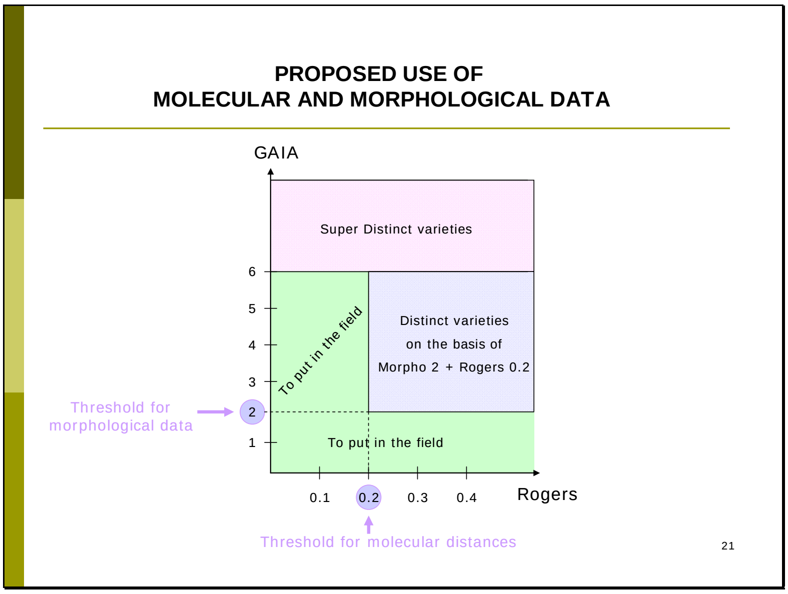


EVALUATION OF THE LEVEL OF CORRELATION BETWEEN MOLECULAR AND MORPHOLOGICAL DATA

Slide 21



PROPOSED USE OF MOLECULAR AND MORPHOLOGICAL DATA

Super Distinct varieties

Distinct varieties on the basis of Morpho 2 + Rogers 0.2

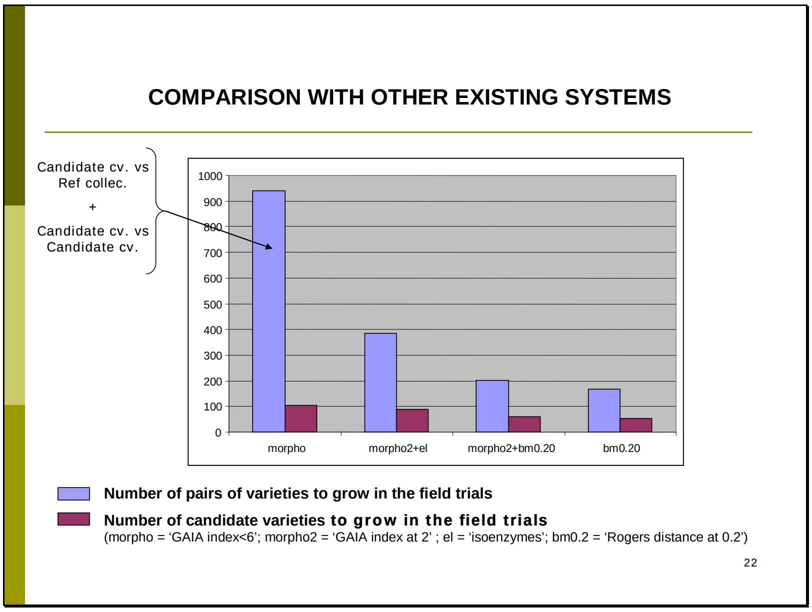
To put in the field

To put in the field

Threshold for morphological data

Threshold for molecular distances

Slide 22



COMPARISON WITH OTHER EXISTING SYSTEMS

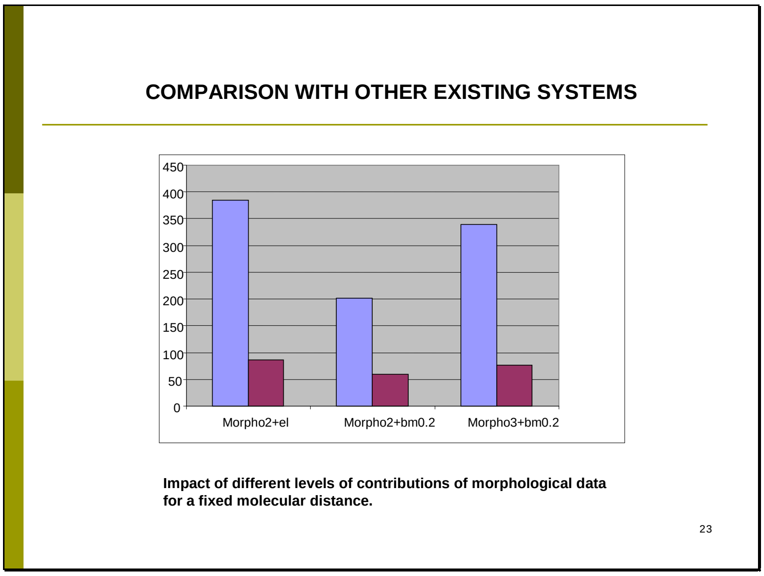
Candidate cv. vs Ref collec.
+
Candidate cv. vs Candidate cv.

Number of pairs of varieties to grow in the field trials

Number of candidate 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’)

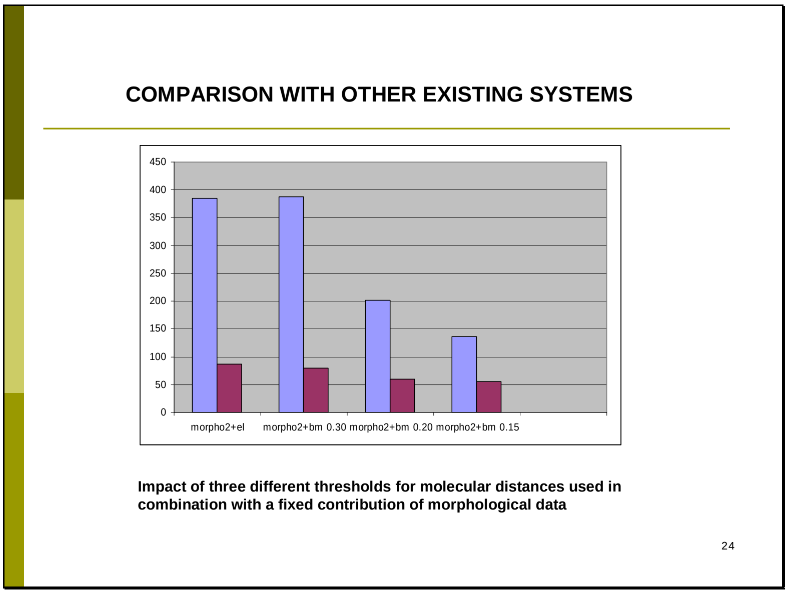
Slide 23



COMPARISON WITH OTHER EXISTING SYSTEMS

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

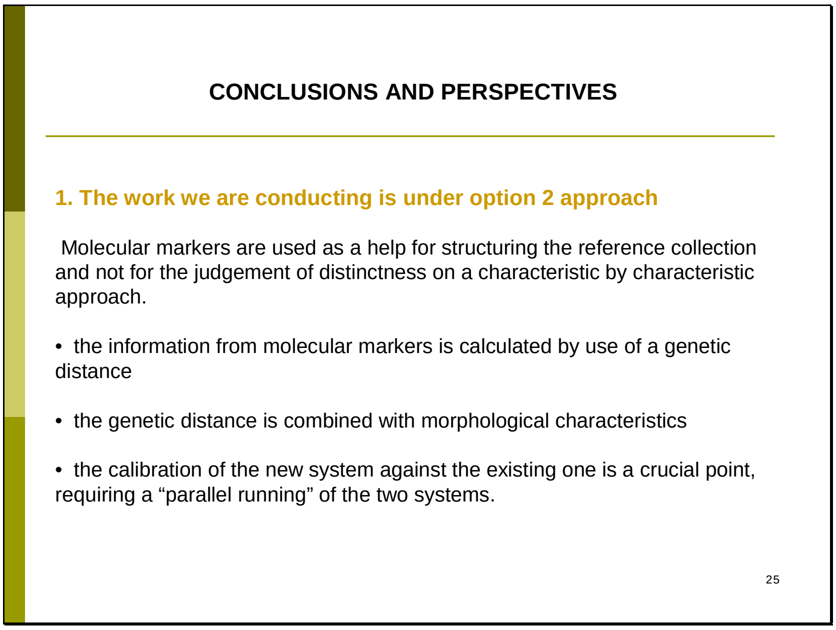
Slide 24



COMPARISON WITH OTHER EXISTING SYSTEMS

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

Slide 25



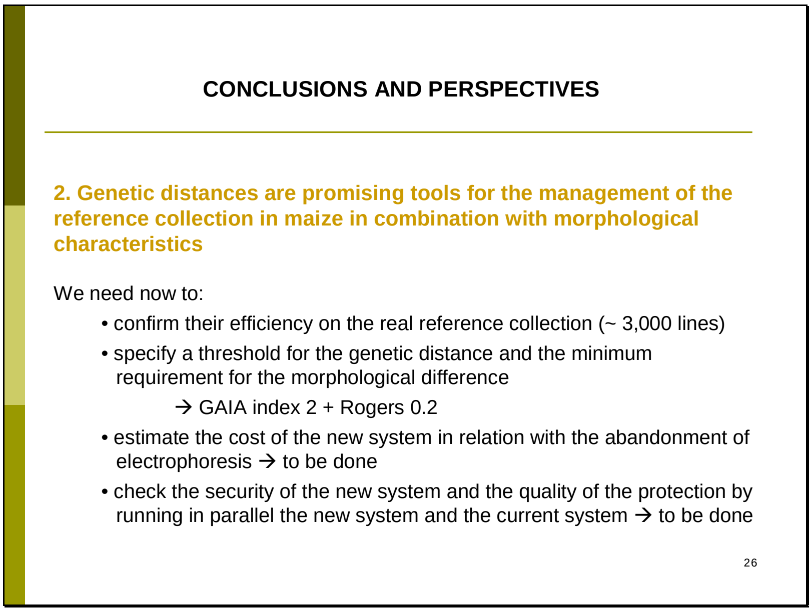
CONCLUSIONS AND PERSPECTIVES

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.

Slide 26



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

Slide 27

THANK YOU FOR YOUR ATTENTION !

GEVES

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