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

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 Françoise Blouet, Cécile Collonnier, Daniel Guérin, Joël Guiard and Joëlle Lallemand – GEVES - France

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*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, 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.

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 (making 836,882 comparisons take a few hours)
- development of a technical cooperation with Spain and Germany; construction of a common database for phenotypic data

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

Main objectives for the development of molecular markers

- Management of the reference collection
- Check of conformity of the formulae
- Identity check

Materials

• Management of the reference collection	930 lines
• Check of conformity of the formulae	28 hybrids +56 parental lines
• Identity check	162 lines

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

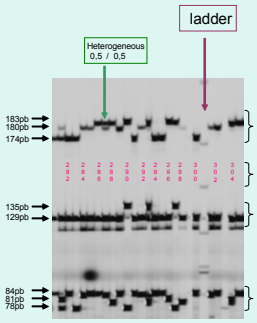
- 50 public microsatellites
- Tri or tetra-nucleotide motives
- Mapped markers

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	152	149	143	Nu1	122	116	107	104	101
105721	3	.	.	.	.	.	.	.	.	.	.	.
138082	4	1	0	0	0	0	0	1	0	0	0	0
157626	5	1	0	0	0	0	0	0	1	0	0	0
143011	6	0	0	0	1	0	0	0	1	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

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

Efficiency of the markers

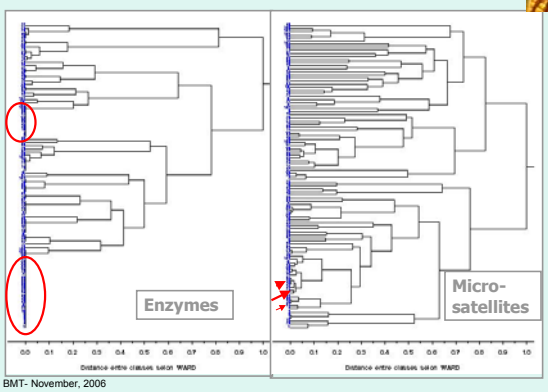
SSRs

- Nb loci : 32
- Nb allèles/locus : 4,1 (max : 7)
- PIC : 0,50 (0,21 - 0,70)

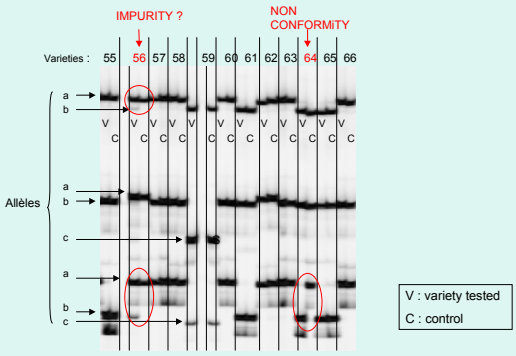
Enzymes

- Nb loci : 16
- Nb allèles/locus : 2,2 (max : 4)
- PIC : 0,16 (0,01 - 0,44)

Efficiency of the markers



Identity check



Identity Check

- 162 seedlots tested

151	conform
8	Non fixation
4	Different for:
1	1 locus
3	2 loci
1	7 loci

Identity check

Markers	phi 084		phi 127			umc 1152			phi 452693			phi 333597		
Alleles	a	b	a	b	c	a	b	c	a	b	c	a	b	c
Sample 1	1		1			1			1			1		
Control 1	1		1			1			1			1	1	
Sample 2	1		1	1			1			1		1		1
Control 2	1			1			1			1		1		
Sample 3	1	1			1	1	1				1			1
Control 3	1				1	1	1				1			1
Sample 4		1		1		1				1				1
Control 4		1		1		-				1				1
Sample 5		1	1				1				1			1
Control 5		1	1				1				1			1
Sample 6		1	1				1			1				1
Control 6	1				1	1					1			1
Sample 7	1		1				1	1		1			1	
Control 7	1		1				1	1		1			1	
Sample 8		1	1			1	1				1			1
Control 8		1	1				1				1		1	
Sample 9	1		1				1				1	1		1
Control 9	1		1				1				1	1		
Sample 10		1		1				1		1				1
Control 10		1		1				1		1	1			1
Sample 11	1			1			1				1	1	1	1
Control 11		1		1	1		1				1	1	1	1

Check of conformity of the formulae

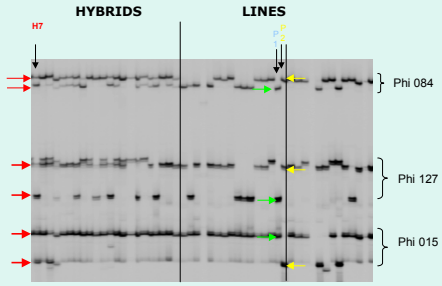
Materials

★ **28 Hybrids**
★ **56 Parental lines**
(Bulks of 30 seeds)

Methods

★ **12 microsatellites**
★ **comparison hybrid profiles / parental lines**

Check of conformity of the formulae



Check of conformity of the formulae

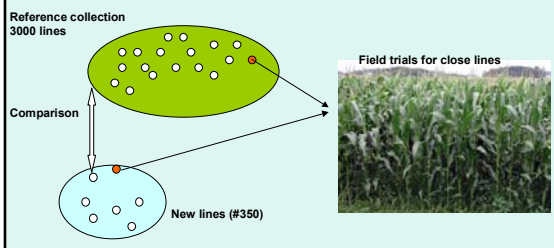
possible cases :

	Markers	phi 084		phi 127			umc 1152			phi 462693			phi 333597		
	Alleles	a	b	a	b	c	a	b	c	a	b	c	a	b	c
F1 Hybrid	Parent 1	1			1					1			1		
	Parent 2		1	1					1	1					1
	Expected Hybrid	1	1	1	1		1		1	1			1		1
3 Ways Hybrid	Parent 1	1			1		1					1			1
	Parent 2	1			1		1					1	1		
	Parent 3	1		1				1		1				1	
	Expected Hybrid	1		1	1		1	1		1		1	1	1	1
non conformity	Parent 1	1			1		1					1		1	
	Parent 2	1			1		1					1			1
	Expected	1			1		1					1		1	1
	Observed	1		1		1	1			1				1	1

Check of conformity of the formulae

- Results
  Concordance enzyme/BM : 22/27 hybrids
  Enzyme C/MM NC : 2/27
- Perspectives
  Elaboration of the complete procedure including the protocol and the decision rules
  (nb of markers; nb of seeds tested; criteria for acceptance, refusal and further study including field checks, etc…)

Management of the reference collection distinctness procedure



MANAGEMENT OF THE REFERENCE COLLECTION

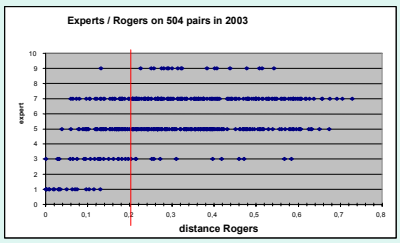
CORRELATION BETWEEN MOLECULAR AND MORPHOLOGICAL DATA ?

- 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)

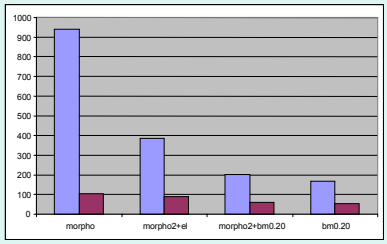
THE EXPERT’S APPRECIATION OF DEGREE OF SIMILARITY/DIFFERENCE BETWEEN 2 VARIETIES

- **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



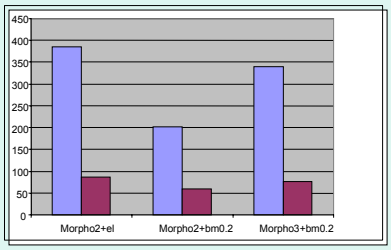
COMPARISON WITH OTHER EXISTING SYSTEMS



Number of pairs of varieties to grow in the field trials

Number of varieties "non super distinct" (index<6)

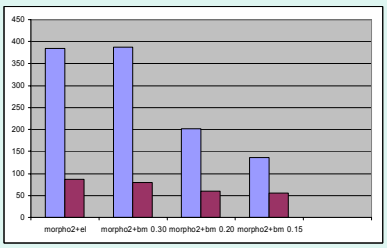
COMPARISON WITH OTHER EXISTING SYSTEMS



Graph 2

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

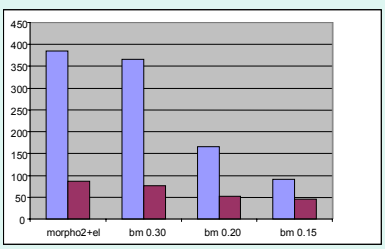
COMPARISON WITH OTHER EXISTING SYSTEMS



Graph 3

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

COMPARISON WITH OTHER EXISTING SYSTEMS



Graph 4

Illustration with only molecular thresholds

CONCLUSIONS AND PERSPECTIVES

1. 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
- 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

2. The set of molecular markers used provide tools for technical checks which are entirely part of the DUS 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
- checking the conformity of the formulae of the hybrids under test

CONCLUSIONS AND PERSPECTIVES

3. 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.