UPOV

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THE USE OF MOLECULAR MARKERS (SNP) FOR MAIZE DUS TESTING: DEVELOPMENT AND OFFICIAL APPLICATIONS TO ASSESS DISTINCTNESS OF HYBRIDS VARIETIES (FRANCE)

Document prepared by an expert from France

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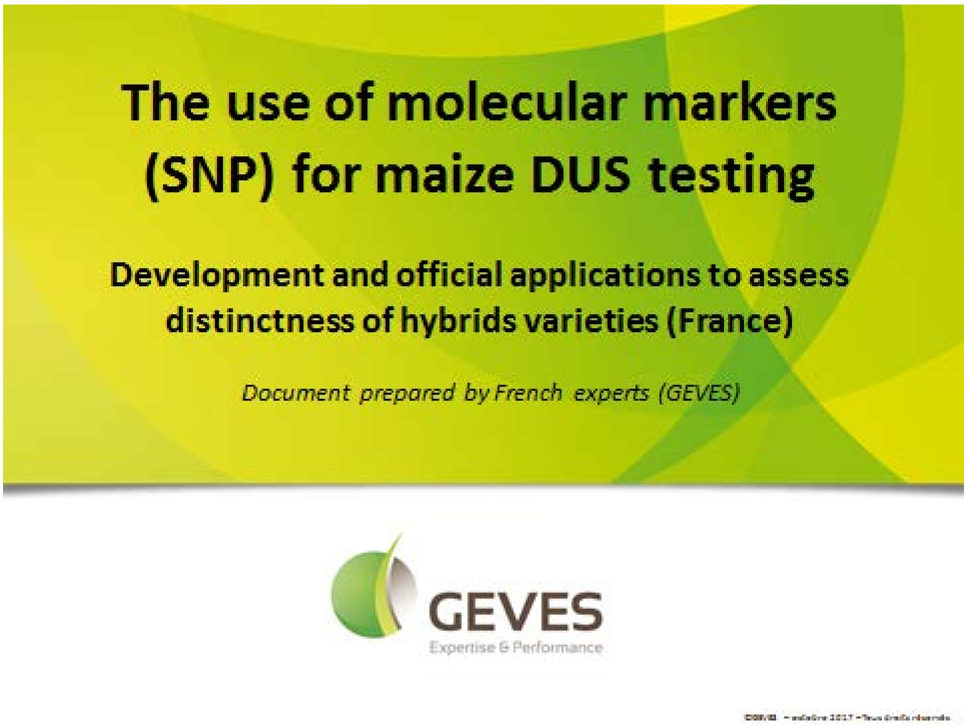
1. According to the recommendations in the Test Guidelines for Maize, document TG/2/7, we follow the required steps to assess the distinctness of hybrid varieties. First, we describe their parental lines and check their distinctness in comparison with our reference collection (5000 lines). At the same time, we check the originality of the hybrid formula in comparison with those of the hybrids of our reference collection (4500 hybrids). Finally, we assess distinctness at the hybrid level for varieties with a similar formula.

2. For the assessment of distinctness of the hybrid lines, we divide our process into different steps in order to identify the similar hybrids (with a similar formula). We have started a study to include molecular data (SNP) in addition to the hybrid formula data and morphological description. After a methodological study, we have been successful in developing a rule taking into account the hybrid formula, morphological data and molecular data.

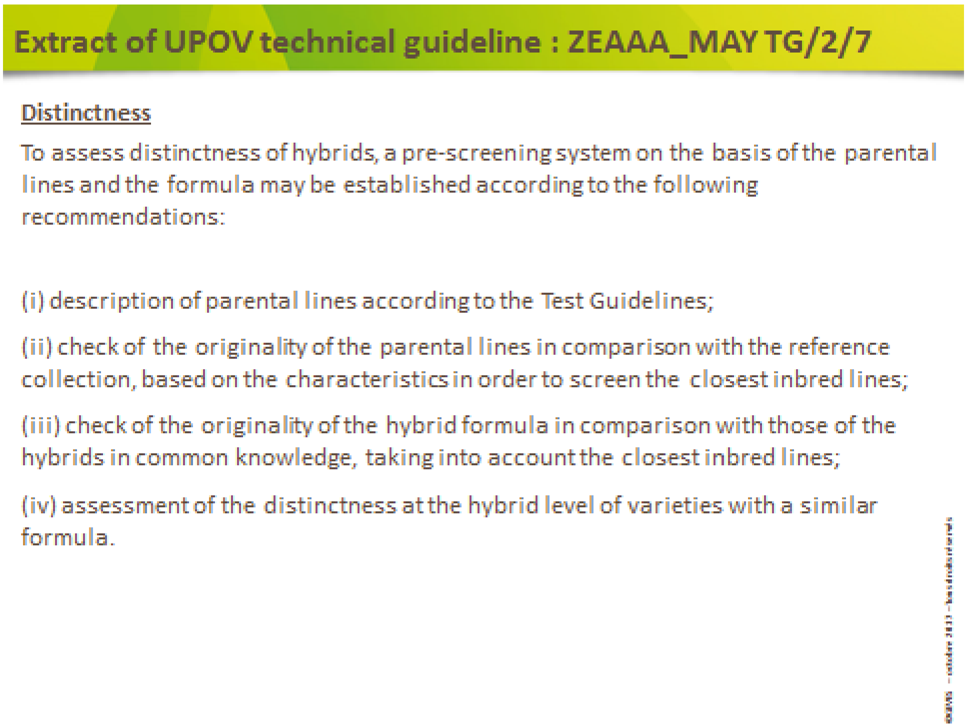
[Annex follows]



The use of molecular markers (SNP) for maize DUS testing

Development and official applications to assess distinctness of hybrids varieties (France)

Document prepared by French experts (GEVES)



Extract of UPOV technical guideline : ZEAAA_MAY TG/2/7

Distinctness

To assess distinctness of hybrids, a pre-screening system on the basis of the parental lines and the formula may be established according to the following recommendations:

(i) description of parental lines according to the Test Guidelines;

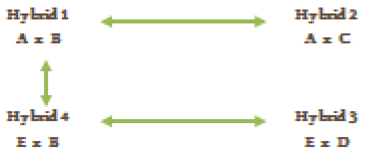
(ii) check of the originality of the parental lines in comparison with the reference collection, based on the characteristics in order to screen the closest inbred lines;

(iii) check of the originality of the hybrid formula in comparison with those of the hybrids in common knowledge, taking into account the closest inbred lines;

(iv) assessment of the distinctness at the hybrid level of varieties with a similar formula.

The 4 steps of our method to identify the most similar hybrids varieties :

1/ Identification of hybrid's pairs with one common parent in the formula



2/ Identification of hybrid's pairs for which the non common parental lines show a morphological distance less than a threshold of 6

Hybrid 1 vs Hybrid 2 : Morphological distance between B and C ≥ 6
Hybrid 1 vs Hybrid 4 : Morphological distance between A and E < 6
Hybrid 3 vs Hybrid 4 : Morphological distance between B and D ≥ 6

The 4 steps of our method to identify the most similar hybrids varieties :

1/ Identification of hybrid's pairs with one common parent in the formula

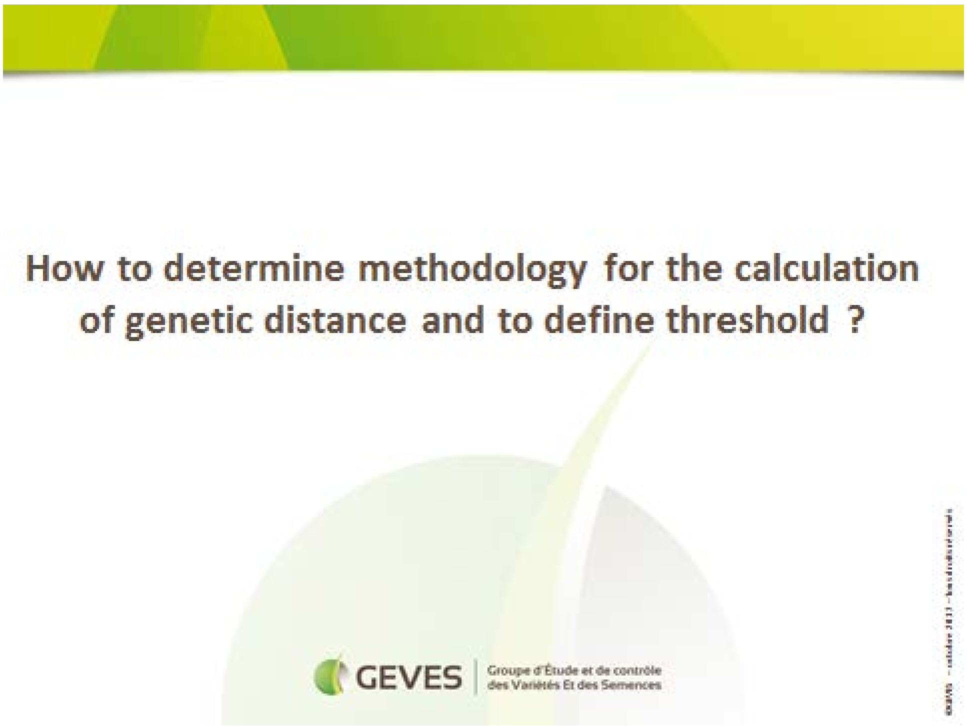
2/ Identification of hybrid's pairs for which the non common parental lines show a morphological distance less than a threshold of 6

3/ Identification of hybrid's pairs with a morphological distance less than the threshold of 6

Morphological distance between Hybrid 1 vs Hybrid 4 < 6

4/ Identification of hybrid's pairs with a genetic distance less than a threshold.

Genetic distance between Hybrid 1 vs Hybrid 4 ?



How to determine methodology for the calculation of genetic distance and to define threshold ?

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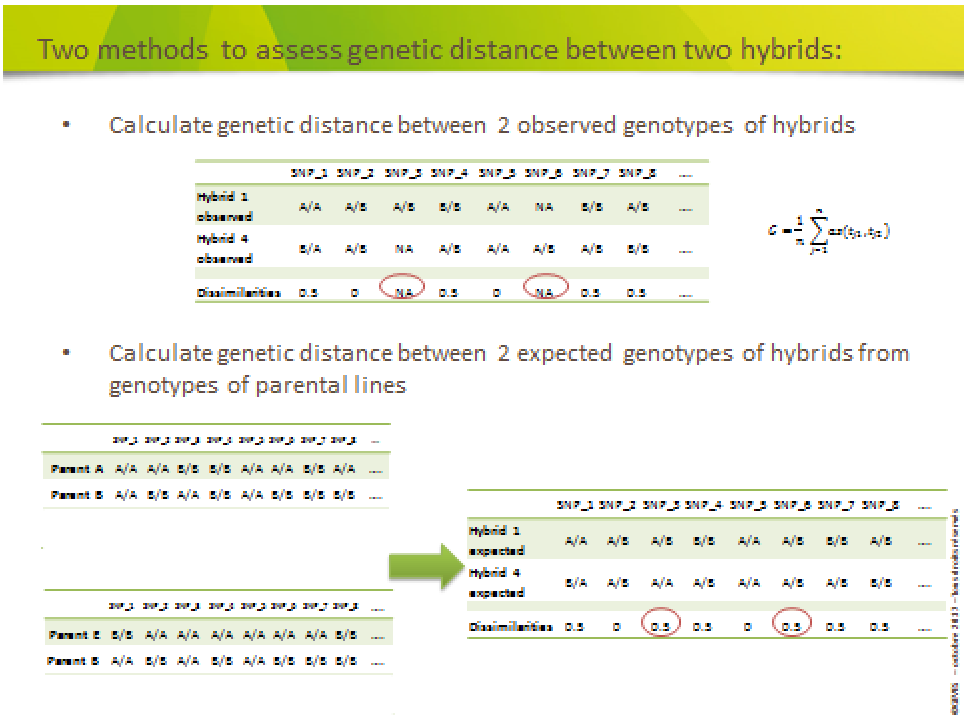


Two methods to assess genetic distance between two hybrids:

- Calculate genetic distance between 2 observed genotypes of hybrids

	SNP_1	SNP_2	SNP_3	SNP_4	SNP_5	SNP_6	SNP_7	SNP_8	....
Hybrid 1 observed	A/A	A/B	A/B	B/B	A/A	NA	B/B	A/B	....
Hybrid 4 observed	B/A	A/B	NA	A/B	A/A	A/B	A/B	B/B	....
Dissimilarities	0.5	0	NA	0.5	0	NA	0.5	0.5	....

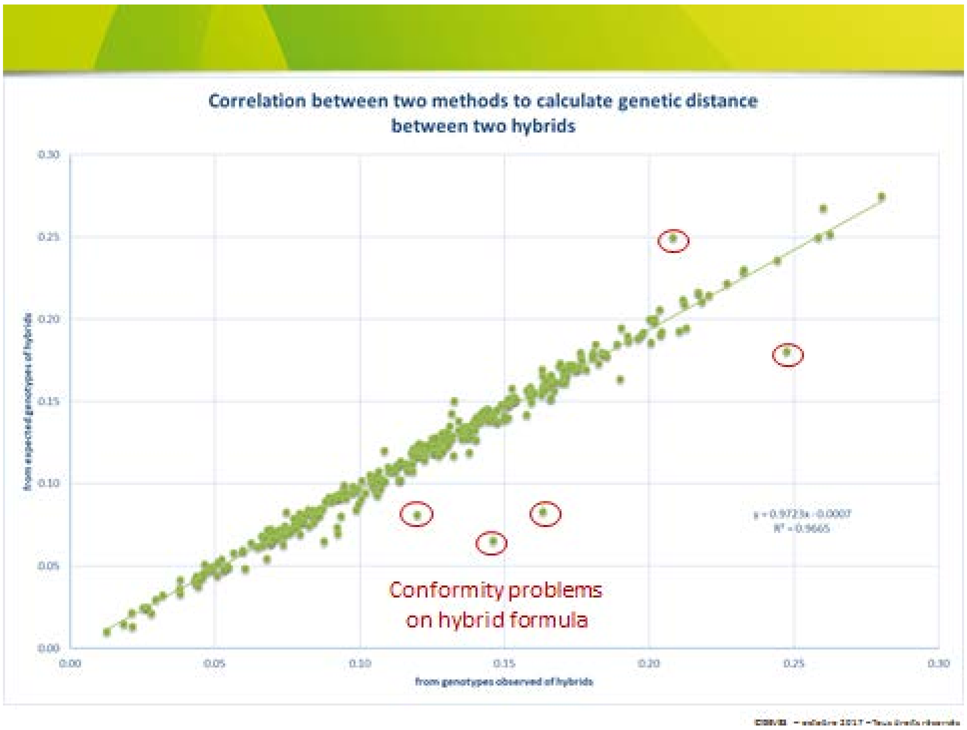
$$G = \frac{1}{n}\sum_{j=1}^{n} ss(t_{j1}, t_{j2})$$

- Calculate genetic distance between 2 expected genotypes of hybrids from genotypes of parental lines

	SNP_1	SNP_2	SNP_3	SNP_4	SNP_5	SNP_6	SNP_7	SNP_8	....
Parent A	A/A	A/A	B/B	B/B	A/A	A/A	B/B	A/A	....
Parent B	A/A	B/B	A/A	B/B	A/A	B/B	B/B	B/B	....

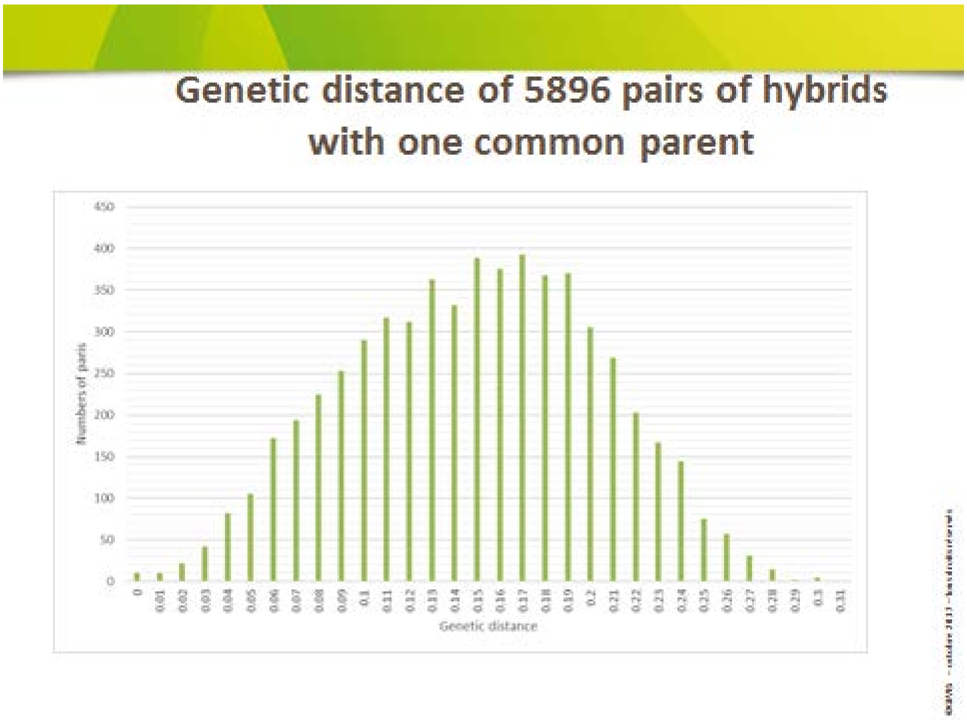
	SNP_1	SNP_2	SNP_3	SNP_4	SNP_5	SNP_6	SNP_7	SNP_8	....
Parent E	B/B	A/A	A/A	A/A	A/A	A/A	A/A	B/B	....
Parent B	A/A	B/B	A/A	B/B	A/A	B/B	B/B	B/B	....

	SNP_1	SNP_2	SNP_3	SNP_4	SNP_5	SNP_6	SNP_7	SNP_8	....
Hybrid 1 expected	A/A	A/B	A/B	B/B	A/A	A/B	B/B	A/B	....
Hybrid 4 expected	B/A	A/B	A/A	A/B	A/A	A/B	A/B	B/B	....
Dissimilarities	0.5	0	0.5	0.5	0	0.5	0.5	0.5	....

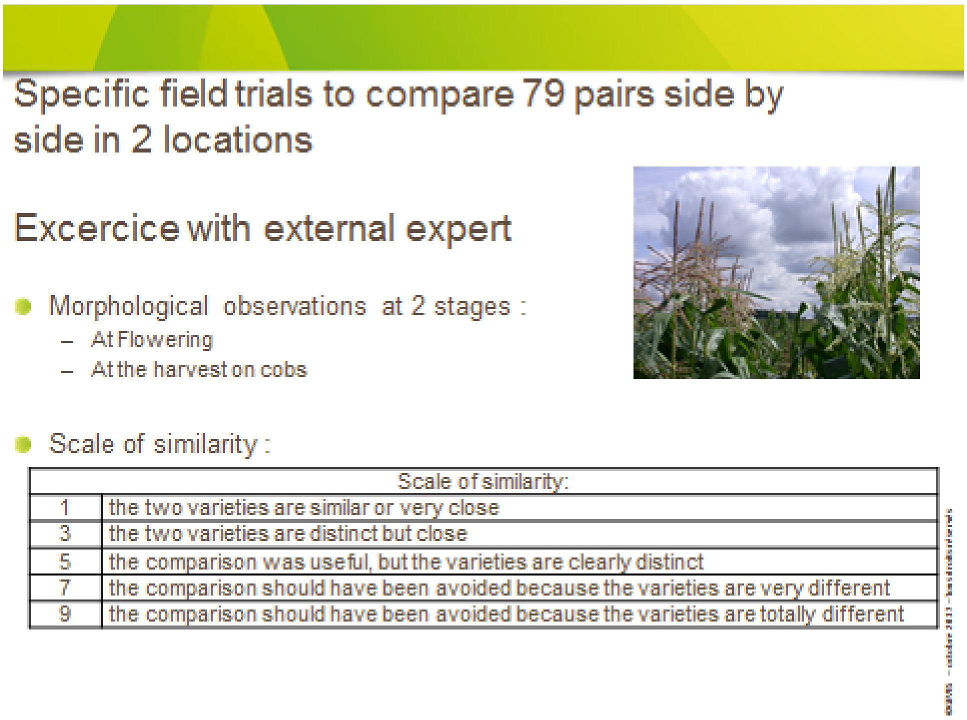


Correlation between two methods to calculate genetic distance between two hybrids

Conformity problems
on hybrid formula



Genetic distance of 5896 pairs of hybrids
with one common parent



Specific field trials to compare 79 pairs side by side in 2 locations

Excercice with external expert

- Morphological observations at 2 stages :
  - At Flowering
  - At the harvest on cobs

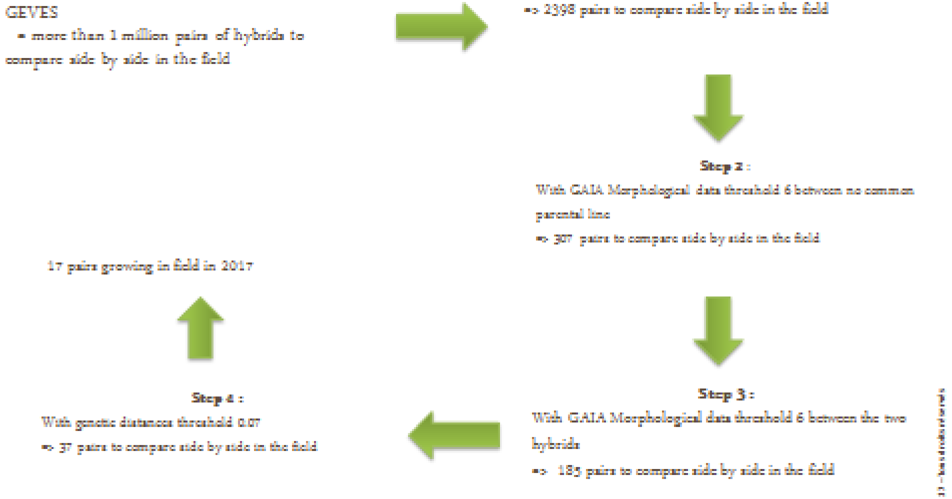
- Scale of similarity :

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

Regarding our experience on inbred lines and the results of our testing in 2016, choice for the Genetic Distance threshold : **0.07**

Relation between the genetic distance and the experts score
on 79 hybrid's pairs observed side by side in the field in 2016

Example with applications of 2016 : 263 hybrids applied



Near 4500 hybrids in the reference collection of GEVES
= more than 1 million pairs of hybrids to compare side by side in the field

Step 1 :
With one common parental in the formula
=> 2398 pairs to compare side by side in the field

Step 2 :
With GAIA Morphological data threshold 6 between no common parental line
=> 307 pairs to compare side by side in the field

Step 3 :
With GAIA Morphological data threshold 6 between the two hybrids
=> 185 pairs to compare side by side in the field

Step 4 :
With genetic distances threshold 0.07
=> 37 pairs to compare side by side in the field

17 pairs growing in field in 2017

Conclusions

- Genetic Distance used into the process to select the most similar hybrids to be compared to the field
- New rules adopted for national listing, thresholds came into force January 2017
- Officially used since 2017 new applications

Benefits :

- Not necessary to genotype all the reference collection of hybrids
- Reasonable number of pairs to check in the field

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Thank you for your attention

November 2017, LA ROCHELLE

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[End of Annex and of document]