The use of SNP markers in maize DUS testing

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Introduction

Maize (*Zea mays* L.) is a cereal crop with important economic value and with a large morphological and genetic variability. The extent of the size of maize reference collection (greater number of varieties of common knowledge and hybrids) has led to an increase of workload and space when conducting DUS (Distinctness, Uniformity and Stability) field trials. *Groupe d'Etude et de contrôle des Variétés Et des Semences* (GEVES) need to implement tools and procedures to continue to manage its reference collection in an efficient way. The previous system used in France to select the varieties to be grown and compared in the field trials used a combination of differences between varieties observed on morphological and electrophoresis characteristics.

The recent developments in high-throughput genotyping provide an opportunity to explore application of new marker technology. Single Nucleotide Polymorphism markers (SNPs) because they are co-dominant, evenly distributed across the genome, reproducible, have become the marker system of choice to study cultivated plant species. The main aim of this study was to test SNPs that have been developed in Maize and to identify a subset of highly informative markers for routine genotyping. Results obtained from SNP markers were used to test the model "Combining Phenotypic and Molecular Distances in the Management of Variety Collection" (UPOV/TGP15/1) and compared with results obtained from electrophoresis.

Selection and validation of the SNP set

50 K SNP chip (UFS – INRA Moulon)

- Quality of the flanked regions
- Evenly distributed throughout the genome
- Minor allele frequency
- High genetic diversity

384 SNP tested on more than 4000 varieties

- SNP primer design, genotyping and alleles call were carried out using KASPar (LGC Genomics) platform
- Over 4000 inbred lines and 400 hybrids

Data analysis & selection of 312 SNP

- Reproducibility
- Ease of scoring
- Low rate of missing data
- Genetic diversity & linkage disequilibrium
- Genetic distances (®)

Threshold estimation

Morphological and genetic threshold

In order to eliminate pairs of varieties prior to the DUS growing trial, there is a need to set a threshold to decide which varieties can be safely excluded. This threshold termed "distinctness plus threshold" has to be calibrated and be robust enough to take a decision without direct comparisons. The system proposed combined genetic and morphological distances (**Figure 1**). The morphological distances are computed with the GAÏA software developed by GEVES, the genetic distances are calculated based on the 312 SNP set.
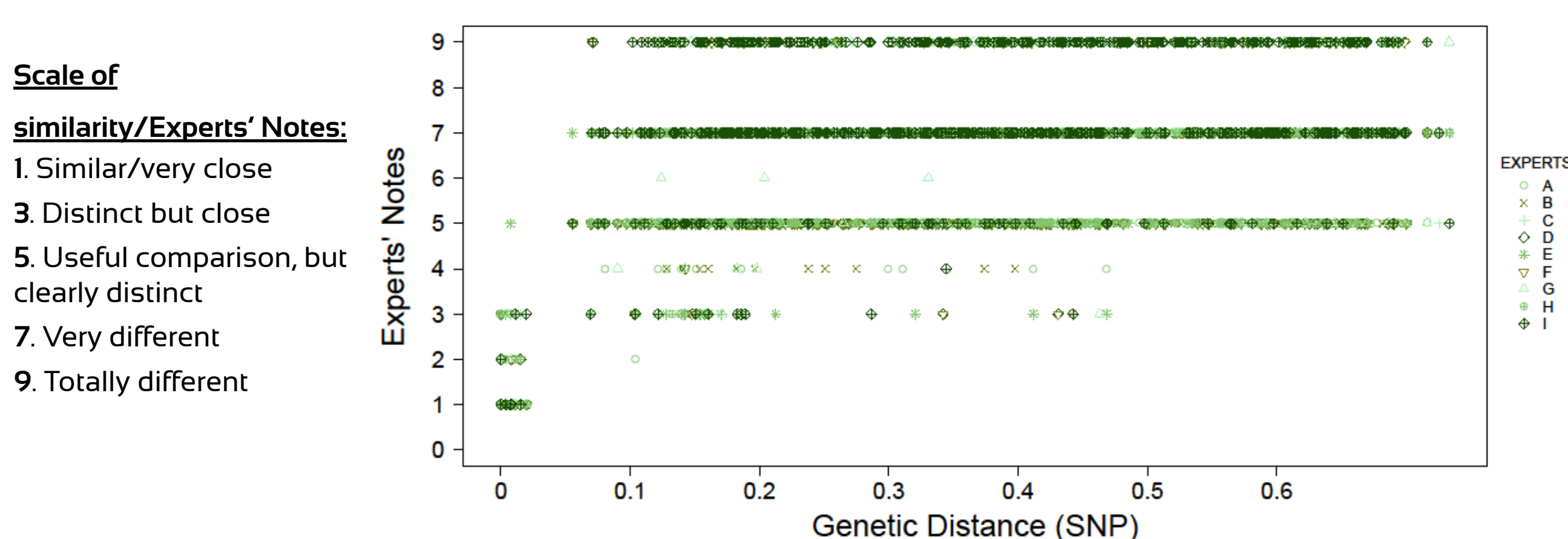

Figure 1: Model combining phenotypic and molecular distances
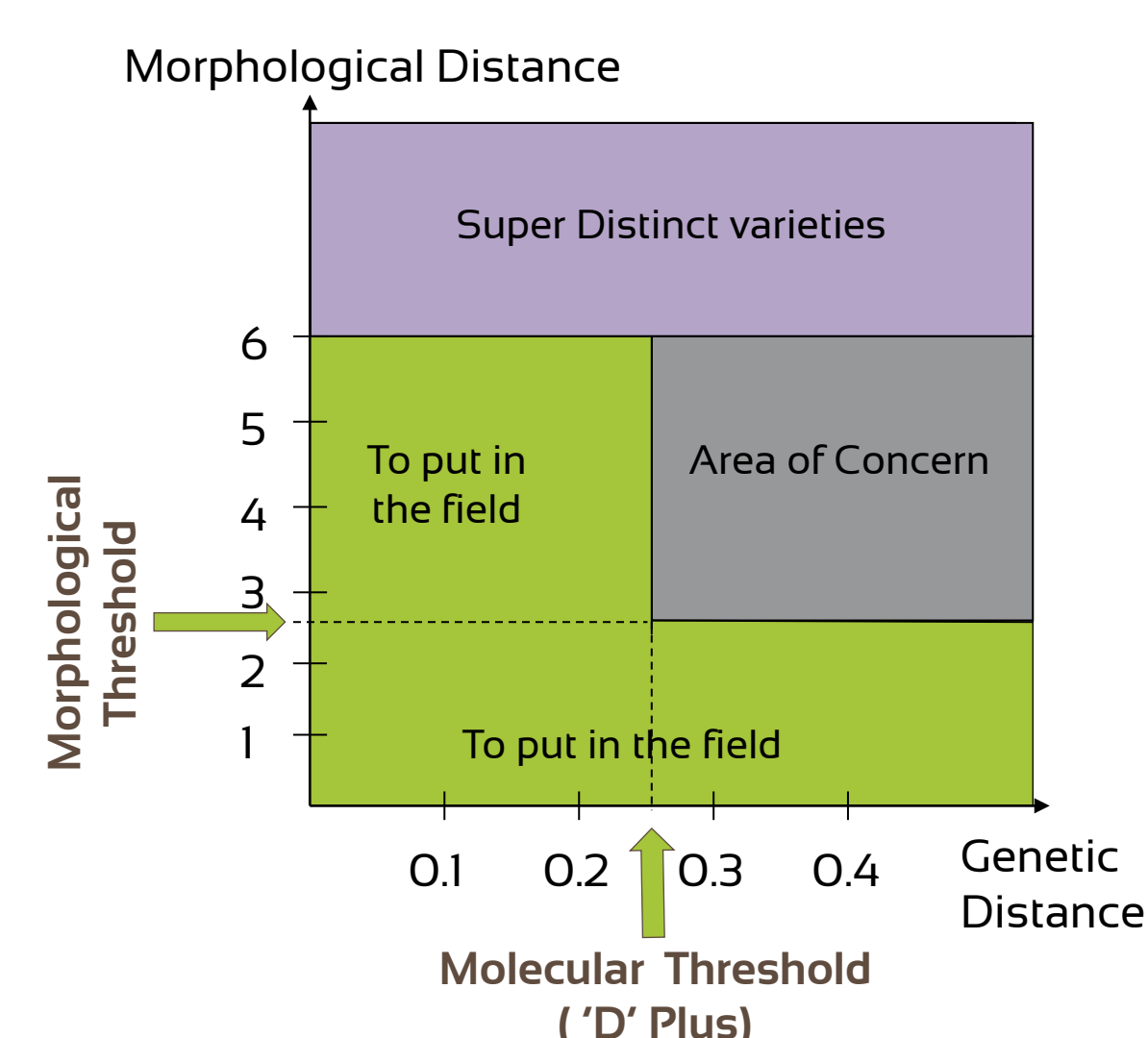
Correlation between molecular and morphological data and threshold calibration

Previous studies showed that the relation between genetic distances and morphological distances is not linear. To define an appropriate way of integrating molecular data into the decision, we used "the expert's appreciation of degree of similarities/differences" between varieties and compare it with the molecular distances (**Figure 2**). Different pairs of varieties were compared successively during years 2011, 2012, 2013.

Figure 2: Correlation between genetic distance and the experts note on 654 pairs of maize of inbred lines observed side-by-side in the field in 2012

Scale of similarity/Experts' Notes:
1. Similar/very close
3. Distinct but close
5. Useful comparison, but clearly distinct
7. Very different
9. Totally different

Over the 3 years, results have shown that no pairs of inbred lines with a genetic distance greater than 0.18 were considered as similar or very close during experts' evaluation. To have a secure and reliable threshold, the threshold of 0.2 genetic distance was confirmed and adopted in combination with morphological distances (GAÏA) between 2 and 6 already in used in the previous decision scheme.

SNP versus electrophoresis

During the 3 years of the study, the number of inbred lines pairs using the model combining morphological distances and genetic distances was reduced compared to the model using isoenzymes. In 2013, decrease was 28% in term of workload reduction. With the new model less varieties would need to be sown in the field and compared side by side (**Figure 3**).

Figure 3: Comparisons of numbers of varieties to be observed in the field according to the model used for management of the reference collection with electrophoresis or SNP.

DUS Trial in 2013

274 candidate lines; 3741 lines in the reference collection
⇒ more than 1 million of pairs to compare side by side in field

Morphological data (GAIA only)
5266 pairwise comparisons to grow side by side

-83%

Morphological data (GAIA) + **Isoenzymes**
905 pairwise comparisons to grow side by side

-28%

Morphological data (GAIA) + **Genetic distances (SNP)**
647 pairwise comparisons to grow side by side

Conclusions

New rules based on the model combining genetic distances (SNP) and morphological distances (GAÏA) were adopted at national official level and this analytical scheme was accepted by the *Community Plant Variety Office of the European Union* (CPVO). The model is now routinely used for the management of the reference collection in the frame of maize DUS test in France. Moreover the SNP set is also used to verify the renewal of reference material and to check the conformity of the hybrid formulae. Using SNP technology for DUS examination is being assessed for other species.

Acknowledgements

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