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COMBINING PHENOTYPIC AND MOLECULAR DISTANCES
IN THE MANAGEMENT OF REFERENCE COLLECTIONS:
APPLICATION TO SPRING BARLEY

Document prepared by an expert from France

COMBINING PHENOTYPIC AND MOLECULAR DISTANCES IN THE MANAGEMENT OF REFERENCE COLLECTIONS: APPLICATION TO SPRING BARLEY

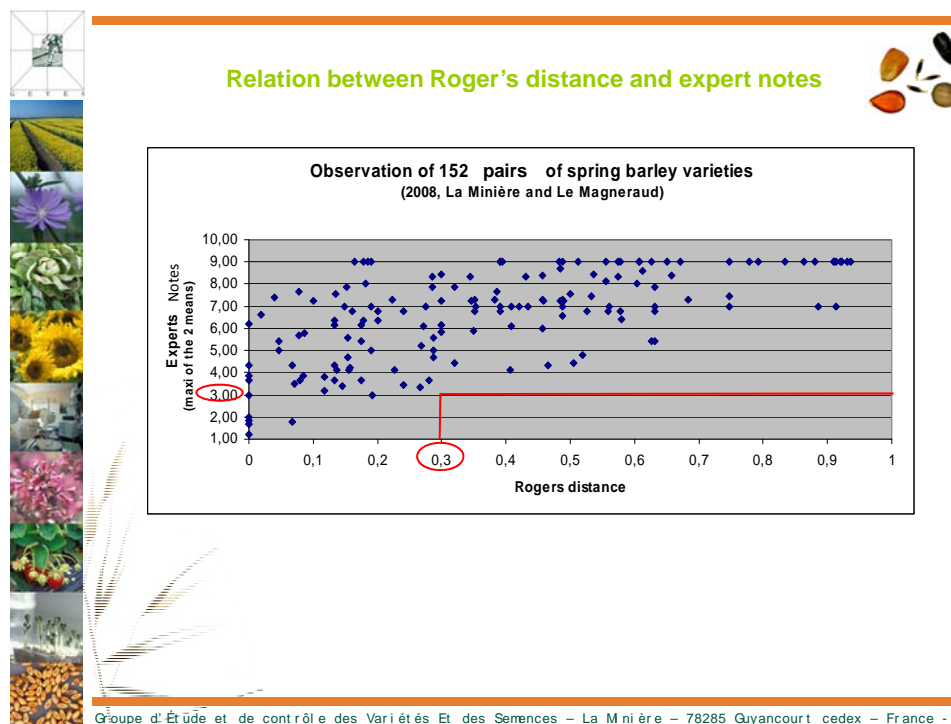
1. *Le Groupe d'Etude et de contrôle des Variétés et des Semences* (GEVES) has developed a new model which combines phenotypic and molecular distances for the management of reference collections. Maize was the model species on the basis of the long experience which was available for molecular markers and because Maize is an important crop in France with a large reference collection and many applications in DUS test each year: there are more than 3000 inbred lines in the reference collection and 150 new candidates each year.
2. The aim was to produce a tool to reduce the number of varieties, that need to be compared in the field by improving the system of selecting the most similar varieties from the reference collection and from the candidate varieties (see Annex).
3. This method has been presented on different occasions in UPOV meetings and to the BMT Review group in October 2009 as follows (see document BMT/12/2, paragraphs 21 to 27 and Annex III):

At its meeting on April 1, 2009, the BMT Review Group:

- (a) concluded that the proposal in the Annex to document BMT-RG/Apr09/2 "Proposal: System for combining phenotypic and molecular distances in the management of variety collections", incorporating the clarifications set out in document BMT-RG/Apr09/3, paragraphs 7 and 8 (Annex III to document BMT/12/2), where used for the management of variety collections, was acceptable within the terms of the UPOV Convention and would not undermine the effectiveness of protection offered under the UPOV system;
 - (b) agreed that the proposal in the Annex to document BMT-RG/Apr09/2 (Annex III to document BMT/12/2) represented a model that might be applicable to other crops provided that the elements of the proposal were equally applicable. In that respect, it noted, for example, that the proposal in the Annex to document BMT-RG/Apr09/2 (Annex III to document BMT/12/2) applied only to maize parental lines and did not extend to other types of maize. The BMT Review Group concluded that it was important to consider on a case-by-case basis whether the model would be applicable; and
 - (c) noted that some of the elements of the proposal in the Annex to document BMT-RG/Apr09/2 (Annex III to this document) were similar to the Option 2 approach "Calibration of threshold levels for molecular characteristics against the minimum distance in traditional characteristics", as set out in documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. However, the BMT Review Group concluded that it would not be appropriate to classify the proposal under Option 2 and agreed that the proposal should be referred to as the "System for combining phenotypic and molecular distances in the management of variety collections".
26. The Administrative and Legal Committee (CAJ), at its sixtieth session, held in Geneva on October 19, 2009, endorsed the recommendations of the BMT Review Group, as set out above (see document CAJ/60/10 "Report on the Conclusions", paragraph 43). The CAJ noted that the TC, at its forty-sixth session, would be invited to express its opinion on the recommendations of the BMT Review Group, in conjunction with the opinion of the CAJ.
 27. The Technical Committee (TC) endorsed the recommendations of the BMT Review Group, as set out in paragraph 25 (see document TC/46/15 "Report on the Conclusions", paragraph 42).

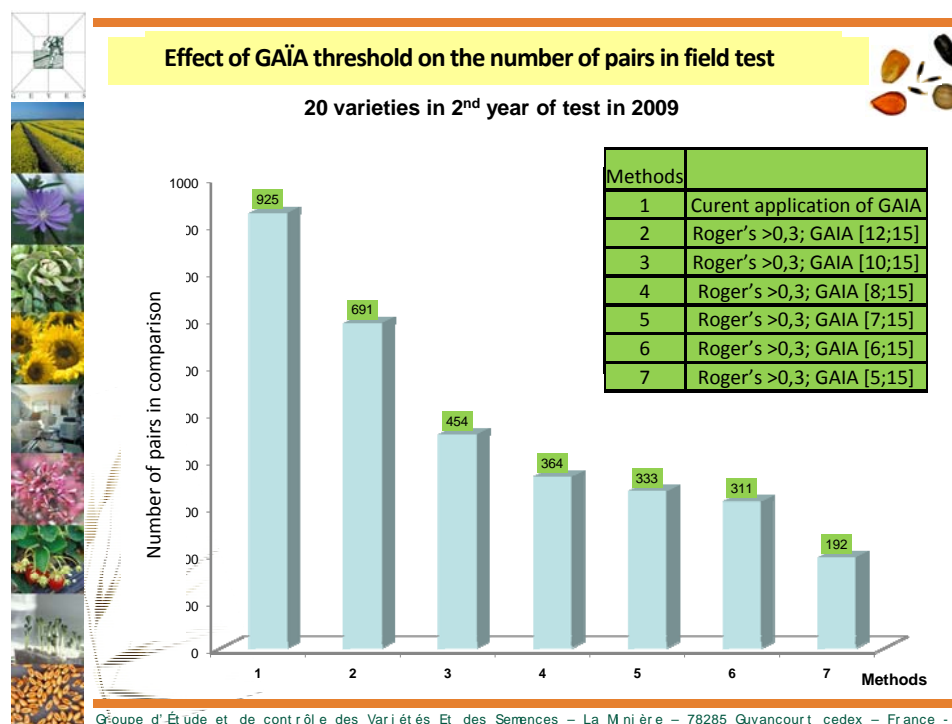
4. On the basis of the positive experience in Maize, GEVES decided to test that model on spring barley, where the reference is also rather large and the number of comparisons in the second year of DUS testing is high. A total of 30 Simple Sequence Repeat (SSR) markers have been tested and used to describe 501 varieties in the reference collection. The level of polymorphism is high, with 234 alleles (average of 7.83 per allele with a minimum of 6 and a maximum of 19). The average Polymorphic Information Content (PIC) value of this set of markers is 0.58.
5. The molecular (Roger's) and phenotypic (GAÏA) distances have been computed for all pairs based on the 501 varieties. 80% of the pairs have a Roger's distance greater than 0.3.
6. The level of heterogeneity has been evaluated at 2.2%, which means that 2.2% of loci are still heterozygous, with approximately 58% of lines having at least one heterozygous locus and 13.8% having at least 2. That is comparable with what was observed in Maize inbred lines.
7. In 2008, specific trials were conducted to study the relation between notes given by experts on similarity between varieties and the molecular distances, in order to define an appropriate threshold, in terms of molecular distance, to select the varieties which need to be grown for direct comparison in the field. This is the same approach as for Maize (see document BMT/12/2, Annex III). A total of 152 pairs of reference collection varieties have been observed by 11 experts.
8. The results showed that the smallest Roger's distances that can be used is 0.3 (see Figure 1). This value has been confirmed in a new trial in 2009 with 30 pairs of candidate varieties.

Figure 1



9. A first evaluation of the reduction in the number of varieties which need to be grown when using a molecular distance of 0.3, in combination with a GAIA distance, has been made in a comparative trial in 2008 with 20 candidate varieties in the second year of DUS testing. It appears that, with a GAIA distance equal to or higher than 12, approximately 25% of the pairs of varieties do not need to be grown together. With a GAIA distance equal to or higher than 10, the reduction is 50%. With a lower GAIA distance, a reduction is still observed but is less significant (see Figure 2).

Figure 2



10. In 2008, two varieties that were rejected for lack of Distinctness in the current DUS testing procedure were included in the specific trial with a Roger's distance lower than 0.3 and a GAIA distance lower than 10.

11. A new test has been conducted in 2009 with 905 pairs including 18 candidate varieties in the second year of DUS testing. The same thresholds have been obtained. A new specific trial has been conducted in 2009 in order to define more precisely the minimum GAIA distance that should be used in combination with the molecular distance. Among the pairs of similar varieties included in the official DUS trial, 5 were selected for each of the following GAIA distances: 5; 6; 7; 8; 9; and 10. A total of 30 pairs were sown. The observations made by the experts confirmed that the GAIA distance must be at least 10.

Conclusion

12. The combination of phenotypic and molecular distances as defined above for spring barley offers the possibility to achieve a significant reduction in the workload in the field (approximately 50% reduction in the number of pairs to be composed in the field). A threshold for Roger's distance of 0.3 and a GAIA distance of 10 seems to be appropriate. The full reference collection has been described with the set of markers. The new model will be tested in 2010 on a large set of candidate varieties before an official proposal to use the tool to manage the reference collection as a part of the French DUS protocol on spring Barley.

[Annex follows]

PROPOSED USE OF MOLECULAR AND MORPHOLOGICAL DATA

