E

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Eleventh Session
Madrid, September 16 to 18, 2008

COMBINING MOLECULAR DISTANCES TO MORPHOLOGICAL CHARACTERISTICS FOR THE MANAGEMENT OF FIELD COMPARISONS IN SPRING BARLEY

Document prepared by experts from France

COMBINING MOLECULAR DISTANCES TO MORPHOLOGICAL
CHARACTERISTICS FOR THE MANAGEMENT OF FIELD COMPARISONS IN
SPRING BARLEY

Document prepared by Cécile Collonnier, Joëlle Lallemand, David Zhang, Christelle Godin, Bénédicte Jaudeau, Julien Duriez, Annie Belliard, Béatrice Bouhedi, Joël Guiard, GEVES, France.

INTRODUCTION

1.   As for most species, registration and protection of spring barley varieties require their comparison with an ever-increasing reference collection, which should in theory contain all relevant varieties of common knowledge. In France, an average of 35 candidate varieties of spring barley are proposed for registration each year. Their comparison to the French reference collection led, for example, to approximately 2,800 field comparisons in 2004 and 4,000 field comparisons in 2005. This growing number of field comparisons is becoming technically and financially unmanageable. Therefore, it is highly desirable to find means of selecting the reference varieties to compare to the candidate varieties, without weakening the quality of plant breeders' rights (PBR).

2.   Up to now, the method used in France to select spring barley reference varieties for DUS testing is based on the accumulation of differences between varieties observed on morphological characteristics (Gaïa software). Those differences are weighted (a difference between more reliable characteristics being given a greater weight than a difference between less reliable ones), and an index is calculated for each pair of varieties. For spring barley, all pairs of varieties with a Gaïa index greater or equal to 15 are considered "super distinct" and are not put in the field for further comparison.

3.   This selection is efficient and reliable, but the number of varieties to be compared in the field remains high. That is the reason why proteic profiles, and later, molecular markers appeared to provide potential help. Thus, SDS or acid gel electrophoresis of hordeins were first tested [1, 2, 3], but, despite the fact that numerous proteic types were encountered among the varieties on the French catalogue, only 15% of these varieties could really be discriminated [3]. Presently, the overall discrimination rate is 11%, with an average Polymorphic Information Content (PIC) of 0.56 (unpublished data). When considering spring barley alone, the discrimination rate improves (20% of the varieties), but still remains insufficient.

4.   Therefore, Simple Sequence Repeats (SSR) markers were investigated for their possible use in the management of the spring barley reference collection and for identification purposes. We report here the molecular characterization of the French reference collection by using these markers and the development of a system based on a combination of molecular and morphological distances for the selection of field comparisons in spring barley.

RESULTS

a)   Molecular characterization of the French reference collection of spring barley

5.   Two sets of publicly available SSR markers, 36 from Ramsay et al. (2000) [4] and 48 published by Macaulay et al. (2001) [5] were first tested on a subset of 12 varieties.

6.   Most of them had di-nucleotide repeats. They frequently showed slippage, but it was possible to select 30 which provided good coverage of the genome and which presented a high level of polymorphism and easy scoring.

7.   These markers were then used for the description of 512 varieties from the French reference collection on bulked samples of 20 seeds. This analysis generated 234 alleles (7.83 per SSR, with a minimum of 6 alleles and a maximum of 19 alleles). The average PIC computed by using the LCDMV software (GEVES) was 0.58. These results enabled discrimination of all varieties except 12 pairs. The majority of varieties displayed heterogeneity on at least 1 locus (58%). Only 13.8% of varieties were heterogeneous on more than 2 loci. The average level of heterogeneity per locus was 2.2%, which is very close to what is observed on maize and can be considered as low.

b)   Calibration of molecular distance with morphological distance

8.   Rogers' distances were calculated using LCDMV (GEVES) [6] and morphological distances calculated by using Gaïa (GEVES).

9.   As expected, no correlation was found between molecular and morphological distances. This also was confirmed by using Euclidian morphological distances computed with Darwin software (CIRAD). However, it is interesting to note that the 12 pairs which had a genetic distance of 0 also had a Gaïa index of 0.

10.   To further investigate the relationship between molecular distances and morphological data, we used a method previously developed on maize (see UPOV document BMT/10/14).

11.   This method is based on a visual appreciation of the global phenotype of the varieties by crop experts, and not on an individual appreciation of the characteristics of the varieties considered one-by-one.

12.   152 pairs of varieties with various Gaïa indices were grown side-by-side in two locations (La Minière and Le Magneraud, France). The trials were visited by 11 crop experts, who individually scored the pairs of varieties on a scale of similarity ranging from 1 to 9:

    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.

13.   For each variety pair, the scores from the 11 crop experts were averaged for each location and the maximum value of these two means was considered as the experts' note for each pair. The experts' notes were then compared with the Rogers' distances.
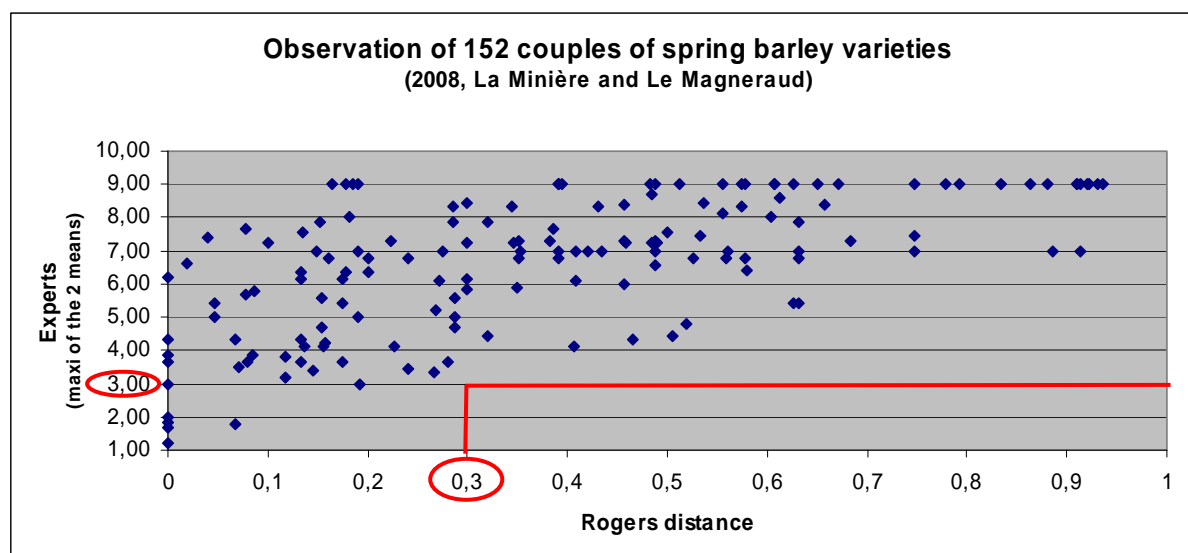
Figure 1: Identification of a molecular threshold applicable for the selection of field comparisons by comparing experts' notes and Rogers' distances for 152 pairs of spring barley varieties.

14.   As shown in Figure 1, all the variety pairs with Rogers' distances greater than 0.3 had experts' notes superior or equal to 3 (meaning "distinct varieties"). A Rogers' distance of 0.3 was thus considered as a potential molecular threshold for the selection of field comparisons in combination with morphological data.

c)   Proposal for an approach combining molecular and morphological distances for the management of reference collection

15.   The approach we propose for the selection of field comparisons in spring barley by using molecular data combined with morphological characteristics is illustrated in Figure 2.

16.   According to the crop experts, a Gaïa index of 5 could be considered as a potential morphological threshold below which all variety pairs should be compared in the field.

17.   With this indication, the procedure can be described as following :
   (a) when the Gaïa index based on morphological data is higher than 15, the pairs of varieties are considered to be super-distinct and do not need to be compared in the field;
   (b) when the Gaïa index based on morphological data is lower than 5, as recommended by the crop experts, the pairs of varieties are systematically compared in the field;
   (c) when the Gaïa index based on morphological data is between 5 and 15:
       (i) if the molecular distance is strictly higher than 0.3 (Rogers'), then the pairs of varieties are considered distinct and do not need to be compared in the field;
       (ii) if the molecular distance is lower than or equal to 0.3, the pairs of varieties need to be compared in the field.
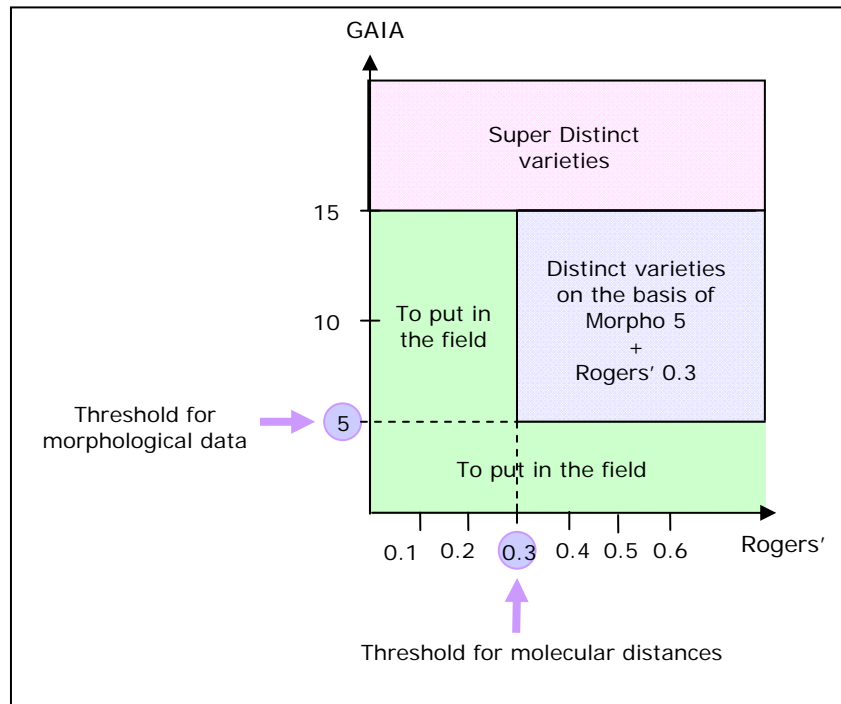
Figure 2: GEVES' approach for the selection of field comparisons in spring barley by using molecular data combined with morphological characteristics.
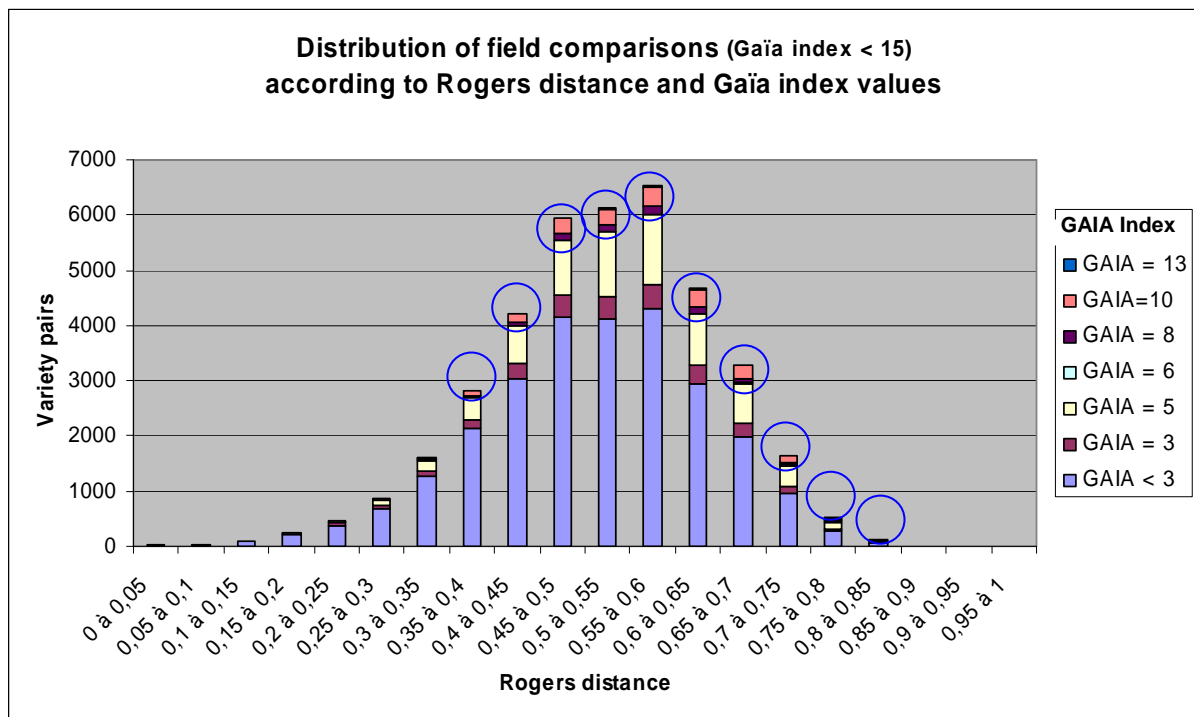

Figure 3: Distribution of Rogers' distances among the variety pairs not discriminated by Gaïa (ie Gaïa index < 15). In blue circles, field comparisons that could be discarded by using the "combined approach" (Gaia > 5 and Rogers' > 0.3).

18.   By applying the morphological and molecular thresholds presented in Figure 2, a significant number of field comparisons could probably be avoided. This number still has to be determined precisely by including candidate varieties in the analysis. As a theoretical example, Figure 3 presents the preliminary results obtained on the varieties of the reference collection only.

CONCLUSION

19.   The results presented here are promising. They support the idea that combining molecular and morphological data for the selection of field comparisons could be a valuable tool for the management of the spring barley reference collection.

20.   The selected markers and the molecular database containing the description of the French reference collection will also be useful for checking variety identity in the frame of seed certification in France.

PERSPECTIVES

21.   The approach presented here now needs to be tested on a set of varieties, including candidate varieties. Its technical advantages, risks and costs will then be evaluated by comparison with the current system, under real conditions of testing.

22.   As a complement, the efficiency of the selected markers will also be tested for use in the checking of variation between successive maintenance seed lots in the reference collection.

REFERENCES

[1] Shewry P.R. et al. (1980) Genetic analysis of barley storage proteins. *Heredity* 44, 383-389
[2] Marchylo B.A. and LaBerge D.E (1980) Barley cultivar identification by electrophoretic analysis of hordein proteins. *Can. J. Plant Sci*. 60 1343-1350.
[3] Lallemand J. and Briand F. (1990) Description variétale des orges par électrophorèse. Description de 280 variétés. *Agronomie*, 6, 447-450.
[4] Ramsay L. et al. (2000) A simple sequence repeat-based linkage map of barley. *Genetics*, 156: 1997-2005.
[5] Macaulay M. et al. (2001) A representative, highly informative 'genotyping set' of barley SSRs. *TAG*, 102:801-809.
[6] Dubreuil P. et al. (2004) User's manual for the LCDMV software (Calculation Software of Molecular Distances between Varieties) for fingerprinting and Genetic Diversity Studies. First edition GEVES.

[End of document]