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USE OF MOLECULAR MARKERS FOR CHECKING SEED LOTS IDENTITY AND HYBRID FORMULAE IN MAIZE

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USE OF MOLECULAR MARKERS FOR CHECKING SEED LOTS IDENTITY AND HYBRID FORMULAE IN MAIZE

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INTRODUCTION

1. The use of molecular markers for DUS testing has been debated within UPOV for several years. Although molecular markers are broadly recognized to have many desirable features such as high polymorphism, independence from environmental bias, reproducibility of results and speed of results, there is a general concern that the level of protection granted to varieties could be decreased by using them for DUS purposes. Some breeders also fear that molecular markers could reveal "too much" heterogeneity at the loci investigated.

2. However, advances in breeding research have promoted molecular markers as an important tool for Marker Assisted Selection (MAS), variety identification and quality control. Therefore, the question is no more whether to use them, but rather how to use them.

3. At the tenth session of the Working Group on Biochemical and Molecular Techniques and DNA Profiling in Particular, held in Seoul from November 21 to 23, 2006, (see document BMT/10/14) we presented an approach for the management of the reference collection combining molecular and morphological markers. Several modalities were investigated with different thresholds for genetic distances combined with morphological data. The data showed that it was possible to propose an appropriate balance of morphological and molecular markers in order to maintain the same level of protection as with morphological markers alone.

4. The objective of this paper is to give additional data to show how the same tool (SSR markers) can be used for the other purposes of variety testing, namely checking the identity of maintenance seed lots and the checking of hybrid formulae in maize.

5. For this purpose it was necessary to estimate the level of residual heterogeneity within the varieties and also of the "natural" variation within a variety during its lifetime (i.e. for different locations and years of production).

6. The results show that it is possible to deal with residual heterogeneity and "natural" variation of the varieties. The problem of using different technical platforms or different sets of markers is also addressed.

Materials and methods

7. Evaluation of the uniformity of varieties was performed using 1,000 maize lines previously described with 36 to 51 SSR markers run on a Licor sequencer. With the ABI capillary sequencer, the estimation was done on 400 lines and 36 SSR markers.

8. Checking the identity of seed lots was performed on 25 lines and a total of 92 seed lots which were described using 38 SSR markers. Reference seed lots from different countries (France, Spain, and Germany) and seed production lots were analyzed. All seed lots were

simultaneously grown in the field and morphological data were produced. The results of the field and laboratory tests were then compared.

9. The hybrid formulae were checked using 12 SSR markers. For this purpose, 83 hybrids and their 183 parental lines were genotyped. Results were compared with those obtained with isozymes. An additional set of 12 SSR markers was used for some of the genotypes studied. All analyses were performed on bulk samples of 10 or 30 seeds. The results were recorded as presence/absence of alleles.

RESULTS

Estimation of the level of uniformity

10. As a result of selfing, residual heterozygoty can be observed in a number of loci depending on the number of generations of selfing. That results in non fixation in some loci. Therefore residual heterogeneity can be observed on a number of lines according to the number and the efficiency of markers used. For the French reference collection, this was observed on a significant number of lines. However, the average level of heterozygosity per locus was only 2% for experiments on Licor. Unexpectedly, this percentage was approximately the same when using the capillary sequencer, despite the difference in sensitivity of the two platforms: 1 “off type” out of 10 seeds was detected with the Licor, whereas detection was effective from 1 “off type” in 30 seeds with the capillary sequencer.

11. This low level of residual heterogeneity reflects the effect of a breeding scheme with selfing and the quality of the quality of plant material delivered. It is also possible to conclude that within-line residual heterogeneity will not impair between-line differentiation. This was indeed confirmed on a collection of 1,000 lines where no pairs of identical lines were observed (except isogenic lines).

Identity control

12. Official seed lots from France, Spain and Germany were compared to each other and also to commercial seed lots. The results are shown in Table 1.

Table 1

Number of lines	Number of seed lots	Results from laboratory	Results from field
15	62	OK	OK
5	14	+/- 1 allele	OK for 13 seed lots for 1 seed lot the plants were taller than the reference
1	1 out of 2	+2 alleles	OK
1 1 1	1 out of 3 1 out of 3 1 out of 4	14 loci different 18 loci different 22 loci different	Mislabeling Mislabeling Homonymy
1	1 out of 4	3 loci different	Plants taller than reference

13. Among the 25 lines (92 seed lots) studied, 15 lines were completely identical for all their seed lots (74 seed lots).

14. From the remaining 10 lines (20 seed lots):

- five lines (14 seed lots) had one difference on one locus (the locus was not fixed in the reference seed lot nor in the commercial seed lot). All these seed lots behaved in the field as expected, except one which displayed a difference in height compared to the official seed lot. This single case of a phenotypical difference appeared to be an effect of the seed quality rather than a lack of stability, or the result of a mistake in providing the right line;

- one line had two differences (the expected alleles were present, but a supplementary allele was observed on two loci);

- three lines showed significant differences between the official seed lots from France, Germany and Spain (respectively 14, 18, and 22 loci displayed unexpected alleles). The results obtained in the field confirmed a mislabeling problem for two of them. The third line was, after enquiry, discovered to be a case of homonymy (2 different lines sharing the same name);

- Finally, one line showed differences for three loci between production and official seed lots. In this case, the expected alleles were absent and a "foreign" allele was observed instead. The morphological results showed a difference for the characteristic "height of plants". Otherwise, the general phenotype of the line was as expected.

15. In conclusion, most of the seed lots were as expected or had only a very small difference to their reference. The three seed lots which were really different were explained by mislabeling or homonymy. The three countries had identical reference seed lots. Only one questionable case (commercial seed lot) remained: the line with three loci showing unexpected alleles. That case would need more investigation to decide if it is non conforming using other tools. A check has already been done with isozymes; the results show a full conformity based on 20 loci. Such a case contributes to the definition of a decision rule for which much still needs to be elaborated.

Control of hybrid formula

16. As shown in Table 2, a good level of concordance was obtained when comparing our results with those obtained with isozymes.

Table 2

Enzymes	Molecular markers	Number of hybrids
Conform	Conform	67
Conform	*Non conform*	*8*
Non conform	*Conform*	*3*
Non conform	Non conform	5

17. 67 hybrids conformed to the formula using isozymes and micro satellites. Five did not conform for both types of markers. This left only 11 hybrids with discordant results. In all these remaining cases, except hybrid 31 and 85, the non conformity was observed on only one locus. Further investigations on these hybrids showed that the isozyme alleles present in the parental lines of hybrid 31 were the most frequent. Therefore, the probability of obtaining the expected isozyme pattern with a "wrong" parent was significant. For hybrid 85 a non

conformity was observed when using a supplementary isozyme locus. These two examples may indicate that, in some cases, molecular markers are more reliable than isozymes.

18. However, it is possible to have a disagreement between the methods when only one locus is concerned. Other discrepancies occurred when the analysis was performed using another set of 12 molecular markers. This suggests that basing non conformity on only one locus could be too stringent.

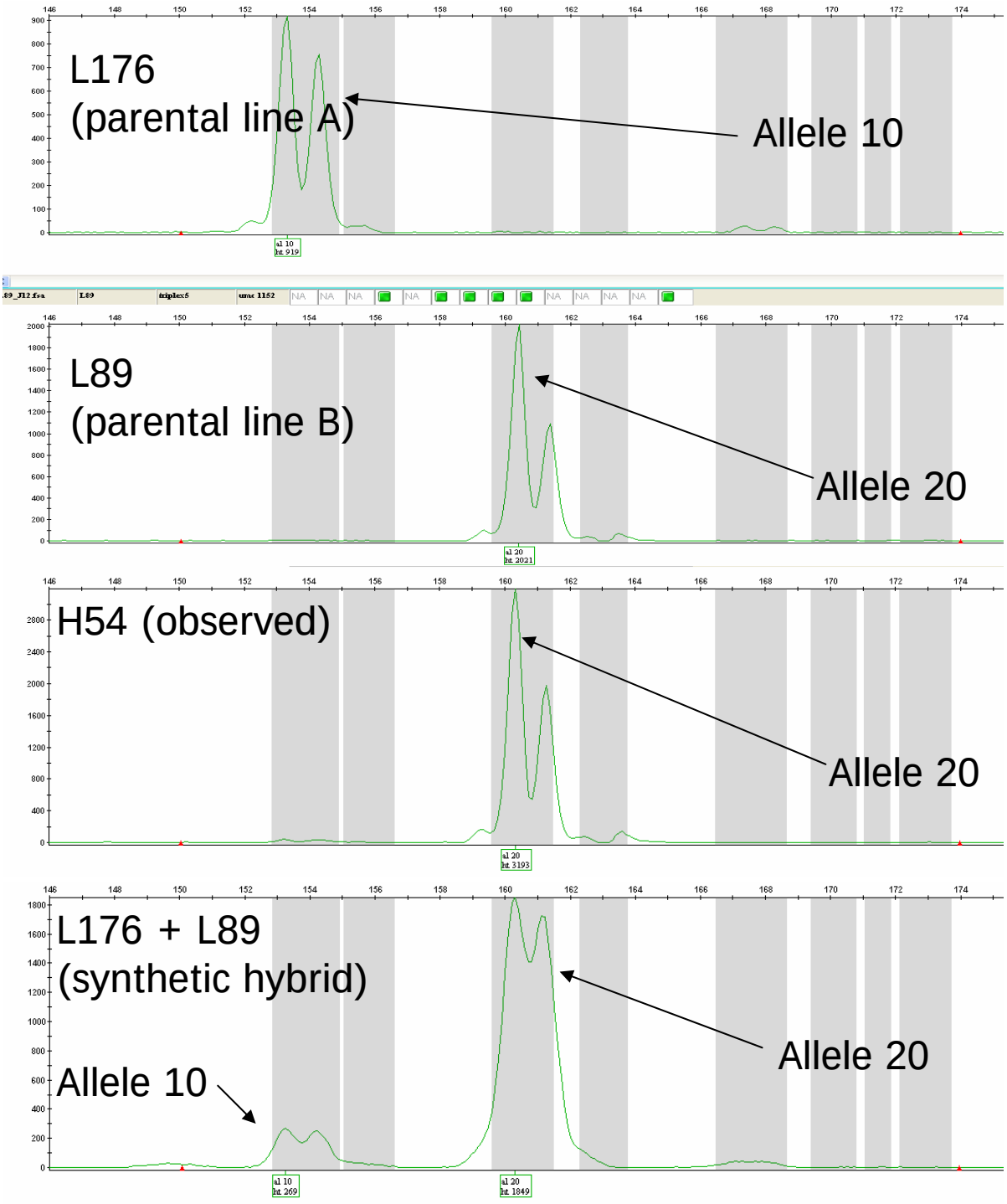
19. With molecular markers, we observed that some loci displayed an “allelic competition”: one of the parental alleles was not present in the hybrid (figure 1). One locus out of 12 (Umc 152) displayed this behavior systematically: the smallest allele always disappeared or was strongly decreased when the other parental allele was present. To decide between two hypotheses (“allelic competition” versus “non conformity”), we performed PCR on “synthetic hybrids” consisting of mixtures of both parental lines. In most cases the smallest allele disappeared again (figure 1). Consequently Umc 152 was removed from the analysis.

CONCLUSION

20. Our laboratory has described over 2,000 genotypes of maize (reference lines, commercial and candidate varieties). The results obtained show that the level of residual heterogeneity is low. This shows that the fixation of the lines during breeding is efficient. Moreover, this enables the use of molecular markers for identification and control purposes without adding any new constraints on uniformity (i.e. uniformity on molecular markers, not subjected to breeding, is not necessary). After three years of checking varieties and seed lots, we have observed that most problems were unequivocal: either the differences were huge and unmistakable or they were trivial. In between, there were only a very small number of cases which required further investigation. The same occurred when dealing with hybrids: most of them were either right or obviously wrong. Dubious cases were not numerous and could be a starting point for a discussion on decision rules.

21. In conclusion, we think that substituting enzymatic markers with molecular markers will be an improvement of the existing system and will increase the reliability of variety testing without increasing requirements on uniformity or stability.

Figure 1



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