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# Gynogenetic haploid plants analysis for agronomic and enzymatic markers in maize (*Zea mays* L.)

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**Summary.** Experiments were conducted to investigate whether selection occurs during the processes involved in the production of doubled haploids. Haploid plants produced from two hybrids, each heterozygous for isozyme markers, were subjected to genetic analysis. The distributions of doubled haploid lines and pedigree lines derived from the hybrid C123 × Oh7 were compared with regard to agronomic character. The results suggest that the populations of haploid plants obtained by in vivo gynogenesis represent a random gametic array. Thus, in order to introduce haploid plants into breeding programmes in maize, maternal haploidy seems to be a very attractive method.

**Key words:** *Zea mays* – Haploid – Gynogenesis – Gametophytic selection – Genetic markers

## Introduction

The use of anther culture as a means of haploid breeding in maize has been limited by relatively low anther response frequencies and the difficulties associated with plant regeneration and chromosome doubling. Improvement can be obtained by selecting for highly responsive genotypes (Dieu and Beckert 1986; Petolino and Jones 1986). Transfer of this genetic aptitude through crossing and breeding programmes for in vitro androgenesis may provide a solution to some of the efficiency problems associated with maize anther culture (Petolino and Thompson 1987). However, this genetic improvement may be counterbalanced by theorical disadvantages such as possible negative gametophytic selection, and introduces complications into a breeding programme that could offset the time-saving effect of doubled haploids.

The spontaneous occurence of haploids may be an attractive alternative method for inbred line development in maize. Despite the lack of satisfactory methods for screening haploid and chromosome doubling, spontaneous haploids have been successfully incorporated into commercial hybrids (Chase 1969). Increased maternal haploid induction frequencies were achieved through the identification and selection of haploid inducing pollinators (Coe 1959; Lashermes and Beckert 1988). As a result, a large number of maternal haploid plants can be produced quite easily.

The present study was designed to determine whether any genetic selection occurs during haploid plant and doubled haploid line production.

## Materials and methods

### *Agronomic observations of inbred lines*

The hybrid C123 × Oh7 was used to derive homozygous lines by pedigree ($F_5$ generation) and doubled haploid methods. Doubled haploid lines were obtained by chromosome doubling of gynogenetic haploid plants produced from $F_1$ plants. The agronomic experimental design was a randomized block with two replications. Ten of the 20 plants per line were measured for the following traits: plant height, ear length, ear diameter, number of seed rows per ear, male and female flowering dates, number of leaves per plant, length and width of the leaf carrying the main ear, ear height and number of tassel branches at Clermont-Ferrand, France in 1986.

The Student *t*-test and the Mann-Whitney U-test were used to determine if the frequency distributions of the doubled haploid and pedigree lines differed in mean and in skewness. Tests of signifiance of an observed value of *t* and U were conducted according to the procedure outlined in Dagnelie (1970). Standard SPAD statistical programmes (Lebart and Morineau 1985) were used to do multivariate analyses and to calculate principal components.

**Table 1.** Chromosomal locations of allozyme loci and parental genotypes

| Enzyme | Locus symbol | Chromosomal location | F101 × W401 allele | F186 × W64A allele |
|---|---|---|---|---|
| Acid phosphatase | Acp1 | 9-60 | 3/2 | 4/2 |
| Isocitrate dehydrogenase | Idh2 | 6L-101 | 4/6 | 4/6 |
| Malate dehydrogenase | Mdh2 | 6L-103 | 6/4.5 | 3/3.5 |
| Phosphogluco-dehydrogenase | Pgd1 | 6L-10 | 4/3 | 3.8/2 |
| Phosphoglucomutase | Pgm2 | 5S-0 | | 4/1 + 8[a] |

[a] The addition of 1 and 8 alleles is justified by the unfixed nature of W64A to Pgm2

**Table 2.** The results of the Student *t*-test and Mann-Whitney test on the frequency distributions of doubled haploid and pedigree lines derived from C 123 × Oh7

| Agronomic trait | Student: *t* value | Mann-Whitney: Significant level |
|---|---|---|
| Plant height | 2.44[a] | 0.48 |
| Ear length | 2.28[a] | 0.38 |
| Ear diameter | 0.35 | 0.67 |
| Seed rows/ear | 0.21 | 0.81 |
| Male flowering date | 1.77 | 0.18 |
| Female flowering date | 1.64 | 0.20 |
| Leaves/plant | 1.20 | 0.47 |
| Leaf length | 1.89 | 0.26 |
| Leaf width | 0.11 | 0.87 |
| Ear height | 2.35[a] | 0.56 |
| Branches/tassel | 0.78 | 0.41 |

[a] Significant at 5% level

**Table 3.** Segregation of genetic markers among maternal haploid-derivative plants from $F_1$ hybrid F186 × W64A and $F_1$ hybrid W401 × F101

| Locus | Alleles from F186 | | Alleles from W64A | | $\chi^2$[a] |
|---|---|---|---|---|---|
| | Allele | No. of plants (%) | Allele | No. of plants (%) | |
| Acp1 | 4 | 61 (44) | 2 | 78 (56) | 2.08 |
| Idh2 | 4 | 73 (53) | 6 | 66 (47) | 0.35 |
| Mdh2 | 3 | 70 (50) | 3.5 | 69 (50) | 0.01 |
| Pgd1 | 3.8 | 57 (52) | 2 | 52 (48) | 0.23 |
| Pgm2 | 4 | 74 (59) | 1 + 8 | 51 (41) | 4.23[b] |
| | | | | total | 6.90 |

| Locus | Alleles from W401 | | Alleles from F101 | | $\chi^2$[a] |
|---|---|---|---|---|---|
| | Allele | No. of plants (%) | Allele | No. of plants (%) | |
| Acp1 | 2 | 71 (55) | 3 | 58 (45) | 1.31 |
| Idh2 | 6 | 69 (53) | 4 | 61 (47) | 0.50 |
| Mdh2 | 4.5 | 70 (54) | 6 | 60 (46) | 0.77 |
| Pgm2 | 3 | 57 (45) | 4 | 70 (55) | 1.33 |
| | | | | total | 3.91 |

[a] Expected ratios were 1:1
[b] Significant at 5% level

*Segregating allozyme loci among maternal haploid plants*

The haploid gynogenetic plants were obtained by pollination with the haploid inducing line "WS 14". The haploid plants in the progeny were identified by ligule-less marker and confirmed by chromosome number counts (see Lashermes and Beckert 1988, for the haploid-production technique).

Starch gel electrophoretic analyses were conducted following established procedures (Cardy et al. 1983), except for extraction, which that was conducted on younger leaves of 2-week-old plants. The extraction solution was as specified by Wendel and Parks (1982).

Maternal haploid plants produced from two $F_1$ hybrids F186 × W64A and F101 × W401 heterozygous at four and five codominant isozyme markers, respectively, were subjected to genetic analysis. Locus symbols and chromosomal locations of loci studied have been previously detailed (Wendel et al. 1985) and are presented in Table 1. The loci segregated independently to each other except Idh2 and Mdh2, which are separated by two map units.

## Results

Thirty-three pedigree and nine doubled haploid lines derived from the hybrid C123 × OH7 were studied for their agronomic characters. Comparisons for the quantitative characters studied did not show any noticeable deviation (Table 2). Distributions of both groups of lines are quite similar. The principal component analysis (Fig. 1) situates the points representing the different lines. The doubled haploid lines were not fundamentally distinguishable by agronomic traits studied.

Segregating allozyme loci among maternal haploid-derivative plants from the $F_1$ hybrids F186 × W64A and W401 × F101 (Table 3) were determined. All plants derived from hybrids were homozygous for the enzymatic marker tested and were thus certainly of maternal origin. The distribution observed at each locus was tested for conformance to Mendelian expectation. The individual segregation ratios at all loci, except for Pgm2 in F186 × W64A, were not significantly different from 1:1. However, the magnitude of distortion observed was very small and finally, no locus showed aberrant segregation. Notably, the haploids obtained from F186 × W64A did not exhibit any distortion in favor of alleles derived from W64A, although the W64A line had clearly more aptitude for gynogenetic development than the F186 line (Lashermes and Beckert 1988).

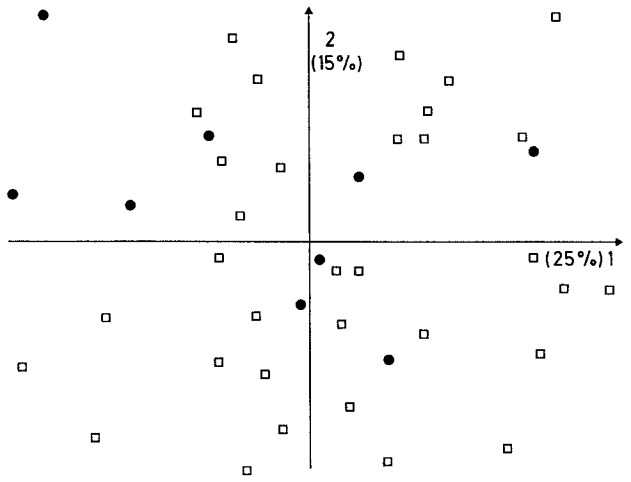


**Fig. 1.** Principal component analysis of inbred lines derived from C 123 × Oh7. 33 pedigree lines (□) and 9 doubled haploid lines (●) are described in this analysis. The first two components describe 40% of the variation, the first component puts in + value the late lines with long and narrow ears. The second component puts in + value lines with thin ears and short leaves

## Discussion

In theory, the distribution for a quantitative character of the two populations of lines derived by doubled haploid or pedigree methods is expected to be the same in the absence of linkage and selection pressure. Our results indicated that the agronomic characters studied had not been subjected to significant selection during maternal haploid production. However, additional investigations including larger numbers of lines and a wider range of genetic origins would be necessary to confirm these results.

The segregation of allozyme loci in the expected 1:1 ratio in gynogenetic haploids indicates that the two alleles of each marker gene have segregated normally during gamete formation, and that there has been no selection among the different macrospore genotypes. Although the ability to detect significant deviations from expectations was clearly due to the large sample sizes employed, the genes controlling the maternal haploid formation are probably not linked with the markers tested.

These results suggest that haploids obtained by in vivo gynogenesis are a random sample of gametes from $F_1$ plants. As far as practical plant breeding and genetic analyses are concerned, the property of randomness is a highly desirable feature of the doubled haploid method. Furthermore, without exception, gynogenetic development was induced in varying degrees in all genotypes. The benefits of the gynogenetic haploid system need not be limited to particular cultivars. The problems that prevent extensive exploitation of haploids in breeding are the lack of a quick and efficient system of screening for haploids, and the absence of a simple and effective procedure for doubling the chromosome number. Although really effective, the use of techniques using recessive markers such as ligule-less or glossy is restricted to the production of haploids for basic studies. An in vitro biochemical detection (Dhaliwal and King 1979) and the introduction of a specific marker by genetic transformation into inducer lines could be alternative procedures. In addition, it may be more efficient to apply colchicine in vitro to embryogenic tissue to improve chromosome doubling.

Despite these problems, maternal haploidy in maize remains a very attractive method. Notably, its potential yield from any genotypes appears greater than in vitro production of haploids by anther culture (Dieu and Beckert 1986): assuming a rate of 600 plated immature embryos per person per day (in vitro biochemical screening), 3% of maternal haploid frequency (Lashermes et al. 1988) and 50% successful selfing, the overall yield would be 9 doubled haploid lines per person per day.

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