Genetic control of maternal haploidy in maize (*Zea mays* L.) and selection of haploid inducing lines

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Summary. The effect of genotype on maternal haploid plant production in maize was studied. The frequency of gynogenetic plants when "Stock 6" was used as pollinator varied according to the female parent genotype. No simple relation was observed between genotypic aptitudes for gynogenetic and androgenetic development, which occured after pollination of "W23" plant carrying the "indeterminate gametophyte" gene. Furthermore, the population NS, a favorably responsive genotype to anther culture, does not exhibit exceptional ability for in vivo gynogenesis. The effect of inbreeding and the influence of maternal haploid origin suggest that specific genes control maternal haploid initiation and development. However, gynogenetic development is not limited to a particular genotype. The frequency of maternal haploids may be increased by using specific pollen parents. Attempts were made to select for a high haploidy-inducing trait and the present study reports the successful development of lines that can be utilized as pollen parents to induce haploids for experimental purposes and breeding programmes. When an inbred line "WS14", derived from the cross W23 × Stock 6, was used as pollen parent, 2%–5% maternal haploids were obtained according to the female parent genotype. A high haploidy-inducing potential is a heritable trait and may be controlled by a limited number of genes. Genetic determination of the haploidy-inducing character was examined in relation to the efficiency of the selecting method and the mechanisms involved in the origin of maternal haploids.

Key words: *Zea mays* – Haploid induction – Gynogenesis – Genetics – Inducer line

Introduction

The development of spontaneous haploids has been reported in many plant species (Kimber and Riley 1963; Magoon and Khanna 1963). Stadler and Randolph first reported haploidy in maize (1929 unpublished, cited by Randolph 1932). A haploid embryo can arise through sporophytic development from a male gamete or from an unfertilized egg or other cell of the embryo sac. The frequency of such occurrences is rather low in natural populations, but variations in the haploid yielding potential of different strains have been noted.

Increased frequencies of paternal haploids through the use of a spontaneous mutation referred to as "indeterminate gametophyte" has been reported by Kermicle (1969).

The frequency of maternal haploidy in a cross is known to be influenced by female and male parents (Chase 1952). Discovery of a genetic strain "Stock 6" by Coe (1959), that, on selfing, produced a haploid frequency as high as 3.2%, opened up possibilities for use of selective pollinators in enhancing haploid frequencies. Inducer lines were developed from this "high haploidy" stock (Sarkar et al. 1972; Aman and Sarkar 1978). Maternal haploidy in maize is concluded to result primarily from failure of fertilization caused by an abnormal condition, either inherent or induced, in a male or female gamete, and subsequent development of the reduced egg into the embryo (Sarkar and Coe 1966). Exact modes of origin of maternal haploids are difficult to establish by cytological analysis and material with higher haploid induction frequency is necessary for such studies.

Various physical, chemical and physiological treatments have been tried by different workers to induce maternal haploids in maize. Increases in the frequency of haploids have been reported through heat treatment

of pollen (Mathur et al. 1980), X-irradiation of pollen (Mathur et al. 1976), treatment of corn silks with maleic hydrazide (Zuoyu and Mingguang 1984) and delayed pollination (Chase 1974), but with limited success.

The purpose of this study was to determine the genetic basis of aptitude for gynogenetical development, and to investigate the potential of genetic selection techniques and the development of genotypes that induce haploids at greater frequencies.

Material and methods

The materials included different strains, inbred lines and hybrids of variable origin maintained at INRA Clermont-Ferrand. The maize stocks used as pollen parent were Stock 6 (the high haploidy line), various inbred lines growing well under Clermont-Ferrand conditions and diverse materials derived from crosses involving Stock 6. Controlled pollinations were made in the field with detasseled female plants. The inbred Wisconsin-23 carrying the "indeterminate gametophyte" gene (Kermicle 1969) was used to estimate genotypic aptitude for androgenetic development. Two simple recessive traits, ligule-less and glossy (conditioned by lg_1, lg_2 and gl_1, respectively) were used as markers for haploid frequency determination. The genotypes whose tendencies for gynogenetic or androgenetic development are examined, are homozygous for either lg_1, lg_2 or gl_1. The haploid plants were identified by the marker in comparison with the normal phenotypes of the true hybrids. The haploid nature of seedlings was confirmed by morphology and the chromosome numbers were checked using root tip squashes. The population CS results from open-pollination of multiple cross hybrids including "Stock 6" and about 20 flint inbred lines (F2, F120, F712, F1444...). The population NS (Dieu and Beckert 1986) was a synthetic consisting of material adapted to anther culture (Hsi ba Tang, Shui Baï, Ill High Oil, Black Mexican Sweet Corn...).

Results

Considerable variations in haploid production potentials were observed between the different genotypes (Fig. 1). The frequency of maternal haploids when "Stock 6" was used as pollinator varied between 0.4%–2.4%; the average was approximately 1%. For example, the population CS was almost 6 times as effective as the hybrid C123 x Oh7 in producing maternal haploids.

No simple relations were observed between genotypic aptitude for gynogenetic and androgenetic development, which occurred after pollinisation of a W-23 plant carrying the "indeterminate gametophyte" gene. The population CS produced concurrently the highest frequency of maternal haploids and the lowest frequency of paternal haploids. Furthermore, the population NS, a favorably responsive genotype to anther culture, did not exhibit exceptional ability for in vivo gynogenesis and androgenesis. F_1 hybrid genotypes F186 x W64A and F1243 x A619 had maternal haploid production potential intermediate or lower than those of their component inbred lines (Fig. 2). No hybrid vigor in ability for gynogenetic development was observed and these results indicate a degree of dominance of factors conferring low production frequency.

A more detailed study was made with the cross C123 x Oh7. Maternal haploid production did not increase significantly during successive self-pollinated generations (Fig. 3). The frequency with which haploid plants occurred in the progeny did not appear proportional to the degree of homozygosity achieved by inbreeding. Nevertheless, some pedigrees showed a high
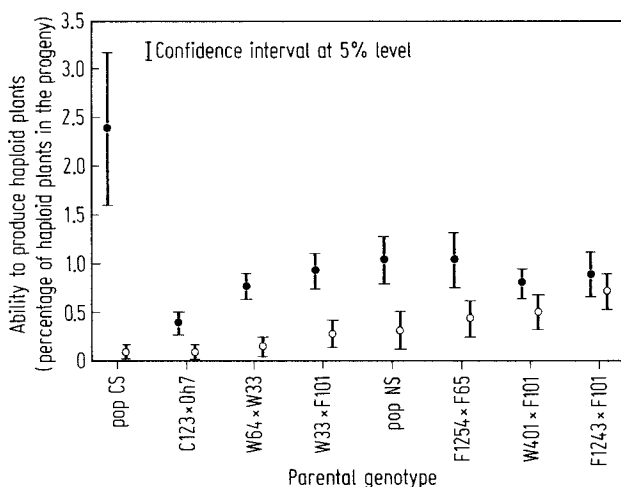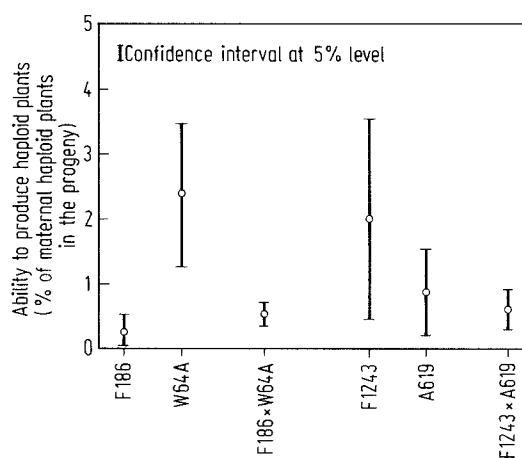

Fig. 1. Comparison of ability of different genotypes to produce maternal (●) and paternal (○) haploid plants; maternal haploids are obtained by pollination with Stock 6 pollen; paternal haploids are obtained after pollination of W23ig plants

Fig. 2. Ability to produce maternal haploid plants of two F_1 hybrids and their parental lines; maternal haploids are obtained by pollination with Stock 6
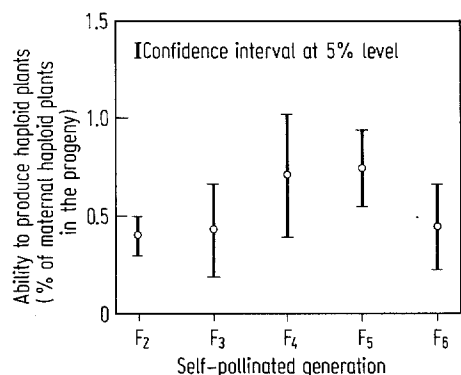
Fig. 3. Comparison of ability to produce maternal haploid plants of successive self-pollinated generations from C123 × Oh7; maternal haploids are obtained by pollination with Stock 6
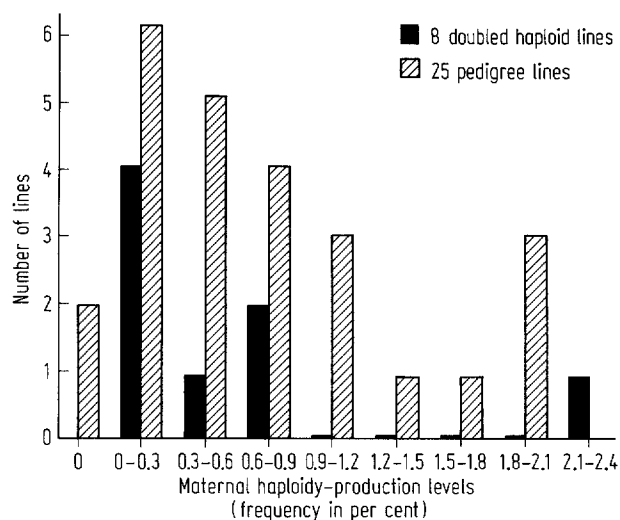

Fig. 4. Distribution of doubled haploid and pedigree lines derived from C123 × Oh7 cross according to ability to produce maternal haploids (haploidy-production level was estimated by observation for each line of 800 plants obtained after pollination with Stock 6 pollen)

haploid production ability. The distribution of 25 pedigree lines according to their ability to produce maternal haploids is presented in Fig. 4. The correlation coefficient between the F_3 and F_4 generation (0.93 with confidence limits 0.75–0.98 at 5% level) indicates that haploid production tendency is a highly heritable character.

Genotypes derived from maternal haploids sometimes show a higher gynogenetic rate than the stocks from which the original haploids were obtained (Table 1). The positive effect of maternal haploid origin results from development of some genotypes (i.e. doubled haploid) favorable to maternal haploid production, and appears very similar to "inbreeding effect". The distribution of doubled haploid and pedigree lines derived from C123 × Oh7 suggests a segregation of genetic factors influencing the ability to produce maternal haploids (Fig. 4).

The haploidy-inducing ability of 29 different inbred lines, including European and American germplasm, was evaluated (Table 2). Pollen from 11 lines induced at least 1 maternal haploid plant in our experimental conditions (750–1000 plants were examined). These inducer lines were derived from populations of different origins including Greece (CM48), Poland (PS20), France (F7, F673) and North-America (ND33). The mean haploid frequency when considering all of the lines tested in this study was 0.07% (16 haploid plants out of 23,300 observed plants). Furthermore, no inbred lines tested showed any exceptional aptitude; the inducer line Stock 6 was almost 10 times more effective than any other line.

The F_1 hybrids involving Stock 6 as female parent showed the same level of haploidy-inducing potential as Stock 6 (Table 3). The results indicate that the haploidy-inducing ability of F_1 hybrids is not dependent upon the cytoplasm source of Stock 6. The exceptional aptitude of Stock 6 appears to be a dominant character with nuclear determination. However, there is a highly significant het-

Table 1. Influence of maternal haploid origin on the ability to produce[a] haploid plants

Parental genotypes			Genotypes derived from doubled haploids	
genotype	type	yields of haploid plants (% of maternal haploid plants in the progeny)	type	yields of haploid plants (% of maternal haploid plants in the progeny)
C123 × Oh7	hybrid	0.38 ± 0.09[b]	hybrid[c]	0.32 ± 0.31
			doubled haploid lines	0.10 to 2.40
F186 × W64A	hybrid	0.62 ± 0.09	hybrid[c]	0.90 ± 0.59
			hybrid[c]	0.24 ± 0.28
DS	synthetic	0.48 ± 0.14	hybrid[c]	1.52 ± 0.68
			synthetic[d]	1.36 ± 0.32

[a] Maternal haploids are obtained by pollination with Stock 6
[b] Confidence interval at 5% level
[c] Result of a cross between two doubled haploids
[d] Result of intercrossing of 40 doubled haploids

Table 2. The ability of different inbred lines to promote gynogenesis in crosses with different F_1 hybrids (F1243 × F101, W401 × F101, W64A × W33)

Inbred line genotypes	Aptitude[a] for gynogenesis induction (+/−)	Pedigree/Origin
A239	−	A347 × A73
A258	−	A73 × Os420 n.
A659	−	Mimnesota Syn. 3. (USA)
AS4	+	(C78 × H) H₂ Golden B. × Hayes Whites
BK34	−	hyb. Dobrudjenka
BK417	−	?
BK418	−	?
CM48	+	pop. from Greece
DU101	−	pop. Bade (West Germany)
Ep1	−	pop. Lizargarote (Spain)
F2	+	pop. Lacaune (France)
F7	+	pop. Lacaune (France)
F32	+	hyb. mv 23
F69A	−	pop. Esterre (France)
F72	−	pop. Laruns (France)
F140	+	F10 × A158
F161	+	Fv1 × V3
F673	+	pop. St. Germain des bois (France)
F1341	−	F21 × F115
F1547	−	F18 × F47
F1575	−	F217 × AK3
MS1334	−	Golden glow × Amargo (USA)
NAY307	−	pop. Butler (USA)
ND33	+	pop. Manitoba (USA)
PS6	−	pop. Wielkopolanka (Poland)
PS20	+	pop. Wielkopolanka (Poland)
Rcw5	+	Rc8 × W182 E
W10	−	L224 × Ill. A48
W15	−	pop. Manitoba (USA)

[a] For each inbred line tested, 750−1,000 plants were examined: an inbred line was noted +(positive) when it was observed in at least one haploid plant

erogeneity in the haploidy-inducing ability of different hybrids. For instance, a remarkable aptitude was noted for Lo32 × Stock 6. The inducer character of Stock 6 was influenced by the genetic background.

Haploidy-inducing abilities of 67 F_2 plants and their F_3 self-pollinated generations of 3 F_1 hybrids Co220 × Stock 6, MS1334 × Stock 6 and W23 × Stock 6 were determined (Fig. 5). F_2 plants of MS 1334 × Stock 6 and W23 × Stock 6 crosses showed a haploidy-inducing ability higher than that of inbred parental line Stock 6 (i.e. the mean frequency of haploids induced by pollen of Stock 6 was 0.8% with the material used in this study). The correlation coefficient between F_2 and F_3 generations is highly significant and suggests that the haploidy-

Table 3. Comparison of haploidy-induction potential of different hybrid genotypes in crosses involving a common female parent F1243 × A619 (no. of plants examined is indicated in parentheses)

Pollen parent genotype		Percentage of haploid plants		Test χ^2
Co220	× Stock 6	0.95	(844)	0.93
F7	× Stock 6	0.70	(1,000)	0.01
F83	× Stock 6	0.20	(1,000)	3.39
F1945	× Stock 6	1.10	(1,000)	2.59
Lo32	× Stock 6	1.15	(2,250)	7.48[b]
ND203	× Stock 6	0.60	(1,000)	0.09
W153R	× Stock 6	0.35	(2,000)	3.20
W182E	× Stock 6	0.52	(963)	0.35
W703	× Stock 6	0.30	(1,000)	2.12
				$\chi_h^{2\,a} = 20.2^{\,b}$
Total hybrids		0.68	(11,057)	$\chi_i^2 = 0.79$
Stock 6		0.51	(2,500)	

[a] Expected number was calculated from the mean frequency of haploids from all the crosses
[b] Significant at 1% level
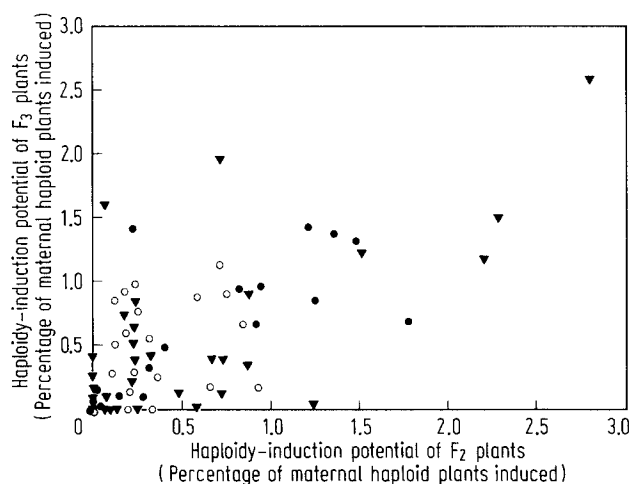
Fig. 5. Relationships between haploidy-induction potentials of F_2 plants and their F_3 progenies for three crosses. (Haploidy-induction potential for each F_2 plant and F_3 line was estimated by observation of 750 plants obtained after pollination with the plant or line tested); o Co220 × Stock 6, ● MS1334 × Stock 6, ▼ W23 × Stock 6; r (correlation) = 0.59 (significant at 1% level)

inducing potential is a heritable trait. Notably, the best F_2 plants confirm their aptitude in the F_3 generation.

Comparisons were made using Stock 6 and an inbred line WS14 (F_4 generation) derived from the cross W23 × Stock 6. WS14 was selected among the F_3 lines and shows a high inducing potential. The frequency of maternal haploids when WS14 was used as pollen parent was found to vary greatly in relation to female parent
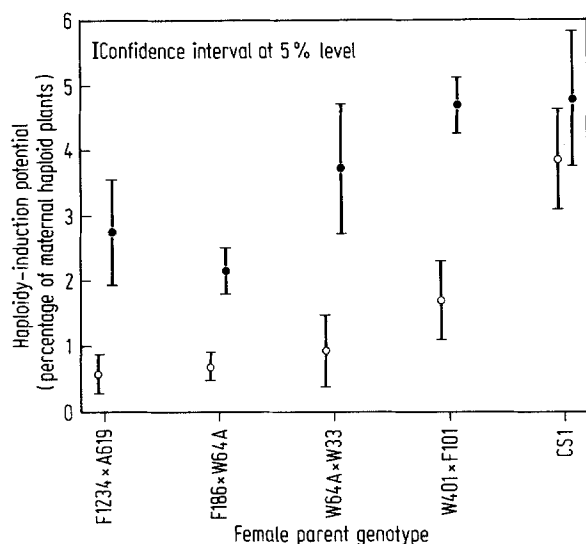
Fig. 6. Comparison of haploidy-induction potential of Stock 6 (o) and WS14 (●) used as male parent in different crosses

genotype; haploid yields ranged from 2%–5% (Fig. 6). A frequency as high as 10% was observed when the haploid yield from individual ears was considered. However, the enhancement attributed to WS14 pollen in comparison with Stock 6 was quite variable: WS14 was found to be 1.2–5.5 times more effective than Stock 6. Finally, haploid yield did not result from a simple addition of genetical male and female effects.

Discussion

These results confirm that the frequency of occurrence of haploids in maize is under genetic control (Chase 1952). Although gynogenetic development seems be possible from all genotypes, differences attributed to genotype of the maternal parent can be considerable. Chase (1969) suggested that the haploid frequency is inversely related to the frequency of lethal and sublethal genes among the female gametes. However, the ability distribution of the inbred lines derived from C123 × Oh7 and the limited inbreeding effect indicate clearly that maternal haploid production must be affected by genes controlling haploid initiation and haploid development. The genetic control of gynogenetic development seems to be distinct for genotypic anther culture response and the aptitude for in vivo androgenetic development. The anther culture response is a complex character including callus or embryo induction, plantlet regeneration and plant development, and probably requires specific components. The differences in intergenotypic variation between the aptitudes for in vivo gynogenetic and androgenetic development is in agreement with the suggestion of specific genes con-

trolling maternal haploid formation. Furthermore, the aptitudes of the hybrid F186 × W64a and its parental lines and the limited influence of maternal haploid origin on the haploid frequency cannot be completely explained by simple gametophytic inheritance. Nevertheless, the aptitude for gynogenetic development appears to be a highly heritable character.

The aptitude for inducing haploidy is a heritable trait of pollen. Although the use of specific pollinators as Stock 6 is favorable, in vivo gynogenesis induction appears to be a general character of pollen in maize. The inducing aptitude of Stock 6 is a transferable character. The data suggest that the capacity for maternal haploid induction is nuclear in origin. This, of course, does not exclude nuclear-cytoplasmic interactions. The exceptional aptitude of pollen from Stock 6 seems to be a character with sporophytic expression. This result is consistent with those of Coe (1959; Sarkar and Coe 1966) but not with those of Sarkar et al. (1972). The limited number of hybrids and progenies observed by these authors is probably responsible for this contradiction. Genetic models that reflect the observations during F_1 generation (dominance) and F_2 and F_3 generation (segregation) have been proposed (Lashermes 1987). A system involving three dominant genes, one complementary and epistatic over the two others, gives a satisfactory explanation. Although involving some untested hypotheses, this genetic study suggested a oligogenic component for the inducer character of Stock 6.

The selection procedure for the inducing aptitude which was employed here, has given a rapid improvement. Inducer lines such as "WS14" used as pollinators greatly increase the frequency of maternal haploids. After hybridization, high inducer genotypes can be selected from the F_2 generation. These exceptional plants occurred with a large frequency and probably resulted from a limited number of genes or recombinations. F_1 crosses such as Lo32 × Stock 6 showing a good inducing ability would be a material favorable for further selection. The heritability and noticeable oligogenic determination component of the inducing haploidy character should facilitate breeding and future improvement. The comparison of haploidy-induction potential of Stock 6 and WS14 suggests that the haploid-producing frequency is the result of genetic interaction between the male and female parents. So, this result must be considered at the time of the choice of female plant genotype for inducing haploidy breeding programmes.

Genotypic aptitude for promoting gynogenesis can be associated with pollen ability for revealing particular predispositions to gynogenetic development within the female reproductive organ. External conditions (Lashermes 1987) or a delayed pollination (Chase 1969) may facilitate the development of these predispositions, otherwise genetically determined. No addition of genotypic

influences of both male and female parents on the frequency of maternal haploid plants is consistent with this interpretation. No structural abnormality such as modifications in the number of pores and nuclei within the pollen population of Stock 6 has been noted (Lashermes 1987; Venkateswaru cited by Sarkar and Coe 1966). Gynogenetic induction does not appear to result from abnormalities at a low frequency within the pollen population but rather from characteristics of the pollen of inducing inbred lines. In vivo gynogenesis in maize appears to be associated with an incomplete fertilization and the egg cell seems to be at the origin of gynogenetic plants (Sarkar and Coe 1966). Pollen genotypes with a high aptitude to promote gynogenesis would be favorable to the non-fertilization of the egg cell. The high frequency of haploid plants observed in the progenies of some crosses should permit further studies to determine the exact mode of origin of maternal haploids.

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