Management of the ig gene for haploid induction in maize

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Agronomie (1991) 12, 247-251

Plant improvement

Management of the *ig* gene for haploid induction in maize

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(Received 1 April 1991; accepted 16 January 1992)

Summary — The effect of the *ig* gene showed the same intensity at the haploid as at the diploid level. This was contrary to the results on the exceptional rate obtained elsewhere, and suggested that haploid manipulation should not be used as a routine procedure. At the diploid level, the genetic background of the *ig* gene has a strong effect in paternal haploid induction. In backcrosses of W23 ig as the initial source with recurrent inbred lines which were more or less related to the Golden Glow OP origin, pleiotropic effects differed according to the genetic background in the offspring. If male sterility observed at the homozygous stage was still present, the other effects were completely lost from the initial cross with lines of the same origin (W25, CG3). On the other hand, they were maintained at a similar level in backcrosses with some non-related lines (F1254 for polyembryony). Due to its earliness the C0220 inbred line, in which the *ig* gene manipulation procedure is of interest, was shown to maintain a fairly good level of haploid induction in backcross and appears to be a promising background.

***Zea mays* L = maize / haploid induction / *ig* gene / *in situ* androgenesis / polyembryony**

Résumé — Adéquation du gène *ig* pour l'induction d'haploïdes chez le maïs. *Au niveau haploïde, l'effet du gène* ig *se révèle de la même intensité qu'au niveau diploïde, infirmant le résultat de taux exceptionnel obtenu par ailleurs. Ceci écarte la manipulation du gène* ig *à l'état haploïde dans un processus de routine. Au niveau diploïde, l'environnement génétique du gène* ig *a une forte influence sur l'aptitude à induire des haploïdes paternels. En rétrocroisements à partir de la lignée W23* ig *avec des lignées récurrentes plus ou moins apparentées à la population Golden glow d'origine, les effets pléiotropiques du gène* ig *se retrouvent diversement. Si la stérilité mâle manifestée à l'état homozygote est toujours présente, les autres effets, dont la polyembryonie, sont perdus dès le croisement initial avec les lignées de la même origine W25, CG3. À l'inverse, ils sont maintenus à un niveau similaire en rétrocroisement avec des lignées non apparentées (F1254 pour la polyembryonie). La lignée CO 220 intéressante pour la manipulation du gène* ig, *par sa précocité, a été détectée comme maintenant un bon niveau d'induction en rétrocroisement et apparaît comme un* background *prometteur.*

Zea mays *L* = *maïs / induction d'haploïdes / gène* ig / *androgenèse* in situ / *polyembryonie*

INTRODUCTION

The instantaneous derivation of homozygous lines is an old challenge in maize breeding first emphasized by Chase (1952). *In situ* androgenesis is possible with the use of the *ig* (indeterminate gametophyte) gene discovered by Kermicle (1969) which enormously increased the occurrence of paternal haploids (1%) contrasting with the natural spontaneous frequency of about 1 per 80 000 observed in maize (Goodsell, 1961).

On the other hand, the rapid production of homozygous lines by *in vitro* androgenesis has been recently improved, but with specific limiting factors of genotypic dependence (Petolino and Jones, 1986; Barloy *et al*, 1989).

The *ig* gene was localized on chromosome 3 by Kermicle and Demopoulos (1980) but its structure remains unknown.

In situ gynogenesis appeared as an alternative method due to its apparent independence from genotype in haploid induction due to the Coe Stock 6 strain (1% on average) – or its derivative SW 14 – (about 3%) (Lashermes, 1987). This procedure could be used if the limiting factor (efficient chromosome doubling) could be overcome and if the detection aspect could be monitored by the use of appropriate markers.

The use of the *ig* gene continues to be of interest in the rapid production of paternal haploids which occurs simultaneously with the recovery of the transferred maternal cytoplasm. It can serve many purposes, *eg* to obtain instantaneous inbred lines or cytoplasmic male sterile counterparts, to provide a rapid evaluation of the ability either for maintenance or restoration of male fertility, to transfer useful cytoplasm. This research was carried out to improve the level of the induction rate by the *ig* gene, and the management of the procedure for countries with lower heat units where the original late genotype W23 is difficult to grow to maturity.

We have noticed that with such use of haploids, the problem of chromosome doubling is solved thanks to direct repeated pollinations of haploid plants by the inbred line to be converted. This ensures the recovery of a few kernels. These successes can be expected because haploids have a better female fertility than the estimate based on the assumption of 10 chromosomes distributed independently at meiosis with an expectation of one normal egg out of 1 024 (Chase, 1949).

The first approach was to check on the exceptionally high rate of induction reported by Chase (1982): 2 paternal haploids were observed among a progeny of 8 seedlings following the pollination of 1 haploid *ig* plant.

A second approach consisted of looking for an earlier genotype than the late W23 inbred line for northern European countries to carry the *ig* gene. The paternal influence of the donor has been well documented by Kermicle (1973) and Chumak (1980) but very limited information is available (Rome and Poneleit, 1979) on the interaction between the induction rate and the female genotype carrying the *ig* gene.

MATERIAL AND METHODS

ig gene effect at the haploid level

The *ig* gene in the W23 background was associated with the recessive alleles of seedling markers, glossy 1 (gl1) or liguleless 1 (lg1) using backcrosses. Plants homozygous for *ig* show the usual male sterility; one of the pleiotropic effets reported by Kermicle (1971).

In order to enhance the production of maternal haploid *ig* plants (the *ig* gene also has a maternal haploid inducing effect), the glossy (gl_1 gl_1 *ig ig*) or liguleless (lg_1 lg_1 *ig ig*) male sterile plants were crossed with the gynogenetic haploid inducer Coe Stock 6, carrying the dominant alleles. From these crosses, within different progenies screened in a greenhouse, 8 liguleless *ig* haploid plants were pollinated by the pollen of F564, an INRA France inbred line carrying the glossy 1 marker and 16 glossy *ig* haploid plants were conversely pollinated by a liguleless 1 hybrid (F186 × W64A background) to detect paternal monoploids. Each plant was pollinated 3 times to ensure maximum fertilization.

We did not control the haploid level by chromosome counting at seedling stage but all paternal monoploids were actually male sterile at flowering time.

Background effect for *ig* gene at the diploid level

ig versions of different inbred lines more or less related to W23 (first cycle inbred lines issued from Golden Glow OP were obtained following 2 or 3 backcrosses (table I) and guessing the presence

Table I. Relation to the original *ig* W23 background.

Inbred line genotypes	*Pedigree/Origin*
A632	(Mt42 × B14) $B14^3$
CG13	Golden Glow OP
CO220	Improved CO109
F7	Lacaune OP
F244	F188 × F186
F252	F186 × CO125
F492	F556 × F575
F1254	F49 × F21
LH38	A619 × L120
Mo17	187-2 × C103
Ms12	(Golden Glow × Maize Amargo) S5 × Duncan
Ms1334	(Golden Glow × Maize Amargo) Golden Glow
Ms71	A619 × R168
Pa54	I11 A × W23
W23	Golden Glow OP
W25	Golden Glow OP
W117	643 × Minn # 13
W64A	Wf9 × 187-2
W401	(W33 × W25) × W670

of *ig* in the backcrossed plants by male sterility and polyembryonic effects detected in the seeds or at the seedling stage (twins, triplets). The choice of the lines to be advanced was based upon the promising results of a given generation.

The pollen of a single cross MBS847 × F564 carrying gl1 was used to detect paternal haploids produced by the male sterile plants within self pollinations of either the first cross or the following backcross. For the naturally gl1 inbred line F7, the pollen came from the lg1 F186 × W64A single cross.

The male sterile plants for testing the induction efficiency within segregations were of course different from the plants used without selection for the backcrosses. The data in each generation were pooled regardless of filiation. In practice, only CO220 for high induction and F1254 for high polyembryonic level have a minimum of 2 backcrosses.

RESULTS AND DISCUSSION

Haploid level

The *ig* gene showed maternal and paternal induction effects of the same magnitude as noted by Kermicle (1969) at the diploid level

Table II. Haploids produced by pollination of *ig* haploid plants.

gl_1 ig plants × ♂ lg_1 Seedlings				*lg_1 ig plants × ♂ gl_1 Seedlings*		
Seed set	*Single*	*Twins or triplets*	*Plant No*	*Seed set*	*Single*	*Twins or triplets*
18	12		1	16	11	1
32	24	1	2	5	4 (1)	
2	0		3	0		
0	0		4	34	24	2
8	4	1 [1]	5	21	16	1 [1]
3	2		6	4	3	
7	3		7	12	6 [1]	
6	2	2	8	6		
16	13		9			
5	4(1)		10	98	64	4
92*	84*	2*	11			
9	7		12			
24	18	1 [[1]]	13	Pooled results[a]		
8	6		14	Paternal haploids (seed set %)		
11	4	1	15	1.2 (0.1 – 3.0)		
0	0		16	Maternal haploids (seed set %)		
				2.5 (0.6 – 5.4)		
149	99	6	Total			

* Plant excluded from statistics (spontaneous doubling?) ( ); [ ]: respectively paternal and maternal haploids; [[ ]]: in a twin, 2 members are haploids; [a] confidence interval at 0.5% level after angular transformation.

Table III. Influence of the *ig* background on the ability to produce paternal haploids within crosses between W23 *ig* and various inbred lines used as recurrent parents.

Genotype	*Generation of backcross*	*No of tested ears*	*Single*		*No of seedling Twins or triplets*	*Polyembryony (%)*
W23		22	3024 (0.8)[a]		158 (0.4)	5.0 ± 0.8[c]
CO220	F2	15	2340 (1.1)	1.3[b]	231 (0.1)	9.0 ± 1.2
	BC1	35	3758 (0.9)	0.2	150 (4)	3.8 ± 1.2
	BC2	27	2692 (0.7)	2.6	162 (6)	5.6 ± 0.8
A632	F2	13	2888 (0)		0	
CG13	F2	14	2002 (0)		23 (0)	0.4 ± 0.3
F7	F2	12	1986 (0)		0	
F244	F2	28	5309 (0.2)		699 (0.01)	11.6 ± 0.8
	BC1	10	3070 (0)		267 (0)	8.0 ± 0.9
F252	F2	31	5289 (0.1)		276 (0)	5.0 ± 0.6
	BC1	23	4186 (0)		108 (0)	2.5 ± 0.5
F492	F2	13	2054 (0)		0	0
F1254	F2	15	2745 (0.1)		312 (0)	10.2 ± 1.1
	BC1	15	1740 (0.1)		286 (0)	14.1 ± 1.5
	BC2	19	3089 (0.3)		253 (0)	7.5 ± 0.9
	BC3	8	1018 (0.6)		81 (0)	7.4 ± 1.5
LH38	F2	14	3080 (0)		21 (0)	0.6 ± 0.3
Mo17	F2	12	2341 (0)		15 (0)	0.6 ± 0.3
Ms12	F2	14	2785 (0.2)		125 (1.6)	4.3 ± 0.7
Ms71	F2	12	3041 (0)		30 (0)	0.9 ± 0.3
Ms1334	F2	13	2535 (0)		0	0
Pa54	F2	14	2520 (0)		3	0.2 ± 0.2
W25	F2	15	2721 (0)		22 (0)	0.4 ± 0.2
W64A	F2	13	2320 (0)		0	
W117	F2	12	2297 (0)		27 (0)	1.1 ± 0.4
W401	F2	14	2352 (0)		48 (0.1)	2.1 ± 0.6
	BC1	8	513 (0)		41 (0)	7.9 ± 2.3

[a] % of haploids are in parentheses; [b] χ^2 not significant at 1% level with the W23 *ig* basis; [c] confidence interval at the 5% level.

and also some pleiotropic polyembryony (table II). The experiment conducted in the absence of an endosperm marker did not allow the evaluation of the intensity of hetero-fertilization. Maternal or paternal haploids were found in single seedlings or twins or triplets. More maternal haploids were produced than paternal haploids. Therefore the preliminary results of Chase (1982) were not confirmed and might be attributable to a chance effect, as they were based on only one plant.

Since the use of haploid *ig* plants requires their prior production, the present results do not support the use of such a procedure.

The high rate of seed set of the haploid plants confirmed the data of Chumak (1980) concerning the use of the *ig* gene in cytoplasmic transfer by pollination with the recurrent parent. The overproduction of viable eggs in comparison with the expected frequency ($1/2^{10}$) in the W23 background can be attributed to a fairly high spontaneous doubling located in fairly large sectors. Plants without seeds might have been affected by the extremely dry conditions of 1990. The *ig* gl1 plant No 11 discarded from the statistics might correspond to a total spontaneous diploidization of the ear and gave an estimate of 4% for this aspect.

Background effects at the diploid level

A strong effect of the background depending on the different doses of W23 genotype (the Golden Glow OP origin), within initial crosses or backcrosses to recurrent inbred lines, is shown in table III. Regardless of the inbred lines used there was a complete lack of haploid induction and polyembryony in CG13 and W25 when male sterility was recovered (first cycle lines related to Golden Glow), as

well as in the non-related lines A632, F7, F492, W64A.

In contrast, some non related lines F244 and F1254 maintained a similar or superior level of polyembryony induction as compared to W23. As far as haploid induction was concerned, CO220 (early Buttler OP origin) could be identified as an earlier and non-related line equivalent to W23 for paternal haploid induction. Lashermes (1987) noticed a variable expression in intensity of the different pleiotropic effects of *ig* in W23 according to the pollinator. These could be expressed differently in other backgrounds. The presence of a large amount of W23 germplasm does not appear to be necessary and thus one may expect to find some more efficient background for *ig* (inbred lines or genetic pool) for *ig* androgenesis.

The *ig* gene does not appear to constitute a simple genetic system but is influenced by modifying genes.

The real falsification of the hypothesis of a compound locus whose functions had been lost in backcrossing would consist of performing a reverse backcross to replace the *ig* gene in its original background.

Acknowledgment

The author is grateful to A Lapierre for her technical assistance.

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