Selective Fertilization in Maize, *Zea mays* L. III. Independence of Gametophyte Factors on Superiority of Pollen Grains from F₁ Plants

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In the previous reports, it was shown that the superiority of pollen grains from F₁ plants in selective fertilization of maize existed. Clarification, however, was needed for possible linkage of any gametophyte factor(s) to the marker gene and for the factor responsible for maintaining the superiority of F₁'s pollen grains. At first, the present study dealt with conducting two kinds of back-crossing, all possible selfing of four F₁s, and all possible crossing among F₁s. The existence of gametophyte factor(s) relating to the marker gene could not be found out in back-crossing used F₁s as pollen parents. In selfing in part, crossing, and another type of back-crossing, however, aberrant segregation ratios of the marker gene were found out in particular pollination combinations. Secondly, pronounced superiority of F₁'s pollen grains was well confirmed in the mixed-pollination experiments (YAMADA 1982). It was, moreover, shown that the superiority of pollen grains from F₁ plants in selective fertilization is due to the grains *per se* which are produced by the sporophyte of F₁ in heterozygosity.

KEY WORDS : Maize, selective fertilization, gametophyte factor, mixed-pollination, superiority of F₁ pollen

Introduction

In the studies of selective fertilization in maize, MURAKAMI *et al.* (1972), investigated selective fertilization by using an inbred line as a female parent and pollen-mixtures involving self pollen and pollen from other inbred lines or F₁s as the pollen parents. Pollen grains from F₁ plants were get still greater advantage in selective fertilization. YAMADA and MURAKAMI (1978) also showed that this superiority was not affected by the genotype of the pollen recipient. However it will be necessary to undertake genetical experiments to determine whether the gametophyte factor(s) linked to the marker gene(s) are involved in selective advantage.

On the other hand, it is well known that selective fertilization in maize is controlled by gametophyte factors, *Ga-s*, *Ga*, and *Ga-m*, of which loci and effect on a marker gene were decided from aberrant segregation ratios in F₂ generation and back-crossing (BIANCHI and LORENZONI 1975). Gametophyte factor(s) are proved to be major genes, or a single gene, and their activity is not controlled by the genotype of sporophyte releasing pollen grains or differentiating macrospores. Therefore, it is important to determine whether gametophyte factor(s) exert any influence on the superiority of pollen grains from F₁ plants.

MANGELSDORF and JONES (1926) who observed aberrant segregation ratios of *Su/su* genotype in F₂ generation of maize pointed out the possible effect gametophyte factors linked to *Su* gene without identification of real *Ga* gene. Three kinds of gametophyte factors have since then been reported : *Ga-s* (NELSON 1952), *Ga-m* (JIMENEZ and NELSON

1965), and *Ga* (recently BIANCHI and LORENZONI 1975). Although these authors argued about the role of gametophyte factors and their linkage to marker genes, they did not pay attention to confirm the existence of selective fertilization in pollen grains *per se* from F_1 plants.

Similarly in other reports on selective fertilization in maize, pollen-mixtures consisting either of pollen grains from two or more than three inbred lines or varieties (HOFMEYER 1958, MOLCHAN 1963, MOLL 1972, and LYUBENOV 1973, 1974), or of pollen grains from two kinds of F_1 s (PFAHLER 1974) were used, but the pollen-mixture consisting of pollen grains from inbred line and those from F_1 plants were not utilized.

Accordingly, the present study was conducted with the following objectives 1) to determine whether there is any gametophyte factor(s) linked to the marker gene used in our experiments and 2) to identify the factor responsible for the superiority of pollen grains from F_1 plants in selective fertilization (This part was reported in YAMADA (1982) insufficiently).

Materials and Methods

Materials

On the basis of the previous results (YAMADA and MURAKAMI 1978), two inbred lines, A 34 and CI 64 (abbreviated as W_1 and W_2) of white dent endosperm genotype $o_r o_r^*$, and two inbred lines, Koshu-564 and Outomorokoshi-143 (abbreviated as O_1 and O_2) of orange flint endosperm genotype $O_r O_r^*$ were used in this experiment (Table 1). Four

Table 1. Original materials used for back-crossing, selfing, crossing, and mixed-pollination experiments.

Abbreviation	Inbred line	Endosperm character		Genotype for kernel color ¹⁾	Origin or original variety in italic
		Color	Type		
W_1	A 34	white	dent	$o_r o_r$	Minnesota (U. S. A.)
W_2	CI 64	<i>do.</i>	<i>do.</i>	$o_r o_r$	U. S. D. A.
O_1	Koshu-564	orange	flint	$O_r O_r$	<i>Koshu</i> (Japan)
O_2	Outomorokoshi-143	<i>do.</i>	<i>do.</i>	$O_r O_r$	<i>Outomorokoshi</i> (Japan)

Note : ¹⁾This genotype is tentative.

F_1 s derived from inbred lines with different endosperm characters, namely $W_1 \times O_1$, $W_2 \times O_1$, $W_1 \times O_2$, and $W_2 \times O_2$, and an F_1 ($W_1 \times W_2$, abbreviated as W_{F_1}) derived from inbred lines with the same endosperm character, were obtained.

Methods

The pollination scheme in this experiment included i) back-crossing, selfing, and crossing in the four F_1 s and ii) mixed-pollination by means of pollen-mixtures consisting of pollen grains from plants with the genotype, $o_r o_r$, and those from F_1 plants with the genotype, $O_r o_r$. In the back-crossing, selfing, and crossing (Table 2), pollen grains from the four F_1 s were pollinated in all possible pollinating schemes as shown in Table 3. In the mixed-pollination experiment, plants having the genotypes, $o_r o_r$, *ie.* W_1 , W_2 ,

* These genotypes, $O_r O_r$ and $o_r o_r$, are not induced from gene-analysis on endosperm character, but are tentatively designated as such.

and W_{F_1} as female parents, were pollinated by pollen-mixture consisting of pollen grains with inbred lines in the same genotype as that of the female parent and pollen grains from four F_1 s with another genotype, $O_r o_r$.

In the mixed-pollination experiment described above, W_1 , W_2 and W_{F_1} as female parents were pollinated by pollen-mixtures. Twenty pollen-mixtures, comprised pollen grains from two female parents with the genotype, $o_r o_r$, designated as Pollen parent I and pollen grains from four F_1 s with the genotype, $O_r o_r$, designated as Pollen parent II in Table 4, were pollinated to three female parents, W_1 , W_2 and W_{F_1} . In summary, the scheme of mixed-pollination was $o_r o_r$ (Female parent) $\times$ [$o_r o_r$ (Pollen parent I) + $O_r o_r$ (Pollen parent II)]. Out of 24 sets of mixed-pollination extending as far as possible (3 Female parent $\times$ 2 Pollen parent I $\times$ 4 Pollen parent II), four sets were not succeeded because of asynchrony in pollen shedding and silking among them.

Pollen grains for pollination were prepared as follows: tassels of pollen sources were covered with parchment-paper bags at 7:00 A.M. after dew disappeared and before pollen shedding started. At 10:00 A.M. pollen grains and anthers together were collected by shaking the tassels covered with a bag. Pollen grains of each line as pollen sources were separated from anthers by a 40-mesh stainless sieve. For mixed-pollination 0.8 ml of pollen grains in each Pollen parent I and II were thoroughly mixed on parchment-paper. Although the size of pollen grains from both Pollen parent I and II were not the same (data are not shown here), the differences among them were not so effective as to need the some modification in the mixing ratio in this experiment. The pollen-mixtures thus prepared were evenly spread with a painting brush on silks covered by parchment-paper bag. The number of ears pollinated were not uniform in all pollinations, though it was attempted to pollinated more than 15 ears of every female parent, since ears having less than 40 kernels at maturity were excluded.

The pollinated ears were harvested at maturity, and the kernels of the ear were classified into two groups on the basis of their endosperm color, colorless ($o_r o_r o_r$ genotype) and colored ($O_r O_r o_r$ or $O_r o_r o_r$ genotype, *ie.* yellow or light yellow) under the light of Toshiba Standard White Illuminator. If the pollen with the genotype O_r originating from O_1 or O_2 fertilized, the color of the endosperm of the female parents turned yellow or light yellow due to xenia. On the other hand, when the pollen with the genotype or originating from W_1 or W_2 fertilized, the endosperm color remained colorless. In the mixed-pollination experiment, the percentage of colored kernels for all the kernels in an ear of female parent was used as a scale for the degree of selective fertilization. The experiment was undertaken at the Kanda Farm, Hiratsuka, Kanagawa in 1976 partly, and at Tsukuba in 1980 and 1981.

Results

The results obtained in two kinds of back-crossing, selfing, and all possible crossing in four F_1 s are shown in Table 2 and significance in χ^2 -test in Table 3, respectively.

In the two kinds of back-crossing, segregation ratio of the colored kernels to the colorless ones was expected to be 1:1, and in the selfing and crossing, moreover, segregation ratios of the colored kernels to the colorless ones were also expected to be

Table 2. Segregation ratios of kernel color in all possible back-crossing, selfing, and crossing in F_1 s of maize inbred lines.

Combination	Colorless	Colored	χ^2 -value	Combination	Colorless	Colored	χ^2 -value
Expected	[1 : 1]			Expected	[1 : 3]		
$W_1 \times (W_1 \times O_1)$ (19)	344 :	353	0.12	$(W_1 \times O_1) \times (W_1 \times O_1)$ (20)	718 :	2,094	0.43
$(W_1 \times O_1) \times W_1$ (7)	1,177 :	977	9.27	$(W_1 \times O_1) \times (W_2 \times O_1)$ (8)	541 :	1,669	0.32
$W_2 \times (W_1 \times O_1)$ (12)	945 :	894	1.41	$(W_1 \times O_1) \times (W_1 \times O_2)$ (10)	1,124 :	3,350	0.04
$(W_1 \times O_1) \times W_2$ (12)	1,931 :	1,712	13.17	$(W_1 \times O_1) \times (W_2 \times O_2)$ (10)	750 :	2,203	0.25
$W_1 \times (W_2 \times O_1)$ (7)	311 :	289	0.81	$(W_2 \times O_1) \times (W_1 \times O_1)$ (7)	675 :	1,634	22.41
$(W_2 \times O_1) \times W_1$ (10)	1,582 :	1,421	8.63	$(W_2 \times O_1) \times (W_2 \times O_1)$ (16)	1,131 :	3,517	1.10
$W_2 \times (W_2 \times O_1)$ (9)	364 :	366	0.01	$(W_2 \times O_1) \times (W_1 \times O_2)$ (10)	650 :	2,315	14.98
$(W_2 \times O_1) \times W_2$ (5)	778 :	549	39.52	$(W_2 \times O_1) \times (W_2 \times O_2)$ (14)	169 :	1,171	109.68
$W_1 \times (W_1 \times O_2)$ (17)	1,004 :	1,049	0.99	$(W_1 \times O_2) \times (W_1 \times O_1)$ (11)	583 :	3,195	184.48
$(W_1 \times O_2) \times W_1$ (5)	738 :	773	0.81	$(W_1 \times O_2) \times (W_2 \times O_1)$ (9)	634 :	2,003	1.29
$W_2 \times (W_1 \times O_2)$ (6)	487 :	475	0.15	$(W_1 \times O_2) \times (W_1 \times O_2)$ (26)	1,621 :	6,153	71.35
$(W_1 \times O_2) \times W_2$ (3)	178 :	171	0.35	$(W_1 \times O_2) \times (W_2 \times O_2)$ (12)	373 :	1,763	64.72
$W_1 \times (W_2 \times O_2)$ (19)	1,384 :	1,236	2.62	$(W_2 \times O_2) \times (W_1 \times O_1)$ (15)	1,028 :	3,833	38.47
$(W_2 \times O_2) \times W_1$ (8)	910 :	1,099	17.78	$(W_2 \times O_2) \times (W_2 \times O_1)$ (13)	430 :	2,576	183.43
$W_2 \times (W_2 \times O_2)$ (19)	1,112 :	1,079	0.50	$(W_2 \times O_2) \times (W_1 \times O_2)$ (14)	452 :	2,231	94.73
$(W_2 \times O_2) \times W_2$ (12)	2,082 :	2,418	24.09	$(W_2 \times O_2) \times (W_2 \times O_2)$ (22)	902 :	5,971	516.01

Note : Figures in parenthesis indicate number of ears observed.

3 : 1, if no gametophyte factor(s) linked to the marker gene existed. In the back-crossing of four F_1 s to the colorless inbred lines, W_1 and W_2 , it was entirely confirmed that there were no gametophyte factor(s) linked to the marker gene in all eight pollination sets, as shown in χ^2 -values between 0.01 of $W_2 \times (W_2 \times O_1)$ and 2.62 of $W_1 \times (W_2 \times O_2)$. On the other hand, in the back-crossing of the colorless inbred lines, W_1 and W_2 , to four F_1 s, three different kinds of segregation ratios were given; i) aberrant segregation ratios with excess number of the colorless kernels in the $W_1 \times O_1$ and $W_2 \times O_1$ as female parents, ii) aberrant segregation ratio with excess number of the colored kernels in $W_2 \times O_2$, and iii) no aberrant segregation ratio in $W_1 \times O_2$.

In the four selfing, χ^2 -values in $W_1 \times O_1$ and $W_2 \times O_1$ were not significant in aberrant segregation ratio from 3 : 1, while χ^2 -values in $W_1 \times O_2$ and $W_2 \times O_2$ were highly significant in the aberrant segregation ratio, showing excess number of the colored kernels. In other words, the F_2 segregation ratios of the kernel color in $W_1 \times O_2$ and $W_2 \times O_2$ deviated

Table 3. χ^2 -test on segregation ratios of kernel color in back-crossing, selfing, and crossing in F_1 s of maize.

	W_1	W_2	$W_1 \times O_1$	$W_2 \times O_1$	$W_1 \times O_2$	$W_2 \times O_2$
W_1	—	—	NS	NS	NS	NS
W_2	—	—	NS	NS	NS	NS
$W_1 \times O_1$	[**]	[**]	NS	NS	NS	NS
$W_2 \times O_1$	[**]	[**]	[**]	NS	**	***
$W_1 \times O_2$	NS	NS	***	NS	***	***
$W_2 \times O_2$	**	***	**	***	***	***

Note : [**] indicates an aberrant segregation ratio exceeding in colorless kernels.

significantly from the expected 3:1, suggesting the existence of any gametophyte factor(s) linked to the marker gene of O_2 .

In 12 combinations of crossing, pollination to $W_1 \times O_1$ by other F_1 s resulted no significant segregation ratio distorting from 3:1 in kernel color, while pollination to $W_2 \times O_2$ by other F_1 s showed highly significant aberrant segregation ratio from the 3:1. Other two F_1 s, $W_2 \times O_1$ and $W_1 \times O_2$, when they were pollinated by alternative F_1 s pollen, showed different results on the significance of χ^2 -test including aberrant segregation ratio and normal one.

Table 4. Mixed-pollination experiments pollinated by equal volumes of pollen grains from $O_r O_r$ and $o_r o_r$ plants.

Female parent ($o_r o_r$ genotype)	Pollen parent I ($o_r o_r$ genotype)	Pollen parent II ($O_r O_r$ genotype)			
		$F_1(W_1 \times O_1)$	$F_1(W_2 \times O_1)$	$F_1(W_1 \times O_2)$	$F_1(W_2 \times O_2)$
W_1	W_1	28.5(13) ^{NS}	31.0(12)**	39.0(18)**	37.5(14)**
W_1	W_2	31.8(8)*	29.4(12)**	—	41.2(12)**
W_2	W_1	43.7(9)**	43.0(9)**	—	45.4(10)**
W_2	W_2	40.6(12)**	35.7(7)*	—	36.9(9)**
$W_{F_1(W_1 \times W_2)}$	W_1	34.7(19)**	47.3(15)**	49.3(16)**	47.4(15)**
$W_{F_1(W_1 \times W_2)}$	W_2	32.2(17)**	33.8(15)**	—	38.9(15)**

Note: Figures in columns show average percentage of yellow or light yellow kernels due to xenia. Figures larger than 25.0 indicate the superiority in selective fertilization of pollen grains from $O_r O_r$ genotype, Pollen parent II. Figures in parenthesis indicate number of ears observed. * and ** are significant at 5% and 1% level, respectively.

Results obtained in the mixed-pollination experiment are shown in Table 4 (YAMADA 1982). The percentages of colored kernels in ears of Female parent in each mixed-pollination are presented with the possible pollinating combinations between Pollen parent I and Pollen parent II. Theoretically the percentages were expected to be 25.0% in the absence of selective fertilization between either pollen grains from Pollen parent I or Pollen parent II. This theoretical expectation was given from the results of back-crossing of four F_1 s to W_1 and W_2 as shown in Table 3, because of four F_1 s as pollen parents having no gametophyte factor(s) in this pollination experiment. As mentioned insufficiently in YAMADA (1982), the percentages of colored kernels in 19 sets except only one for $W_1 \times [W_1 + (W_1 \times O_1)]$ among 20 mixed-pollination sets conducted were significantly higher than 25.0%, suggesting that pollen grains from F_1 s plants were superior in selective fertilization.

Discussion

The first purpose of the present study was to investigate the existence of any gametophyte factor(s) linked to the marker gene O_r or o_r of the materials (Table 1) used in the previous mixed-pollination experiments (YAMADA 1982, YAMADA and MURAKAMI 1978, 1983). The study was carried out by testing the segregation ratios in the genotype of kernel color (Table 2 and 3). The results in the back-crossing to homozygous female parents were consistent in showing the lack of the linkage of gametophyte factor(s) to the marker gene, but in reciprocal back-crossings to heterozygous parents there was linkage of the marker genes to gametophyte factor(s) regarding as active ones either

in the colored genotypes or in the colorless ones. Linkage of gametophyte factor(s) to the colored kernel genotypes, moreover, was more remarkable in an inbred line, O_2 , from selfing in four F_1 s, whereas no aberrant segregation ratios both in $W_1 \times O_1$ and in $W_2 \times O_1$. Linkage of gametophyte factor(s) acting in female parents to the marker gene was proposed in maize by NELSON (1952). In this experiment, similar gametophyte factor(s) in O_1 , O_2 , and even W_1 or W_2 , seemed to be detected, although complete gene-analysis was not carried out, and it has been strictly confirmed there is no gametophyte factor acting in male parents and in pollen grains. On the other hand, NEUFFER *et al.* (1968) reviewed gametophyte factors, Ga_1 , ga_2 , and so on, sited on chromosome 1, 3, 4, 5, and 9, and MIRJUTA (1971) suggested that ga_1^r in Risovaya 645 interacted with Ga -s in female lines to result aberrant segregation ratios. And recently PODOLSKAYA (1981) found out the consistence of Ga -s, Ga -m, and Ga_1 as the alleles of gametophyte gene of fourth chromosome. From these papers, it might be remained to examine more precisely on results obtained here.

The second purpose of this experiment was to confirm whether the superiority of pollen originated from F_1 plants in selective fertilization or in gamete competition is governed by the heterozygous sporophyte or other gametophyte factor(s) well-known in selective fertilization by means of F_2 segregation ratio method. The fact which was pointed out previously with some lack of data in YAMADA (1982), was supported by results obtained here, —the superiority of pollen from F_1 plants *per se*, irrespective of the existence of gametophyte gene (s) linked to the markers.

There are some papers dealing with contributions of gametophytic and sporophytic genotypes to gamete competition in maize. JOHNSON and MULCAHY (1978) and MULCAHY (1975) proposed the interpretation that the competitive ability of pollen was determined, at least in part, by the gametophyte genotype. And OTTAVIANO *et al.* (1982) have not always agreed with our thought, from which plant vigor in heterozygote reflected to the competing ability of pollen, based upon their results obtained in the mixed-pollination in maize. They also, however, would not have shown directly the fact that competing ability of pollen in the mixed-pollination was controlled by the genotype of gamete only.

In conclusion from the data here, existence of the superiority of pollen grains originated from F_1 plants in gamete competition would be confirmed irrespective of gametophyte factor(s) in linkage to the markers. Of course, further studies on genotype of each single pollen grain from F_1 plants relating to gamete competing ability will be required to weigh the contribution of gametophytic genotype.

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トウモロコシの選択受精に関する育種学的研究Ⅲ.

F₁ 個体花粉の有利性と配偶体因子との関係

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これまでの一連の実験(村上ら 1972, 山田・村上 1978, 山田 1982)で, トウモロコシの F₁ 個体に生じる花粉が選択受精上有利であり, この現象が特定の系統にのみ見られる特異な現象でなく, 普遍的なものであることを示した。本報では, この現象が広く知られている配偶体因子と関係しているか否かを明らかにしようとした。

材料は山田・村上(1978)でも用いられた粒色白のデント種2自殖系統, A 34 (W₁ と略記。以下同じ), CI 64 (W₂) とその F₁ (W_{F₁}), 粒色オレンジのフリント種2自殖系統, 甲州 -564 (O₁), 大玉蜀黍 -143 (O₂), およびこれらの中で胚乳形質が異なる自殖系統間の F₁ 4 組合せである。O₁ および O₂ に由来する粒色遺伝子(仮に O_r とする。以下同じ)を持つ花粉が, W₁, W₂, W_{F₁} (o_ro_r) に受精すると, キセニアにより粒色は黄色ないし淡黄色となり, その花粉の受精が確認できる。

標識とした粒色遺伝子 O_r, o_r と配偶体因子との連鎖の有無を知るため, 上記組合せの F₁ について, 正逆2通りの戻し交雑 16, 自殖 4, すべての可能な交雑 12 の計 32 の授粉実験を行った。さらに, W₁, W₂, W_{F₁} を雌親とし, W₁ または W₂ の花粉と上記 4 組合せの F₁ 個体の花粉との等量混合花粉による授粉実験を行っ

た。収穫後、40粒以上着粒した雌穂について、着色粒（遺伝子型 $O_r O_r O_r$, $O_r O_r o_r$, $O_r o_r o_r$ ）と白色粒（ $o_r o_r o_r$ ）との割合を調べた。

4組合せの F_1 を花粉親とした戻し交雑8通りでは、すべて着色粒：白色粒=1：1であって、標識遺伝子に連鎖した配偶体因子の存在は否定されたが、4組合せの F_1 を雌親とした戻し交雑では、 $W_1 \times O_1$ と $W_2 \times O_1$ では白色粒多、 $W_2 \times O_2$ では着色粒多、 $W_1 \times O_2$ では1：1となり、雌親側において作用する配偶体因子の存在が示唆された。さらに F_1 を自殖すると、 O_1 を含む F_1 2組合せでは着色粒：白色粒=3：1に適合したが、 O_2 を含む F_1 2組合せでは着色粒多の分離比異常を認め、雌親側において分離比異常を起こす配偶体因子は、 O_2 においてよく発現することが推察された。一方、4組合せ F_1 の間の交雑において、 $W_1 \times O_1$ を雌親とした場合には分離比異常は認められず、 $W_2 \times O_2$ を雌親とした場合はすべて着色粒多の分離比異常を示し、 $W_2 \times O_1$ と $W_1 \times O_2$ とを雌親とした場合は様々な結果をえた。すなわち、標識遺伝子に連鎖した配偶体因子は雌親側で発現し、しかも O_2 に由来する配偶体因子の作用が顕著であると思われた。

4組合せの F_1 と白色粒の2自殖系統との混合授粉実験は、粒色遺伝子型で示すと $o_r o_r \times [o_r o_r + O_r o_r]$ で、上述の F_1 を花粉親とする戻し交雑の結果から、着色粒の期待値は25%である。Table 4の結果（山田 1982）は明らかに25%より有意に着色粒率が高く、 F_1 個体に生じる花粉の選択受精上の強勢は、接合体におけるヘテロ接合性によるものであって、配偶体因子は関係しないと結論できた。