Superiority of Pollen from F_1 Plants of Maize in Selective Fertilization

By MINORU YAMADA

Department of Physiology and Genetics, National Institute of Agricultural Sciences
(Yatabe, Ibaraki, 305 Japan)

Many studies on selective fertilization in maize, *Zea mays* L., have been reported since it was detected that genetic factor(s) linked to the *de* gene is responsible for distortion of segregation in F_2.[2]

On the other hand, some applications of the selective fertilization to maize breeding, i.e. prediction for combining ability of inbred lines or F_1 seed production without using cytoplasmic male sterility, were proposed. In addition, some papers showed that selective fertilization played a role in the occurrence of genetic drift caused by non-random fertilization in random population from the results of mixed-pollination experiments. However, all of these studies are concerned with genic effects of particular genes in gamete or in homozygous sporophyte, irrespective of any zygosity of plants producing gamete, especially pollen.

The present review shows an approach to selective fertilization of maize pollen derived from heterozygous F_1 plants showing heterosis and implication of such kind of heterosis in gamete competition.

Apparent heterosis in selective fertilization

Studies on selective fertilization have so far been composed of two experimental techniques: 1) genetical analysis of gametophyte factor(s) and 2) gamete competition among pollen grains originating from different genotypes in mixed-pollination and so on. Difference in degree of selective fertilization between pollen from an inbred line and pollen from an F_1 plant can be confirmed indirectly by the method of mixed-pollination. In this method, a pollen-mixture composed of an equal volume of pollen grains from a given female line and pollen grains from each of pollen parents, either inbred lines or their F_1 plant, is pollinated to ears of the given female line. In this case, a female line with recessive homozygosity (*yy*) in its genotype for endosperm color, and pollen parents, either inbred lines or their F_1 plant, with dominant homozygosity (*YY*) are selected for the use.

Therefore, the mixed-pollination using a pollen-mixture composed of pollen grains from different genotypes showing different kernel color results in the induction of xenia in the pollinated ears.

Accordingly, if the ratios of xenia kernels in the pollinated ears differed from the ratio of pollen grains from different sources in the pollen mixture, it would be possible to determine whether or not selective fertilization, or gamete competition, among pollen grains from different genotypes occurred.

At first, it was found out that[5] pollen from F_1 plants were superior to pollen from their respective parental lines in achieving fertilization. As shown in Fig. 1, it is apparent that pollen from F_1 plants showed outstandingly high ratios of yellow kernels, and hence very high degrees of selective fertilization as compared with pollen of most inbred lines and parental lines of each F_1 plant.

However, data shown in Fig. 1 may represent a specific case of mixed-pollination, because pollen of the female parent itself were used in combination with other pollen to be tested. Therefore, another pollination experiment was carried out[7] with a complicated

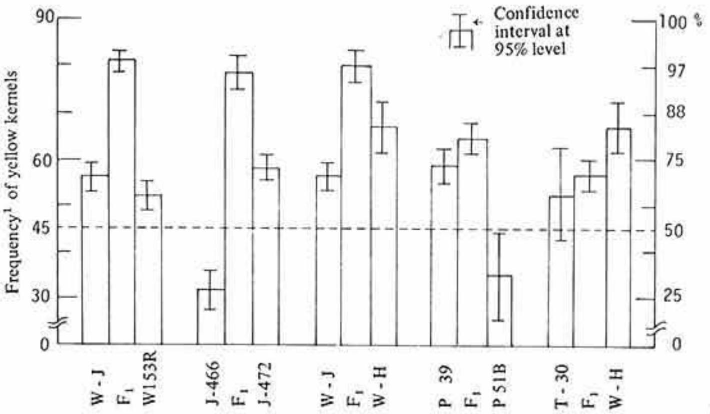

Fig. 1. Percentages of yellow kernels produced on ear of the female parent, A34 (*yy*), which was pollinated pollen-mixtures composed at an equal amount of pollen of A 34 + pollen of F_1 plant, or its pollen or female parent[5]

Note: 1: Percentage of yellow kernels is shown in arc-sine transformed value. There is no selective fertilization when it is 45.

Table 1. Female parents and pollen-mixtures to be pollinated to the female parents in Set I and Set II experiments[7]

Female parents	Pollen-mixtures to be pollinated to female parent		
Set I			
W_1	(W_1+O_1), (W_1+O_2) $(W_1+O_{F_1})$	(W_2+O_1), (W_2+O_2) $(W_2+O_{F_1})$	$(W_{F_1}+O_1)$, $(W_{F_1}+O_2)$ $(W_{F_1}+O_{F_1})$
W_2	*do.*	*do.*	*do.*
W_{F_1}	*do.*	*do.*	*do.*
Set II			
O_1	*do.*	*do.*	*do.*
O_2	*do.*	*do.*	*do.*
O_{F_1}	*do.*	*do.*	*do.*

Nine combinations of pollen-mixtures from specific white dent and orange flint pollen parents were pollinated to female parents in Set I and Set II.

mixed-pollination design as shown in Table 1. In the experimental design, all kinds of factors consisting of female parent, pollen parents and alternative pollen parents, both composed of two inbred lines and an F_1 between them were involved, giving a total of 27 pollinating combinations in each of Set I and Set II in Table 1. Although actual data are not shown here, 10 cases out of 12 in the Set I and 7 cases out of 12 in the Set II demonstrated definitely the superiority of pollen from F_1 plants in selective fertilization.

In Table 2, figures for P/S and P/D alone were highly significant, but figures for another main effect and all interactions were not significant. This means that under mixed-polli-

Table 2. Analysis of variances[1] on percentage of yellow kernels in selective fertilization experiments (Set I and Set II)[7]

S. V.	D. V.	Set I		Set II	
		M. S.	F	M. S.	F
Female parent (F)	2	2,593.9591	4.083	219.0992	<1
Pollen parent with the same endosperm characters to female parent (P/S)	2	14,290.6558	22.492**	3,047.0255	6.464*
Pollen parent with the different endosperm characters to female parent (P/D)	2	26,718.4669	42.0592**	8,967.6341	19.024**
(F)×(P/S)	4	1,023.6065	1.611	703.7912	1.493
(F)×(P/D)	4	751.0070	1.182	482.6529	1.024
(P/S)×(P/D)	4	205.5989	<1	491.2667	1.042
(F)×(P/S)×(P/D)	8	635.3704		471.3858	

1) Analysis of variances was done after arc-sine transformation of the percentages.

Table 3. Mixed-pollination experiments using equal volumes of pollen grains from o_ro_r and O_ro_r plants[6]

Female parent (o_ro_r genotype)	Pollen parent I (o_ro_r genotype)	Pollen parent II (O_ro_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	47.3 (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)**※※

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_ro_r genotype of pollen parent II. Figures in parenthesis indicate number of ears observed.
* and **: difference from the expected percentage, 25.0%, is significant at 5% and 1% level, respectively.
※※ difference from expected percentage, 29.1%, is significant at 1% level.

nation including pollen from F_1 plants, any kind of pollen sources, whether of homo- or heterozygosity, are effective in selective fertilization.

The superiority unrelated to gametophyte factor(s)

In general, gametophyte factor(s) controls aberrant segregation ratios in F_2 and BC_1 in maize. An experiment[6] given in Table 3 was conducted to examine whether or not the superiority of pollen from F_1 plants shown in selective fertilization is induced by any gametophyte factor(s), or whether or not the heterozygosity in sporophyte inducing heterosis is supporting better competing ability in mixed-pollination. In Table 3, percentages of colored kernels in $W_1\times O_1$ and $W_2\times O_1$, both showing no aberrant segregation ratio on kernel color in F_2 and BC_1, were expected to be 25% in mixed-pollination, while those in

$W_1 \times O_2$ and $W_2 \times O_2$, both showing aberrant segregations on kernel color were expected to be 29.1%. Without any exception, however, percentages of colored kernels exceeded the expected percentages in every pollinating combinations (Table 3). It is possible to say that the superiority of pollen from F_1 plants was controlled by heterozygosity in sporophyte, a kind of heterosis, regardless of the existence of gametophyte factors(s) linked to the marker(s).

Relationship between the superiority of pollen from F_1 plants and vigor of growth of sporophytes was investigated with 20 F_1 hybrids among eight inbred lines, and also with F_2 generation of another cross. For the purpose of elucidating the relationship, these materials were examined on degree of selective fertilization by mixed-pollination and on some growing traits, i.e. plant dry weight, grain yield, culm length, and leaf length. Vigor in plant dry weight was significantly correlated to the pollen superiority, while other traits were correlated only partially but not significantly.

The relationship in F_2 generation was also recognized as shown in Fig. 2. From these results it was pointed out that the superiority of pollen from F_1 plants or heterozygous ones in selective fertilization is caused by the heterosis of these plants : the heterosis enhances the competing ability of pollen for fertilization.

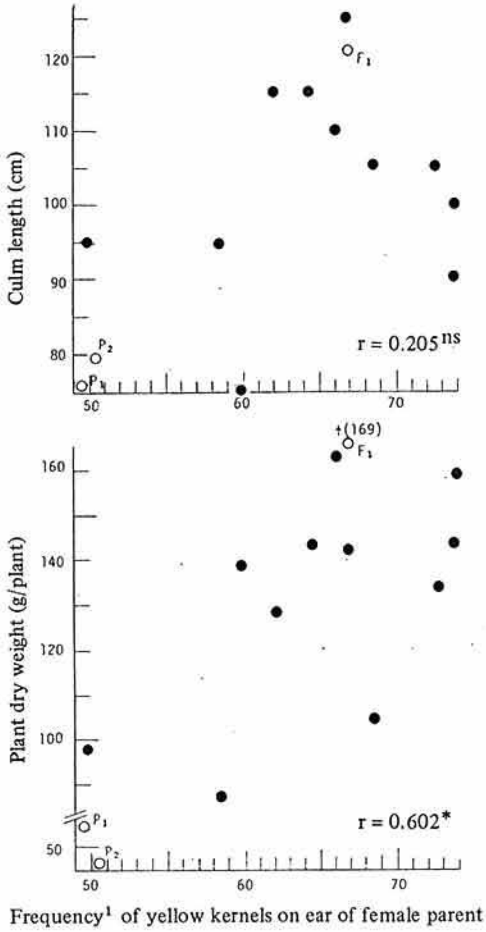

Fig. 2. Relationship between the frequency of yellow kernels on ears of female parent (*yy*) and plant dry weight or culm length in F_2 generation of (J 466 × J 472) used as pollen parent[6]
Note: 1: *cf.* Fig. 1.

The superiority of pollen from F_1 plants as expressed by pollen germination

Some reports indicated that gametophytes originating from F_1 plants having widely different genotypes at haploid phase showed wider variations of pollen tube growth than those from inbred lines[4]. In this connection, the superiority of pollen from F_1 plants, already showed above, was ascertained at first from the point of pollen germination *in vitro*. An *in vitro* germination experiment was carried out using a part of materials shown in Table 3. In fact, the superiority of pollen from F_1 plants was expressed by germination percentage and/or length of germinated pollen tube within three hr after inoculation[9]. Next, with a particular F_1 and its parental lines germinability of pollen grains was traced for 1 hr on an artificial medium[1] (Fig. 3).

Germination of pollen grains from the F_1 plants started earlier than that from inbred lines, which suggests longer pollen tubes developed in the former that the latter. This result indicates that the superiority of pollen

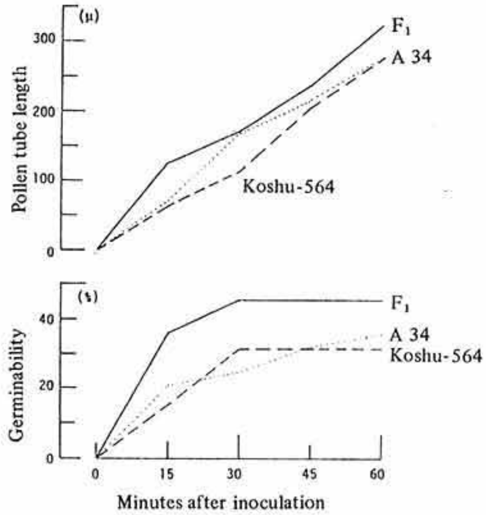

Fig. 3. *In vitro* pollen germination traced for one hr after inoculation. Pollen of inbred lines, A34 and Koshu-564, and of their F_1 plant was used[9]

from F_1 plants is expressed at the first stage of pollen germination.

Conserving heterozygosity in open-pollinated population of maize

The superiority mentioned above was observed by the artificial mixed-pollination experiments. However, there remains a problem whether or not the same superiority of pollen from F_1 plants or heterozygous plants can occur in open-pollinated populations of maize.

Therefore, this problem was examined under the condition of random pollination among every plants in open-pollination in small isolated fields (not-open-fields) where both inbred lines and their F_1 plants were mixed-planted. As the result, it was confirmed that non-random fertilization and the superiority of pollen from F_1 plants in the isolated fields actually occurred due to selective fertilization among different genotypes. Then a theoretical approach was made to know whether or not the superiority of pollen from F_1 plants or heterozygous plants is effective in conserving heterozygosity of an open-pollinating population such as a synthetic variety. A simple case with A_1 and A_2 genes in a locus was postulated. Three kinds of genotypic frequencies of Syn 3 with combinations among all possible genotypes of Syn 2 with their corresponding selective fertilization coefficients effective only for pollen parents are as follows;

$$A_1A_1 = sf_1P^2 + (1/2)\ (sf_1 + sf_2)\ PH + (1/4)\,sf_3H^2$$
$$A_1A_2 = (sf_1 + sf_2)\,PQ + (1/2)\ (sf_1 + sf_3)\,PH + (1/2)\ (sf_2 + sf_3)\,QH + (1/2)\,sf_3H^2$$
$$A_2A_2 = sf_2Q^2 + (1/2)\ (sf_2 + sf_3)\,QH + (1/4)\,sf_3H^2$$

Selective fertilization coefficient, sf_3, for pollen grains originating from A_1A_2 genotypes was assumed to be superior ($1.0 > sf_3 > 0.5$) to that of the others.

This superiority enables to conserve the genotypic frequency of A_1A_2 with 0.5 or ca. 0.5 value irrespective of genotypic frequencies of Syn 2 (Fig. 4), even in earlier generations after synthesizing.

The mechanism, which induces the superiority of pollen grains from heterozygous plants, plays an important role in selective fertilization in conserving the heterozygosity of out-crossing crops, in general.

Concluding remarks

In this review, the author pointed out that heterosis was maintained in gamete (haploid) derived from zygotes (diploid) and this phenomenon was effective in conserving heterozygosity in out-crossing crop populations. From the fact that pollen grains derived from F_1 plants contain all possible genotypes for F_2 zygotes, and moreover they are influenced by heterozygotic effect during gamete formation, the occurrence of pollen superiority can be maintained free from the effects of zygotic (both interallelic and non-interallelic) and gametic (non-interallelic only) interaction control. However, the pollen superiority described in this paper is in terms of the average of all pollen composed of those showing the superiority and the others which may not have the superiority. If the genotype of each single pollen grain derived from F_1 plants could be identified by some ways, it is

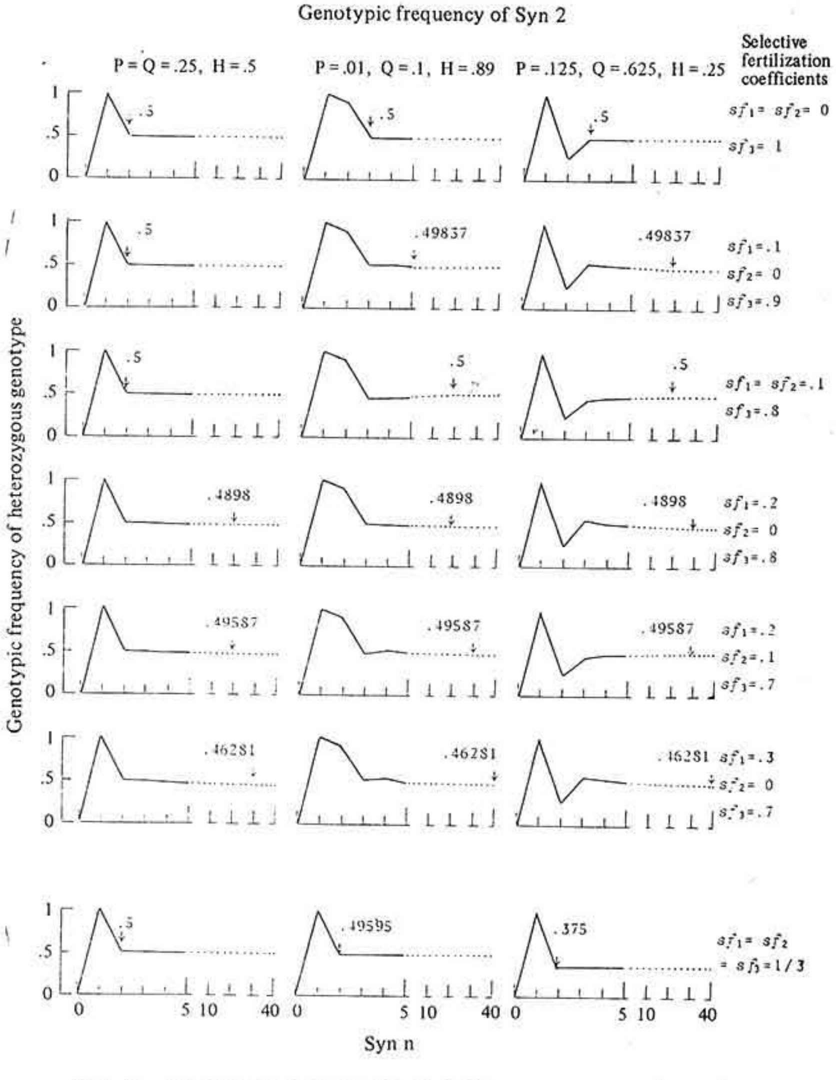

Fig. 4. Genotypic frequency of heterozygous genotype in successive generations of a synthetic variety (Syn n) under selective fertilization in open-pollinating species[9]

possible to link the vigor of a single pollen grain directly to the genotype of that pollen grain[8]. Recently, zymogram of a single pollen grain from interspecific F_1 in genus *Cucurbita* was analyzed[3]. So that, in the future zymogram genotype will be identified for individual pollen grain and the genotype, as the marker in the gamete level, will be used for revealing a kind of heterosis appearing in gamete competition.

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