Competitive Ability of Maize Pollen. Intergametophytic Effects

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Summary. Intergametophytic influences on pollen competitive ability were studied by means of the pollen mixture technique: mixtures of pollen from different genetic sources, marked for the presence of the normal or the mutant allele of the opaque-2 gene, were used to pollinate o2o2 female plants. The variation of relative frequency of one of the two kernel types from apex to base of the resulting ear, made it possible to measure the competitive ability by regression coefficients.

The effect of the pollen marker on the character was estimated by analyzing the progeny of heterozygous o2/+ plants self-pollinated through four generations. The o2 allele-bearing pollen revealed a slower germination rate than the dominant counterpart; no differences between the two pollen types were observed with regard to tube growth rate.

The occurrence of intergametophytic interactions was confirmed: the competitive ability of a line varied according to the genotype of the competing pollen. Thus, this component has to be taken into account in considering male gametophyte fertilization ability.

Key words: Maize pollen — Competitive ability

Introduction

Male gametophyte fertilization ability is a complex character, resulting from different components: it is due in some measure to sporophytic gene expression and in some measure to the genes of gametophyte itself (Brink and MacGillivray 1924; Pfahler 1965; Sari Gorla et al. 1975; Mulcahy et al. 1975; Johnson et al. 1976); it is also conditioned by the mutual influences of the pollen tube and the stylar tissue where it grows (Jones 1928; Pfahler 1967; Heslop-Harrison 1975; Sari Gorla et al. 1976), and it can be affected by the composition of the pollen population competing in the same style (Ottaviano et al. 1975).

Interaction effects between male gametophytes are particularly clear in incompatibility phenomena; in these cases, it has been shown that incompatible pollen, mixed with irradiation- or chemically-killed compatible pollen ('mentor'), is able to grow until the ovules are fertilized (Stettler 1968; Knox et al. 1972a, 1972b; Pandey 1974; Sree Ramulu et al. 1979).

Apart from the case of incompatible crosses, effects due to interaction between genetically-different male gametophytes, though they are less conspicuous, have been observed. Jones (1928) reported on experiments of mixed pollination in cotton: self pollen revealed greater competitive ability than foreign pollen only when used in pollen mixtures; this effect was not found if the two pollen types were separately placed on different parts of the stigmatic surface. Effects due to pollen population composition have been reported in maize (Ottaviano et al. 1975): pollen competitive ability has been observed to vary according to the genotype of the competing pollen. The effects were very clear; however, owing to the complexity of the character, they might depend also on the stylar genotype and the environmental conditions.

The purpose of this study was to verify these aspects, evaluating the effects of the intergametophytic interactions in quantitative terms. Fertilization ability was studied by means of the pollen mixture technique, where the genetic sources of the two competing pollen types is revealed by a genetic marker, in this case the opaque-2 gene. For this reason, it was considered worth while to make accurate estimates of the influence of this gene on pollen competitive ability.

Materials and Methods

The effect of the o2 locus on pollen competitive ability was studied by analyzing the self pollinated progeny of heterozygous plants. These were the result of four selfing generations of a population derived from the cross between an opaque and a normal line. In each generation, heterozygotes were selected by means of

progeny test in order to maintain this condition at the *o2* locus, while the residual genetic background tends to homozygosity.

The ears from the last self-fertilization generation were transversely divided into three segments; the opaque and normal kernel frequencies in the apical and basal segments were computed. The data were analyzed by means of the X^2 test, in order to statistically test departures from the expected ratio of 3 normal : 1 opaque, the relative frequency variation of normal and *o2* kernels between the apex and the base of the ear, and the heterogeneity of the samples.

The male gametophyte competitive ability was studied by means of the pollen mixture technique. The pollination mixtures included approximately equal quantities of pollen from two genetic sources, marked for the presence of the normal or mutant allele of the *o2* gene. The presence of normal or opaque kernels on the resulting ear make it possible to recognise the genetic origin of the pollen which effected fertilization. The ears were transversely divided into five segments; in each, the frequency of normal and opaque kernels was computed: the relative competitive ability of the two pollen types was then measured as the increase in the fertilization frequency of one of the two pollen types from the apex to the base of the ear, where the styles are longest.

Three inbreds : WF9, RNY and C123, were used as pollinator lines, in the two versions opaque and normal. The female parent was the hybrid W64A*o2* X A69Y*o2*.

Pollen mixtures were made in all two by two combinations, including reciprocals (normal version of one line and opaque of the other, opaque version of the first and normal of the second) and, as controls, the two versions of the same line. Thus the design included nine combinations; for each of them, from eight to ten ears were pollinated, in two complete replications. The experiment was carried out twice, in two different years.

Pollen competitive ability was measured as the regression coefficient of the relative fertilization frequency on the ear segments of one of the two competing genotypes. Since the original data were expressed as proportions (number of normal or opaque kernels in each ear segment), the estimate of regression coefficient was carried out according to the method proposed by Maxwell (1971), based on the procedures discussed by Yates (1948), Armitage (1955) and Cochran (1954). This method is proper to contingency tables where there exists a natural order of the categories. The values obtained for each replication, which represent the measurement data of the character under study, were submitted to analysis of variance. The error used in statistical tests derives from the variance between replications, within cells. It may be that the test is not very effective owing to the number of the error degrees of freedom, which was much smaller than the number of ears included in the experiment. However, it should provide a good protection level.

Results

The results from mixed pollinations of the two versions of the same line did not reveal effects of the genetic marker (*o2*) on competitive ability. However, the genetic differences between the two versions of a line might not be confined to the *o2* locus, since a residue of the genetical background of the *o2* source used for conversion of the normal line could be present. Thus the effect of the *opaque-2* gene on pollen fertilization ability had to be ascertained more precisely.

The ears from 15 self-pollinated heterozygous *o2*/+ plants were transversely divided into three segments; in each, the kernels were classified as normal or opaque. The proportions of the two kernel types in the first and third segments were considered. In fact, differences of germination rate should be expressed as deviation from the 3 : 1 ratio in all the segments of the ear, while different growth rates can be revealed by comparing the two kernel type frequencies between the apex (first segment) and the base (third segment) of the ear.

The statistical analysis of the results is reported in Table 1. The first three items test the deviation from the expected ratio respectively per ear, in the first and in the third segment; the last item tests heterogeneity between the proportions in the two segments. A significant deviation from the 3 : 1 ratio was observed: *o2* kernels were fewer than expected both in the first and the third segments and in the ear. Differences between the apical and the basal ear segments as regard opaque and normal kernel frequencies were not significant.

In view of the experimental design, it is possible that the differences observed are due to the presence of a different allele at the *o2* locus. In fact, the portion of genome closely linked to this gene in the original population should have been randomly reassorted during the selfing generations. Thus, even if the effect of genes closely linked to the *o2* locus cannot be excluded, *o2* allele-bearing pollen is likely to have a lower germination rate than the normal (*o2*) allele-bearing pollen, but this gene does not affect the pollen tube growth rate.

Table 1. Test of the differences between expected and observed values of opaque and normal kernels.

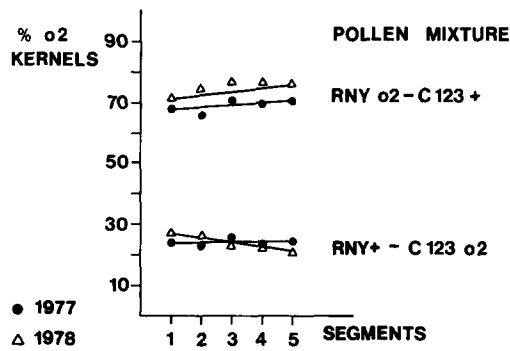|	Observed		Expected		Deviation from 3:1 ratio	Heterogeneity
	+	o2	+	o2		
1) Total	3665	1081	3559.5	1186.5	$x^2_{[1]} = 12.51^b$	$x^2_{[14]} = 13.99$
2) Within 1. segment	1767	519	1714.5	571.5	$x^2_{[1]} = 6.43^a$	$x^2_{[14]} = 34.5^a$
3) Within 3. segment	1898	562	1845	615	$x^2_{[1]} = 6.09^a$	$x^2_{[14]} = 17.99$
4) Between 1. and 3. segment						$x^2_{[1]} = 0.01$

[a] $P < 0.05$
[b] $P < 0.01$

Fig. 1. RNY-C123 comparison

For each line pair combination, four pollen mixtures were made: 2 reciprocal comparison and 2 controls (the two versions of the same line). In this last case, equal proportions of normal and opaque kernels are expected in all the ear segments, while the competition between different genotypes is revealed by the increased frequency of the kernels resulting from the more competitive pollen type, from the first (apex) to the fifth segment (base of the ear), with an opposite trend in the two reciprocal combinations. In all cases, equal proportions of the two kernel types are expected in the first segment, where differences in growth rate are not expressed, owing to the short journey needed to reach the ovules.

This last assumption is not always confirmed by the experimental findings; this may be due to experimental errors (not perfectly equal proportions of the two pollen types in the mixture) or to systematic causes, such as differences in germination rate or in pollen grain size in the two genotypes. This is the case in the RNY-C123 comparison: (Fig. 1) the kernel proportion from RNY pollen, both in normal and opaque versions, is about 70% in the first segment, in both the 1977 and the 1978 experiments. The same result in two versions and two different years should not be accidental; it is more likely that

the RNY germination rate is greater than that of the C123, or that the RNY pollen grains are smaller than those of the C123. This aspect will be investigated.

Differences in germination rate and experimental error in pollen mixture preparation do not bias the competitive ability estimate, which was quantitatively measured as regression coefficient, and thus measures only the fertilization ability component based on pollen tube growth rate.

In Figure 2 the results of line pair comparisons are summarized: the relative fertilization frequency of one of the two competing lines, according to ear segments, are reported. Fertilization frequencies were obtained adding opaque and normal kernel numbers obtained with the two reciprocal mixtures; b values and total number of kernels scored for each comparison are also indicated.

Figure 2a and b shows that the competitive ability of the WF9 line is slightly greater than that of the RNY (b_{1-2}=0.8), and the competitive ability of RNY slightly greater than that of C123 (b_{2-3}=1). Thus, the WF9-C123 comparison (c) is expected to reveal an advantage in favour of the WF9 line, quantitatively estimable in an order of magnitude equal to the sum of b_{1-2} and b_{2-3}. The WF9-C123 comparison reveals a clear advantage in relative tube growth rate in favour of WF9: the increase of the relative frequencies of the WF9 source kernels from the first to the fifth segment is greater than the sum of the relative increases of the other two comparisons (b_{1-3}=3.56). The difference between WF9 competitive ability directly evaluated in relation to C123 and the competitive ability of this line expected on the basis of the previous comparison ($b_{1-2} + b_{2-3}$) was statistically significant.

The analysis of variance (Table 2) shows significant differences in competitive ability only in the comparison (1+2 vs. 3) and no differences between controls, between the two reciprocal combinations of each comparison and between the experiments of the two years.
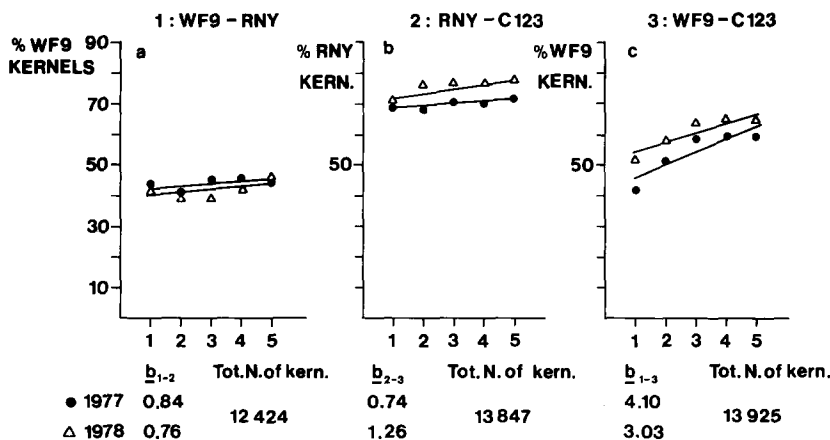

Fig. 2a-c. Competition between lines

Table 2. Analysis of variance

Source of variation	d.f.	M.S.
Comparison	2	10.51[a]
(1 + 2) vs. (3)	1	19.98[b]
(1) vs. (2)	1	1.05
Reciprocals	3	2.94
Controls	2	0.60
Years	1	7.92
Error	18	2.18

Only the more relevant items are reported

[a] $P < 0.05$

[b] $P < 0.01$

Discussion

Analysis of the ears obtained from self-fertilization of heterozygous o2/+ plants, revealed differences as regards the germination rate of dominant and recessive allele-bearing pollen, in favour of the dominant type. Differences in germination ability due to genetic background (Pfahler and Linskens 1973) and to single genes (Pfahler and Linskens 1972) have been reported, but the responsible mechanism is not easily interpretable: in some cases, such as waxy locus, it may be that chemical composition of the grains makes them more sensitive to imbibing water and thus causes them to germinate faster or adhere together in groups more easily.

However, while the opaque-2 gene has some effect on pollen germination, it does not affect the tube growth rate. Thus, the observed between-line differences for this character are not biased by the presence of the mutant or normal allele at the opaque-2 locus.

The results of this study confirm the influence of the pollen-competing genotype on male gametophyte fertilization ability, first observed by Ottaviano et al. (1975). In the earlier study, more conspicuous effects were observed; in fact the WF9 line fertilization ability changed its sign according to the competing pollen genotype. In these experiments the phenomenon has been evaluated in statistical terms.

A quantitatively-variable expression of intergametophytic interaction is due to the complexity of the processes involved. Fertilization ability depends on different components, each of which can be affected by several factors: germination rate and tube growth rate depend on the pollen-producing plant genotype, on the pollen grain genetic content, on environmental effects, on the stylar genotype where the tube grows, as well as on the quality and quantity of the pollen-competing population. In this case, the observed quantitative differences between the two experiments are probably attributable mainly to the difference in the female plant used, the genotype of which

can modulate the expression of the phenomenon, and also to environmental differences between years. The inbred which shows an intergametophytic interaction, even if displaying good combining ability, furnishes pollen which is practically unable to grow on artificial media. For this genotype, it appears that the physiological dependence of pollen tube on stylar tissue is absolute. It may be that its competitive ability is strongly affected by variations of the stylar genotypes where it develops.

Interaction between pollen from different genetic sources are particularly evident in intra- and interspecific incompatibility crosses: certain normally incompatible combinations can be made compatible by mixing killed compatible pollen with normal incompatible pollen at the time of pollination (Stettler 1968; Knox et al. 1972a, 1972b; Pandey 1974; Sree Ramulu et al. 1979). This effect, known as 'mentor pollen effect', may be due to information transfer from one pollen type to the other, thus mobilizing a response from the style, or to release of growth factors lacking in incompatible pollen.

Pandey (1977, 1978) observed a removal of the incompatibility barrier obtained by mixing incompatible pollen with another killed incompatible pollen type. The effect is quantitatively dependent on the genetic combinations used as pollen sources and as maternal plant. Thus the phenomenon can be induced even when 'recognition materials' are not involved, and for this reason it is not directly linked to the incompatibility reaction.

The complexity of the observed interactions in maize is likely to be correlated to the complexity of the physiological processes which characterize the male gametophyte development. After a first autotrophic growth phase, when the reserve materials of the grain are exhausted, it shifts to a heterotrophic, style-dependent, nutrition. It is possible that in this phase the physiological relationships which are established between pollen and style involve processes of stylar activation by the pollen tube, and of the pollen genome as an answer to stylar protein production. In this case, difference in pollen competitive ability could be due to differences in ability to induce metabolic responses in the style or to utilize products released by the style.

Acknowledgement

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