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Male Gametophytic Selection in Maize

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Summary. There is evidence that male gametophyte selection is a widespread phenomenon in higher plants. The pollen tube growth rate is one of the main components of gametophyte selective value; genetic variability for this trait, due to the effect of single genes or to quantitative variation, has been described in maize. However, indication of gametophytic selection has been indirectly obtained; its effect was revealed by the positive relation observed between gametophyte competitive ability and sporophyte metrical traits.

This paper considers the results of selection applied to gametophyte populations produced from single plants. The competitive ability of the lines was evaluated in comparison with that of a standard line by means of the pollen mixture technique. Sporophytic traits were measured in the hybrid progeny obtained by crossing selected S_3 and S_4 families with an unrelated single cross and an inbred line. Gametophyte selection produced inbred lines with high gametophyte competitive ability. In view of the selection procedure adopted, this result was interpreted as an indication of haploid expression of genes involved in the control of pollen tube growth. Moreover, this gametophytic trait was positively correlated with sporophytic traits (seedling weight, kernel weight and root tip growth in vitro), indicating that both groups of characters have a common genetic basis.

Key words: Maize – Pollen – Gametophytic selection – Competitive ability

Introduction

The evidence indicates that selection at the haploid phase is a widespread phenomenon in higher plants (Harding and Tucker 1969; Hartl 1972; Clegg et al. 1978, Ottaviano and Sari Gorla 1979) and its evolutionary importance was stressed many years ago (Darwin 1877; Jones 1928; Haldane 1932).

The phenomenon concerns mainly the male gametophyte (Pfahler 1975); this is because a single plant produces a large number of pollen grains, pollen is directly exposed to environmental factors and usually many pollen grains are competing with each other in the same style. Fertility, germination time and tube growth rate are the main components of male gametophyte selective value. If it is assumed that pollen tube growth is regulated by genes expressed in both the sporophytic and the gametophytic phases, the tube growth rate appears to be a very important characteristic, which in combination with insect pollination and closed carpels, plays a significant role in the evolution of the angiosperms (Mulcahy 1979).

Genetic variability in pollen tube growth rate, resulting from the effects of single genes involved in the regulation of the breeding behaviour of the species, has been described in the case of some systems of self-incompatibility (Ascher 1975) and cross-sterility in maize (Mangeldorf and Jones 1926).

Quantitative variation due to the action of polygenic systems may play an important role in the effect of the gametophytic selection on sporophytic traits. It has been proposed that this variability is under the control of genes with haplo-diploid expression (Mulcahy 1971) affecting basic functions common to both phases of the life cycle (Ottaviano et al. 1980). In this case gametophytic selection would bring about a correlated response in the quality of the sporophyte. In maize, quantitative variation has been studied in terms of tube growth in vitro of different inbred lines and F_1 hybrids (Pfahler 1970; Pfahler and Linskens 1973), and it has been shown that it is affected by genes expressed in the gametophytic generation (Sari Gorla et al. 1975). A large part of this type of variation is also revealed by the competitive ability of pollen comparing inbred lines (Jones 1928; Mulcahy 1971) or hybrid material (Pfahler 1965). In all these cases, the pollen tube growth rate was the main component of the competitive ability of the gametophyte; moreover, the phenomenon was affected by the interaction between pollen and style (Pfahler 1967; Sari Gorla et al. 1976).

The effect of gametophytic selection on the haploid generation has been shown in *Petunia* and *Dianthus* (Mulcahy et al. 1975, 1978) and in other plants (TerAvanesian 1978): when gametophyte competition is increased by means of an increase of pollination intensity or of the length of the stylar tissue between pollen and ovules, the response in the progeny population is revealed by a reduction of variances and an increase of the mean values of some sporophytic metrical

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traits. In maize, the effect of gametophytic selection on gametophytic and sporophytic traits is indirectly shown by the fact that male competitive ability reveals genetic variation when inbred lines are compared, and that this variability is positively correlated to sporophytic metrical traits (Mulcahy 1971, 1974; Ottaviano et al. 1980).

This paper illustrates the results of an experiment carried out to test the effect of selection acting directly on the gametophytic generation of an open-pollinated population. The evaluation concerns the main components of gametophytic competitive ability and correlated responses as regards sporophytic traits.

Materials and Methods

Gametophytic selection for competitive ability is based on the dfferent length of the silks due to the position of the flower on the ear: the distance decreases from the base flowers upwards. Thus the probability of fertilization of the most competitive gametophyte, especially if this is due to the pollen tube growth rate, increases from the apex downwards. The Long Ear Synthetic population (BSLE) was used as basic material. From full open fertilized ears, two samples of 40 kernels were taken: one from the apex and the other from the base. The remaining kernels were discarded. The lines from the first group were reproduced using top kernels for four selfing generations, while those of the second group were developed using base kernels. The choice of plants for reproduction was random: selection for plant traits was carefully avoided. Hereafter the lines of the two selection groups will be referred to as "apex" or "base" lines.

Lines produced by selfing (5 of the apex and 7 of the base) were evaluated for pollen competitive ability at the S_3 generation. This evaluation was repeated at S_4, using the least competitive lines of the first group and the most competitive of the second. For each of the S_3 progenitors two sister lines were used. The evaluation of pollen competitive ability was carried out by means of the pollen mixture technique. Pollen of each line was mixed with an equal volume of pollen of an inbred (W22) with coloured aleurone, which was taken as a standard. The mixture was used to pollinate plants of an unrelated F_1 (A632×Mo17) with uncoloured aleurone. Ten fully fertilized ears of each line were considered. Each of these was divided into five segments of equal length (8 kernels per row) and scored for aleurone colour.

The competitive ability in comparison with that of W22 of each selected line was evaluated as variation of the frequency of uncoloured kernels from apex to base of the ear. It is expressed as the coefficient ($b_{P/S}$) of linear regression of the proportion (P) of uncoloured kernels on ear segments (S). Taking into account the binomial distribution of the data, a weighted statistical analysis was carried out (Armitage 1955). However, owing to the large number of observations for each segment (about 1,200 kernels), this method furnished the same values of $b_{P/S}$ as those of unweighted analysis.

The pollen diameter and pollen sterility percentage of each line were evaluated according to the method described by Johnson and Mulcahy (1978). Pollen tube growth rate in vitro of one of the S_4 apex lines and of one of the base lines was also studied. Fresh pollen from active shedding tassels was inoculated on to the surface of solid medium prepared according to Pfahler (1970) and modified as indicated by Cheng and Freeling (1976). After incubation for three hours at 27 °C, it was fixed using Farmer's liquid and coloured with a solution of 1% aniline blue and 50% lactic acid in distilled water. The length of the tube was measured on drawings obtained by using microprojection (120×).

Several sporophytic traits were evaluated in the hybrid progeny of the cross between each S_3 and S_4 line and the unrelated F_1 (A632×Mo17). Trials including $F_1 \times S_3$ hybrids were performed in 1979 and those with $F_1 \times S_4$ in 1980.

Uncoloured kernels from mixed pollinations were sown in the greenhouse for the evaluation of seedling weight at two weeks from emergence and used for a field trial to evaluate mean kernel weight (50 kernels wt.), number of kernels per row and number of rows per ear. S_4 lines were also evaluated in hybrid combination with the inbred W23. For all these experiments, three completely randomized blocks with plots of ten plants in the greenhouse and twenty plants in the field trials were used. In the field trials data were collected from five guarded plants per plot.

The progeny of each line and the F_1 were also used for the evaluation of growth rate of root tips in vitro. The general procedure was that adopted by Ottaviano (1969): root tips of 1 cm, from sterilized kernels germinated in Petri dishes, were grown in a modified White (1963) medium with 7 g per liter of agar added. Length (cm) of roots was measured two weeks after transplanting.

Results

Table 1 shows the values of the selected S_3 lines with regard to gametophyte competitive ability and sporophytic traits in hybrid combination. Positive values of the regression coefficient indicate a gametophyte competitive ability of the selected lines greater than that of the standard (W22). Six of the seven S_3 lines obtained from the base of the ear proved to be more competitive than the standard and one less competitive. A different situation is observed with regard to the lines obtained from apical kernels: competitive ability of S_3 lines was less than that of the standard in two cases, in another two about the same, while only in the case of one line was it slightly greater.

Clearly, choice of kernels from the base and from the apex of the ear leads to the formation of two groups of lines differing in male gametophyte competitive value: the long styles of the basal flowers enable the gametophyte with the higher tube growth rate to be selected. The genetic determination of differences between lines as regards to pollen competitive ability is confirmed by the results obtained from the S_4 experiment (Table 2, Fig. 1), which clearly show that the parental values have been inherited. Although only two lines, one in each group were tested, a further confirmation is obtained from the evaluation of pollen tube growth in vitro (Fig. 2): the mean values of the apex and of the base lines were 318.8±9.2 and 642.7 ±22.5 μm respectively.

Differences in the regression intercept, simply indicated in the table as proportion of kernels from selected lines at the apex of the ears, may be the result of a

Table 1. Gametophytic competitive ability and sporophytic value of S_3 lines

		Gametophytic traits			Sporophytic traits in hybrid combination	
	Lines	Pollen ∅ (μm)	Y_1	b × 100	10-Seedlings dry weight (g)	50-Kernel weight (g)
Base	B1	83.4	0.44	5.39^b	1.11	19.1
	B2	92.5	0.59	3.86^b	1.18	19.5
	B3	104.7	0.45	3.63^b	1.04	19.2
	B4	91.9	0.48	2.39^b	0.91	18.1
	B5	–	0.57	-3.45^b	0.93	15.7
	B6	85.7	0.21	2.43^b	0.78	20.0
	B7	83.5	0.18	2.40^b	0.91	19.7
	m	90.3	0.42	2.38	0.98	18.7
Apex	A1	88.6	0.70	-3.47^b	0.68	18.2
	A2	76.2	0.74	-3.28^b	0.98	16.4
	A3	83.4	0.26	0.54	0.96	17.1
	A4	–	0.48	1.65^b	1.03	16.7
	A5	91.7	0.70	−0.20	0.91	16.6
	m	85.0	0.64	−0.95	0.91	17.0
	d			3.33	0.07^a	1.7^a

Y_1 = proportion of uncolored kernels at the apex of the ear; m = means; d = differences
$^{a, b}$ = significant and highly significant differences ($P < 0.05$, $P < 0.01$ respectively)

Table 2. Gametophytic competitive ability and sporophytic value of S_4 lines

		Gametophytic traits			Sporophytic traits in hybrid combination			
	Lines	Pollen ∅	Y_1	b × 100	10-Seedl. dry Wt (g)	50-Ker. Wt (g.) (♀ A632/Mo17)	50-Ker. Wt (g) (♀ W23)	In vitro root length (cm)
Base	B11	87.5	0.37	6.04^b	1.24	19.60	13.35	9.34
	B21	92.5	0.36	3.95^b	1.34	18.66	14.27	11.25
	B31	105.0	0.43	4.34^b	1.34	17.79	12.94	12.24
	B41	94.2	0.17	3.97^b	1.21	16.41	13.43	10.16
	B42	95.0	0.20	2.35^b	1.04	17.61	12.95	10.76
	B43	86.7	0.10	4.64^b	1.34	17.99	14.69	11.16
	B61	84.2	0.11	3.27^b	1.21	16.61	12.29	9.22
	B62	87.5	0.16	3.00^b	1.14	18.07	13.69	8.85
	B71	83.3	0.17	2.36^b	1.09	17.17	11.88	8.21
	m	90.6	0.23	3.77	1.22	17.80	13.30	10.13
Apex	A11	86.7	0.27	−0.17	1.14	16.86	12.06	8.89
	A12	90.0	0.19	1.66^b	1.24	15.06	11.35	8.54
	A21	73.3	0.16	0.38	1.09	16.76	12.18	8.11
	A22	79.2	0.24	−0.89	1.26	14.68	11.16	10.81
	A31	–	0.07	-1.38^b	1.34	16.87	11.89	9.66
	A32	83.3	0.16	0.14	1.29	17.45	12.60	8.58
	A51	92.5	0.39	−0.13	1.26	17.30	13.92	6.96
	A52	90.8	0.51	−0.45	1.19	17.46	13.11	8.22
	m	85.1	0.25	0.24	1.23	16.60	12.30	8.72
	d			3.53	−0.01	1.20^a	1.00^a	1.41^a

Y_1 = proportion of uncolored kernels at the apex of the ear; m = means; d = differences
$^{a, b}$ = significant and highly significant differences ($P < 0.05$, $P < 0.01$ respectively) Pedigrees relating S_3 and S_4 lines are indicated in the standard manner

number of different factors, such as: the proportion of the two types of pollen in the mixture, because of differences in pollen size, and differences in germinability, germination time and sterility. As the two groups of selected lines did not reveal clear differences in pollen diameter and sterility and a large eror in the proportion of pollen mixture can be ruled out, the variability of this trait may be considered to be the result of the between-line difference in germination time.

The chi square test for heterogeneity with regard to the proportion of kernels at the apex of the ears (Y_1 in Tables 1, 2) shows significant differences ($P < 0.05$) between apex lines and between base lines; moreover, high and low values of the same order are found in both groups. Therefore it would seem that pollen germination time was not affected by the selection procedure adopted. Furthermore, S_3 (Table 1) and S_4 (Table 2), which were evaluated in two different seasons, revealed large differences with regard to this trait. It is very unlikely that this could be due to the inbreeding difference between the two generations. Probably it is due to environmental factors, which altered the relative competitive ability of the selected lines and the coloured standard line (W22).

The hybrid progeny of the base S_3-line groups proved to have greater kernel and dry seedling mean weights (Table 1). This indicates a positive association between differences in gametophyte competitive ability and differences in sporophytic traits. These results are confirmed by those relating to the S_4 lines (Table 2), where differences between the two groups of lines were found also with regard to the growth rate of root tips in vitro. As regards kernel weight, the difference observed was the same when the lines were evaluated in combination with an hybrid tester (A632×Mo17) or with an inbred line. In all experiments differences as regards number of rows per ear and number of kernels per row were not significant.

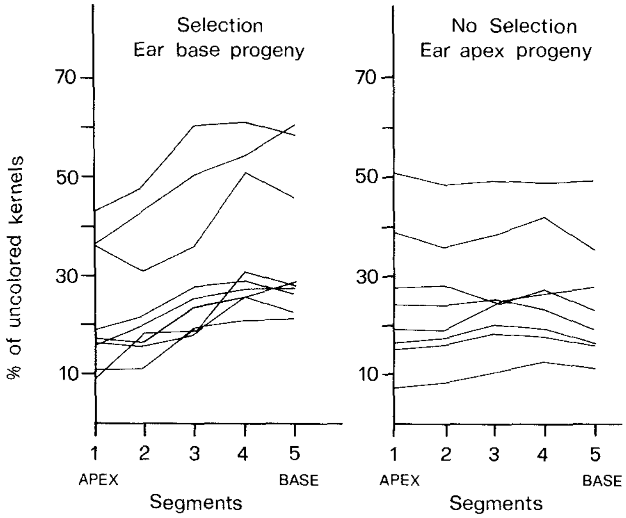

Fig. 1. Gametophytic competitive ability of S_4 lines from ear base and ear apex progeny

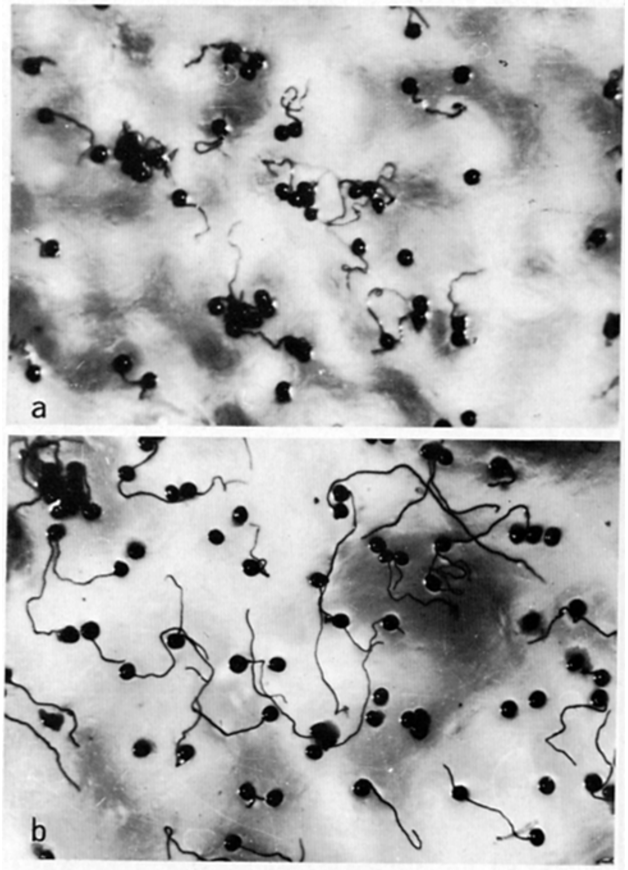

Fig. 2a and b. In vitro pollen tube growth (about 30×): **a** A22 line (ear apex progeny), **b** B31 line (ear base progeny)

Discussion

The results obtained clearly demonstrated that selection for highly competitive gametophytes is effective, and that, in addition to the great effects of gametophyte factors (Schwartz 1950), there is a quantitative genetic variation of the pollen tube growth rate. The variability released in four generations of selection and selfing should represent only a fraction of that existing in the population. In fact, the selection procedure adopted concerns only half of that variability: the selected progeny (basal kernels) were derived from a selected sample of male gametophytes and from a random sample of female gametophytes. Moreover, selection acted substantially in one direction, on the progeny of kernels at the base of the ears: the short distance the pollen tubes had to grow to reach the flowers at the top reduced the chances of selection.

The base population (S_0 ears) was open pollinated, i.e. each ear received pollen from several plants of the same population, while the ears of the following generations were produced by selfing. That is, the breeding method adopted operated on between and within plant variability in the first generation and essentially on within-plant variability thereafter. If it is assumed that not all the observed response was due to the selection intensity applied on S_0 ears, the results obtained indicate the existence of gametophyte within-plant quantitative variation and, consequently, haploid expression of genes controlling pollen competitive ability. This interpretation is substantiated by the fact that the S_0 ears were produced in a standard corn-field, where on average only 13 pollen grains land on individual silk (Kiesselbach 1948) and therefore competition selection intensity is much lower than in the case of artificial pollination (Pfahler 1975).

Pollen germination time and tube growth rate are the main components of gametophyte competitive ability. According to the method used in this work, differences regarding the former component are estimated from the values of the intercept of the regression lines, while differences of the latter are revealed by the slope. The results obtained showed that selection mainly affected the pollen tube growth rate. On the other hand, selfed lines within each group differed as regards germination time, indicating that the genes controlling this component were fixed at random. It is difficult to explain this result on the basis of the data of this work. However, if it is possible to prove the effect of selection on within-plant variability this can be taken as an indication that germination time is mainly regulated by the sporophyte.

Selection for gametophyte competitive ability gave rise to a positive correlated response with regard to the sporophytic traits of the progeny (kernel weight, seedling mean weight and root tip growth). This provides very strong support to the view that male gametophyte competitive ability and various sporophytic traits have a common genetical basis (Mulcahy 1971; Mulcahy et al. 1978; Ottaviano et al. 1980). Previous information essentially related to experiments based on crossing several established inbred lines: the gametophytic trait was that of the inbreds, while the sporophytic traits were measured in the resulting F_1's. Given the material used in those studies, the association was to be interpreted as being due to genetic causes arising from linkage and/or pleiotropy. The present study was based on selection applied in the gametophytic phase, using an open pollinated population; therefore it is very unlikely that linkage played an important role in determining the association between gametophytic and sporophytic traits.

It may seem difficult to justify this association on physiological grounds, because the variables relate to quite different phenomena: gametophytic competitive ability and the size of the sporophytic parts. However, as has been suggested in a previous paper (Ottaviano et al. 1980), it seems quite reasonable that the main metabolic activities, such as those involved in energy production, starch synthesis and wall building, which control characters relating to plant growth (seedling weight, kernel weight, root length) and those which control gametophytic traits, among them pollen tube growth, should be the same. In this respect, the most significant correlated selection response is that observed with regard to root growth in vitro. In fact, root growth in vitro, because of the absence of photosynthesis and indirect effects due to plant and ear architecture, as in the case of endosperm growth, is the most suitable for comparison with pollen tube growth.

Apart from the implications relating to population structure and evolution of higher plants, the correlation between the sporophyte and the gametophyte suggests the possibility of developing very efficient methods of selection based on gametophyte evaluation. This can be applied to very large populations and be performed in a very short period.

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