

## Comparison between responses to gametophytic and sporophytic recurrent selection in maize (*Zea mays* L.)

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**Summary.** In order to evaluate the response at both the gametophytic and sporophytic level of a selection based on the pollen competitive ability and to compare its effects with those obtainable from a conventional sporophytic procedure, three recurrent selection plans were developed in maize starting from the same  $F_2$  population. Two gametophytic recurrent selection procedures at high (GSH) and low (GSL) selection intensity were performed by utilizing, to advance the populations, kernels taken from the base (GSH) or apex (GSL) of ears obtained from pair-crosses of randomly chosen plants. The third scheme was a sporophytic full-sib recurrent selection procedure (SS); the only selection criterion was the machine-harvestable grain yield of the families. In a six-year period of selection, six cycles of both GSH and GSL and three cycles of SS were performed. The source and the selected populations (16 entries) were tested for pollen performance and for sporophytic traits. The selection cycles advanced through GSH showed a progressive increase, as compared to GSL, in pollen tube length measured at 4 h of in vitro culture. The SS cycles response was intermediate at 4 h whereas at 2 h it exceeded both GSH and GSL. A slight decrease in pollen diameter was evidenced in populations advanced with GSL procedure. The SS selection caused a marked increase for grain yield, lateness, leaves per plant and plant height. No response was shown by gametophytic selection for grain yield. The GSH procedure, however, led to an increase in kernel weight and to a decrease in kernel moisture, leaf number and plant height, as compared to GSL. Though gametophytic selection showed limited effects on sporophytic traits, it can be considered an efficient tool to supplement conventional sporophytic selection.

**Key words:** Maize – Gametophytic selection – Pollen – *Zea mays* L.

### Introduction

The extent of the response to selection for a quantitative trait in a population is related to the selection intensity and heritability, with the former depending on the population size and the latter depending on both the relative amount of the dominance component and the accuracy of genotype evaluation.

An ideal population, therefore, should be characterized by a very high number of plants or families whose genotypic differences are not affected by dominance. The factor that mainly reduces the efficiency of conventional breeding methods is the practical difficulty of matching these two prerequisites.

An attractive approach to overcoming these limits appears to be a selection applied to gametophytic generation. The theoretical advantages of such a procedure, especially if male gametophytes are considered, are the larger size of the population and the haploid state, which avoids the masking effects of the dominant over the recessive alleles (Ottaviano and Mulcahy 1986). Since the improvement of sporophytic traits is the first aim of a breeding plan, two conditions are required to apply a gametophytic selection (Mulcahy and Mulcahy 1975): first of all, the gametophytic traits (pollen tube growth rate and fertilization ability) must be controlled by genes expressed in the haploid phase and, second, such genes must also be active in the sporophytic generation (genetic overlap).

Evidence supporting these premises was obtained in studies on variability of pollen tube growth in vitro (Sari Gorla et al. 1975), and the enzymatic activity present in both pollen and sporophyte (Mok and Peloquin 1975; Mascarenhas et al. 1984; Sari Gorla et al. 1986).

Hypotheses on genetic overlap can be inferred from the observed correlations between sporophytic and ga-

metophytic performance for the same trait, such as disease resistance (Laughnan and Gabay 1973) and tolerance to heavy metals (Searcy and Mulcahy 1985) or herbicides (Smith 1986), as well as between different traits, such as yield or its components and pollen tube growth rate (Ottaviano et al. 1980; Landi et al. 1986). It should not be ruled out, however, that these correlations can arise merely from an extension of the sporophytic to the gametophytic generation, as emphasized by Mulcahy and Mulcahy (1975).

One of the most convincing demonstrations regarding genetic overlap was given by experiments of gametophytic selection applied to a pollen population produced by the same heterozygous plant. Such a selection was carried out through different approaches: by subjecting the pollen population to stress during microspore development or microgametophyte growth (Zamir et al. 1982; Sacher et al. 1983; Searcy and Mulcahy 1986; Mulinix and Iezzoni 1988), or by increasing the competition level among growing tubes without a specific stress induction. The latter procedure can be accomplished either by increasing the pollination intensity (Mulcahy et al. 1975; Ter-Avanesian 1978) or the length of the style through which the pollen tubes have to grow to reach the ovules (Ottaviano et al. 1982; McKenna and Mulcahy 1983).

The evaluation of the correlated response to the gametophytic selections has allowed the detection of significant variations for many sporophytic traits. In particular, an increased adaptability of the plants to environmental stresses for which the gametophytes were selected was found, whereas when the pollen competitive ability was the selection criterion, an improvement in yield per plant or of its components as well as seedling weight, plant vigour and plant height were reported in the above studies. Ottaviano et al. (1982, 1986), by comparing progenies derived from moderate or intense gametophytic selection in maize, found that the latter were characterized by higher seedling dry weight, longer roots and heavier kernels.

A selection procedure based solely on gametophytic competitive ability must be carried out by making manual pollinations with pollen collected from single plants (selfing or pair mating). The available information on the response to this kind of selection has been too limited up to now to assess its practical value.

To enhance information on the effects of a gametophytic selection carried out over the long term, a recurrent selection program based on a pair-mating procedure was undertaken. From a breeding point of view, the questions that must be answered involve not only the detection of a correlated sporophytic response to a gametophytic selection, but also the relative extent of such a response as compared to that obtained with conventional methods (Pfahler 1983; Ottaviano and Mulcahy 1986). Accordingly, a conventional full-sib recurrent selection

program, starting from the same material, was undertaken as well, in order to compare the effects of the gametophytic and sporophytic procedures.

## Materials and methods

The source population was the  $F_2$  generation of the single cross A632  $\times$  Mu195. Mu195 differs from A632 in a greater earliness at flowering (about 9 days), fewer leaves per plant, shorter stalk and lower grain yield as a result of a lower number of kernels per plant; but it is similar in silking-maturity interval and kernel weight. Insofar as the gametophytic traits are concerned, it should be stressed that Mu195 exhibits faster pollen tube growth *in vivo* (Landi and Frascaroli 1986).

### Selection procedures

The selection program began with 100 crosses between randomly chosen plant pairs. For each of the 100 ears obtained, three seed samples were taken: 25 kernels at the extreme apex (A), 25 at the extreme base (B) and the remainder (C).

As reported by Ottaviano et al. (1982), when several pollen tubes grow within the same style, the probability that the most competitive gametophyte will fertilize the ovule increases according to stylar length. Therefore the flowers of the ear base, whose silks are longer than those of the apex, are most often fertilized by the faster-growing pollen tubes. Given this premise, samples A and B can be considered as derived from the gametophytic selection (GS) of low intensity (GSL) and of high intensity (GSH), respectively.

The kernels of samples A and B were separately bulked in order to obtain the first cycle (C1) of GSL and GSH. In the following years, 100 crosses between pairs of randomly chosen plants were made within each population. The corresponding ears were shelled by taking 25 kernels from the apex only for GSL and 25 from the base only for GSH, to advance the populations according to a bidirectional procedure. Six cycles of gametophytic recurrent selection for both GSL and GSH were carried out. As this procedure needs 1 year/cycle, the selection work lasted 6 years.

By utilizing sample C (remainder share of the initial 100 ears), a conventional full-sib recurrent selection was simultaneously started for sporophytic selection (SS). The 100 corresponding families were tested in a field trial with three replications; plots were single rows 5 m long, each including 23 plants for a density corresponding to 60,000 plants/ha. The only selection criterion was machine-harvestable grain yield. The 15 superior families were grown in the successive year, then 100 pair crosses of random plants belonging to different families were made to obtain the C1 cycle. The subsequent cycles of SS were carried out following the same methodology. As this procedure requires 2 years/cycle, three selection cycles were completed during the same six-year period.

### Response to selection

For a correct evaluation of the realized responses to these selection procedures, the source population (C0), the six GSL (from C1 to C6), the six corresponding GSH and the three SS (C1–C3) cycles were reproduced by crossing, within population, about 100 plant pairs chosen at random. An equal number of kernels per ear was randomly taken. The same methodology as described above was adopted, to avoid any possible bias due to different seed age and size, e.g. the ear base kernels are larger than those at the apex.

The 16 populations were evaluated in 1987 for both pollen and plant traits. An estimate of the pollen characteristics was performed by randomly sampling in the nursery approximately the same pollen quantity from 90–100 tassels per population. Care was taken in collecting the same number of tassels per population daily to compare their pollen performance, without bias due to climatic effects. Nineteen pollen lots, each harvested from five plants, were collected per population. The pollen of each lot was inoculated on a solid medium, prepared according to Cheng and Freeling (1976), in two petri dishes: one was fixed with Farmer's liquid after 2 h and the other after 4 h of incubation at 27°C. For each of the 608 inoculated dishes, the following traits were detected: (1) germinability on 200 grains; (2) grain diameter on 20 grains; (3) pollen tube length on 40 germinated grains. Traits 2 and 3 were measured by a MINI-MOP image analysis system. The percent of germination was transformed to angular value ( $\arcsin \sqrt{p}$ ) before running the analysis of variance.

The evaluation of the sporophytic characters was made on the basis of field research conducted in two locations (Granarolo and Ozzano). The layout was a balanced lattice design (5 replications); plots consisted of two rows of 23 plants each at a corresponding density of 60,000 plants/ha. The field techniques were those usually adopted in practice and the same as those used in advancing each cycle of the sporophytic selection. The traits evaluated were: (1) days from July 1 to silk emergence (50% of plants at this stage); (2) leaves per plant and (3) plant height, both recorded on 20 plants per plot; (4) kernel moisture at harvest; (5) grain yield; (6) ears per plant; (7) kernel weight on a sample of 500 kernels per plot. Traits 5 and 7 were adjusted to 15.5% moisture. Statistical analysis was carried out separately for each location, then a combined analysis over locations was made, since error variances proved to be nonheterogeneous.

## Results

### Pollen traits

The pollen diameter and germinability showed no significant changes in relative performance of the 16 popula-

tions when measured at 2 and 4 h (population  $\times$  incubation time interaction not significant). The relative behavior of the populations as regards pollen tube length was, in contrast, affected by the time of in vitro growth. Therefore, the trends shown in Fig. 1 are referred to on a mean basis for the two former traits and separately for the latter.

No effect of either gametophytic or sporophytic selection was detected as far as germinability is concerned. As to grain diameter, no appreciable differences among populations were found up to the third selection year; successively, the GSL procedure caused a slight but significant decrease as compared to GSH, which in turn performed similarly to SS.

The trends of the tube length measured after 2 h of growth showed a general superiority of the pollen produced by the populations selected on a sporophytic basis (SS); the responses observed for both GSL and GSH were similar, with intermediate cycles showing the lowest values. The picture of the response to selection changes notably when the tube length after 4 h is taken into account. The two gametophytic selection intensities (high and low) caused a progressive differentiation between the derived populations. An upward response was detected in the GSH up to the third cycle, with a subsequent tendency to level off, whereas only moderate changes were noticed for GSL. The SS populations showed an intermediate performance between the former two, although it was closer to GSL. The observed trends indicate that the choice of kernels from ear base or apex can modify the pollen grain diameter and in vitro growth characteristics.

The variability within populations for pollen diameter and tube length, as evaluated by coefficients of varia-

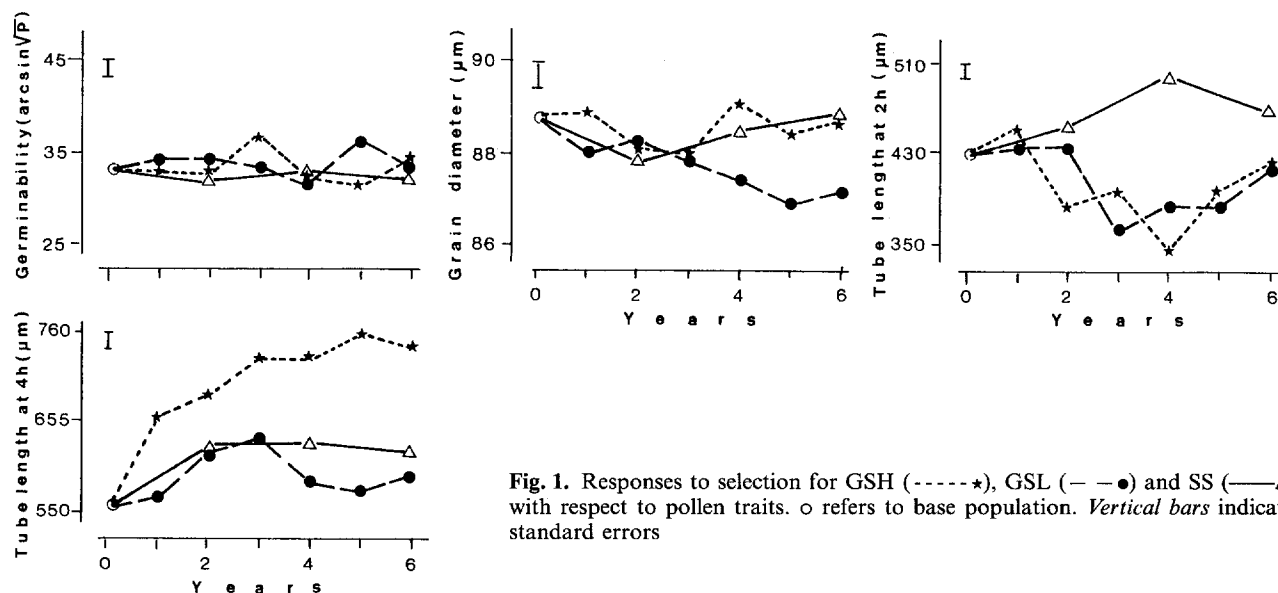


Fig. 1. Responses to selection for GSH (-----\*), GSL (—●) and SS (—△) with respect to pollen traits. ○ refers to base population. Vertical bars indicate standard errors

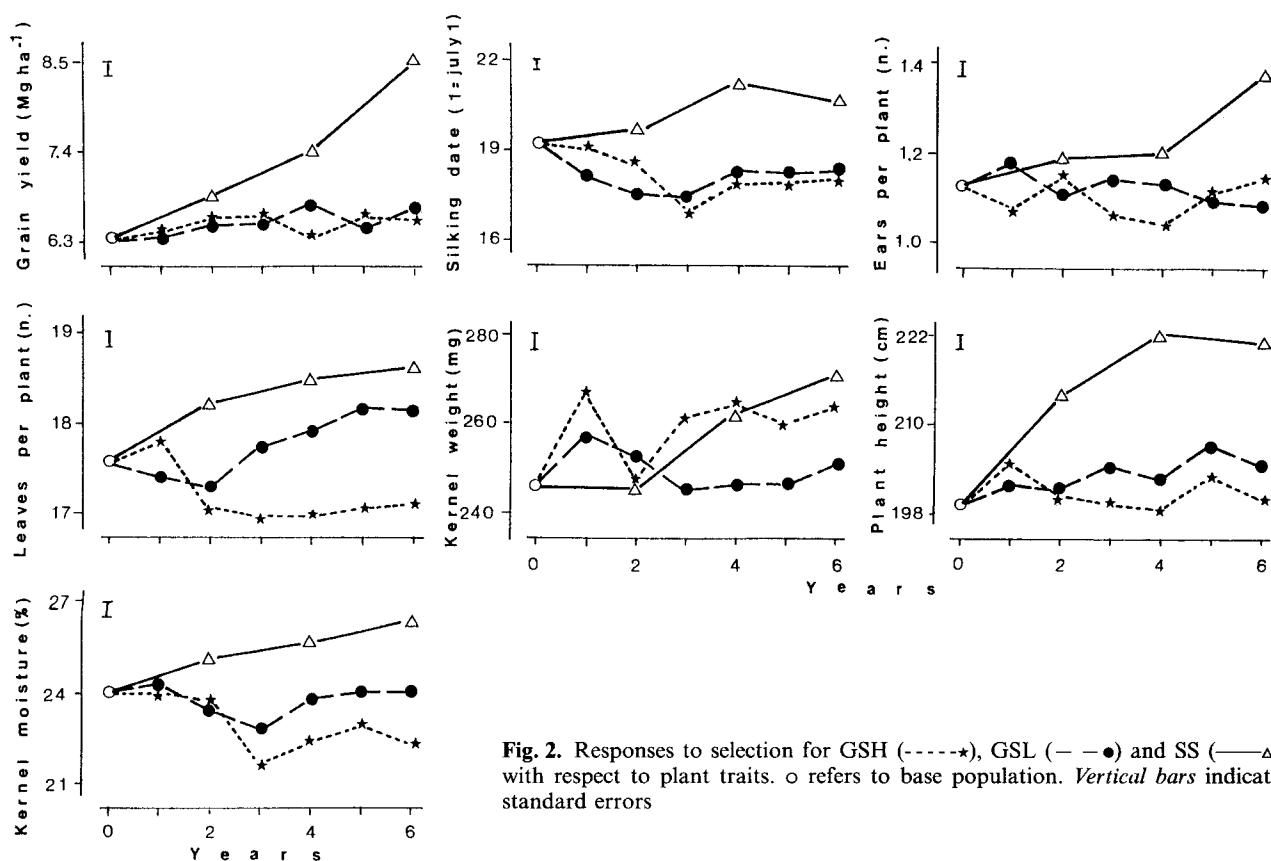


Fig. 2. Responses to selection for GSH (----\*), GSL (—●) and SS (—△) with respect to plant traits. o refers to base population. Vertical bars indicate standard errors

tion (CV) on an individual basis, is reported in Table 1. No statistically appreciable differences among successive selection cycles or methods were found for diameter. The CV values obtained for tube length at both 2 and 4 h differed significantly; nevertheless, they did not show a clear effect of selection on variability. The comparison between GSL and GSH populations in the last selection cycle shows a slight decline in the latter; this result is consistent with the theoretical expectation that a more intense selection should act to reduce the genetic variability. The observed trends, however, indicate that in addition to selection, other factors may have played a role in the extent of variation.

#### Plant traits

The variation coefficients of the errors for the studied traits in both field trials were generally lower than 7% (data not shown), thus indicating a good precision level of the experiments. Population  $\times$  location interactions were not significant, with the exception of ears per plant. Thus, the response to selection was analysed considering the means over locations. The *F* ratio for the population effects was calculated on the pooled experimental error (90 DF) for all traits except ears per plant, where the population  $\times$  location interaction was used.

Differences among populations were highly significant for all the investigated characters, and responses are presented in Fig. 2.

The conventional procedure (SS) caused a significant and noticeable increase with respect to grain yield, which was the selection criterion. The observed total gain after three cycles of recurrent selection was  $2.2 \text{ Mg ha}^{-1}$ , or a 35% increase. This response was obtained through both ears per plant and kernel weight. The gain in yield performance was accompanied by an increase in lateness, at both flowering and maturity, in leaf number per plant and in plant height.

The gametophytic selection at the higher intensity did not show any appreciable grain yield response in absolute terms or in comparison to that furnished by lower intensity. The GSH and GSL procedures, however, acted differently on the yield components. The GSH led to an appreciable kernel weight increase as compared to GSL starting from the third selection cycle, whereas the differences between GSH and GSL populations were negligible for ears per plant. To explain the absence of response at the yield level, it should thus be assumed that the non-measured component, i.e. kernels per ear, behaved in an opposite manner with respect to kernel weight.

**Table 1.** Pollen traits: coefficients of variation (%) within populations of successive selection years

Pollen trait	Selection years			
	0	2	4	6
Grain diameter				
GSL	5.32	5.17	4.97	5.11
GSH	5.32	4.93	5.10	4.87
SS	5.32	5.11	5.01	5.13
<i>F</i> -test: NS <sup>a</sup>				
Tube length (2 h)				
GSL	23.4	30.0	30.4	25.1
GSH	23.4	29.6	31.3	21.9
SS	23.4	24.7	25.7	20.4
L.S.D. (0.05) = 2.6				
Tube length (4 h)				
GSL	31.1	37.9	33.5	33.4
GSH	31.1	34.4	34.3	29.8
SS	31.1	26.0	28.9	33.0
L.S.D. (0.05) = 3.2				

<sup>a</sup> NS – not significant

The advancing of populations through gametophytic selection at the higher intensity resulted in a reduction of leaves per plant and of kernel moisture at harvest, whereas no response was found for plant height. In contrast, the GSL cycle showed an increase in leaf number and in plant height, but no appreciable change in grain moisture. It is worth noting that for these three traits the differences between GSH and GSL populations increased progressively according to the selection cycle, even though they only reached a significant level for kernel moisture and leaves per plant. As regards silking date, both GSH and GSL showed similar trends, with intermediate cycles being the earliest.

## Discussion

These results show the efficiency of a selection for competitive ability of the male gametophyte in modifying pollen quality based solely on the choice of seed from the ear base. Similar findings were reported by Ottaviano et al. (1982, 1986), who evaluated, by means of an analysis of pollen tube growth in vivo, the responses to different gametophytic selection programs. The data from our experiment differ from theirs, as ours derive from an in vitro analysis. A relationship between in vitro and in vivo performance was, however, reported by Ottaviano et al. (1982). Since the pair-mating procedure was adopted to advance populations in the present study, our results should not be affected by interactions between the style

and pollen of the same plant, as may occur when selfing is imposed (Johnson and Mulcahy 1978).

In vitro pollen tube growth is very limited and its performance is linked to the first phase (autotrophic) of gametophyte development (Mulcahy and Mulcahy 1982), which depends on the amount of reserve materials in the grains. To the extent that the resources stored in the grains depend on the characteristics of the pollen mother plant, an effect of the sporophyte on in vitro pollen performance cannot be ruled out. This is reflected in the tube length data recorded after 2 h of in vitro growth, which pointed out a clear superiority of the pollen grains from more vigorous plants selected on a sporophytic basis (SS). The differences between the two gametophytic selection intensities were not appreciable in this phase but became noticeable after 4 h of growth, when the initial advantage of the SS populations was lost. These results suggest that the tube growth in this second phase is mainly controlled by the haploid genotype. This hypothesis is supported by the intermediate behaviour of the SS cycles. As far as gametophytic response is concerned, the selection intensity exerted on the SS populations can be considered as intermediate between the GSH (base kernels) and GSL (apex kernels); the SS cycles, in fact, were advanced using kernels taken from the whole ear.

The reduction in pollen diameter as a result of the choice of ear apex kernels could be accounted for by a negative relationship between grain size and germination rate, as the latter trait can be considered the main component of the pollen's ability to fertilize the apical flowers (Ottaviano et al. 1982).

The marked response to selection for grain yield achieved through the sporophytic procedure indicates a large amount of additive genetic variability in the source population for this trait. On the other hand, no effect on grain yield was evidenced as a result of gametophytic selection. These response patterns should not be ascribed to inbreeding effects, as the population size for advancing the GS cycles was greater than that of the SS cycles; therefore, negative effects due to inbreeding should be assumed to be more marked for the conventional procedure.

The lack of a yield response to a gametophytic selection can be explained in that, as argued by Pfahler (1983), yield is a very complex trait affected by environmental factors acting throughout the whole sporophytic phase, whose duration is much longer than that of the gametophyte. It is, therefore, reasonable to suppose that a conspicuous number of environmental effects influencing the yield performance does not involve the short gametophytic phase.

An appreciable correlated response was, on the contrary, observed when the kernel weight, whose genetic control is less complex, was taken into account. The

increased value obtained for this yield component as a result of intense gametophytic selection is consistent with the responses found by Ottaviano et al. (1982, 1986), who worked on different populations following different selection procedures, and with the findings of Mulcahy (1971) and Ottaviano et al. (1980) on the tube growth rate-kernel weight relationship.

Even though no differences between gametophytic selection procedures were found at silking, a greater earliness at harvesting was achieved with higher selection intensity. This result can be related to a higher rate of grain filling and or moisture loss. As the two parental lines of the source cross did not differ regarding kernel weight and silking-maturity period, the observed correlated responses to selection should not be ascribable to linkage between the genes controlling tube growth rate and those controlling the above characters. More likely, these responses could be due to genetic overlap.

A correlated response due to linkage might be assumed for leaf number and plant height, as the parent with higher pollen competitive ability was characterized by smaller size and fewer leaves per plant. However, such an hypothesis should be ruled out even for these two traits, because a correlated response due only to linkage would be expected to be more pronounced in the first selection cycles than what, to the contrary, was actually observed.

The efficacy of gametophytic selection in modifying the examined plant characters proved to be rather low if compared to that of the conventional procedure. However, if we recognize that the former can be easily and inexpensively integrated with the latter, the effects of a selection based on the pollen competitive ability in affecting some traits, such as kernel weight and moisture, should not be neglected.

It should be emphasized, finally, that our gametophytic selection was carried out under favorable growing conditions so as to have a good seed set, even in the ear apex, for proper bidirectional selection. As stress conditions can increase the relationship between gametophytic performance and grain yield (Landi et al. 1986), it should not be excluded that a gametophytic selection can be more effective when carried out in more adverse environments such as those found in applied breeding.

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