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Review

Pollen selection

J.I. Hormaza [1,]* **and M. Herrero** [2]

[1] Mediterranean Agronomical Institute of Zaragoza (CIHEAM), Apartado 202, Campus de Aula Dei, E-50080 Zaragoza, Spain
[2] Pomology Unit, SIA-DGA, Apartado 727, Campus de Aula Dei, E-50080 Zaragoza, Spain

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Contents

Summary. In recent years a large number of reports have been published hinting about the possible role of selection at the male gametophyte level in natural conditions and its possible use as a tool in plant breeding programs. In this work, the evidence that has so far emerged on gametophytic selection is reviewed, and its relationship to pollen competition under natural conditions is analyzed. Information supporting an overlap in the genetic expression between the gametophytic and sporophytic phases is also reported, mainly in terms of isozymes and RNA hybridization. The correspondence between both phases is reviewed through their common response to different external agents such as temperature, salinity, metals, herbicides, fungal toxins, and other factors. Finally, the implications that pollen selection may have in plant breeding are evaluated.

Key words: Gametophyte – Pollen – Selection – Haploids

1. Introduction

A general phenomenon in plants is that they have two phases, sporophytic or diploid and gametophytic or haploid, which alternate in the life cycle. However, if we move forward along the evolutionary line, we can see that the gametophytic phase is diminishing in terms of both size and length compared to the sporophytic phase (see Heslop-Harrison 1979 for a review). The reduction in the gametophytic phase is maximum in angiosperms, where the male gametophyte consists of three haploid nuclei and the female gametophyte of six haploid cells plus a binucleate cell.

Research in plant breeding has been directed towards the sporophytic phase, where we can observe the outcome in angiosperms. The influence of the gametophytic phase is often forgotten, usually playing a "passive" role in most plant breeding methods. There are, however, many reports that deal with the physiology of the angiosperm gametophyte from pollen germination and tube growth up to fertilization (for reviews see Knox 1984; Mascarenhas 1989, 1990). It has only been in the 1970s that the possibility of gametophytic selection in plant breeding was put forward. The key moment was in 1979 when Mulcahy suggested the importance of gametophytic selection in the evolutionary progress of angiosperms: insect pollination and closed carpels could enhance the selection pressure against the male gametophyte. Since then, the use of gametophytic selection in plant breeding has developed, first by studying the overlap in the genetic expression between the sporophyte and the gametophyte and, later, by assessing the similarity in behavior between

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* Current address: Department of Pomology, University of California, Davis, CA 95616, USA

the two phases in relation to different external stresses (Mulcahy 1984; Ottaviano and Mulcahy 1989; Ottaviano et al. 1990).

The basis of the gametophytic selection theory is that selection among haploid and heterogeneous male gametophytes could be positively correlated with changes in the following sporophytic generation (Mulcahy 1979; Hoekstra 1983; Ottaviano et al. 1988 b, 1990; Ottaviano and Mulcahy 1989). Therefore, pollen would not only act as a transmission vector for the genome, but also be an independent organism expressing its own genetic information.

If we look at two features of the male gametophyte such as large population size (Miller and Mulcahy 1983; Frankel and Galun 1977; Ottaviano and Mulcahy 1986, 1989; Ottaviano et al. 1990), and direct expression of recessive traits due to haploidy (Zamir 1983; Ottaviano and Mulcahy 1989; Ottaviano et al. 1990), we find that selection in the haploid phase may actually be more intense than in the diploid phase (Ottaviano et al. 1988 b, c, 1990; Ottaviano and Mulcahy 1989). These features are also common to microorganisms, which have the greatest adaptability of all living organisms (Mulcahy and Mulcahy 1987).

If each phase of the plant life cycle expresses its own genotype, then selection for a specific genotype in pollen would lead to changes in the next gametophytic generation. However, if some of the genes are expressed in both phases, gametophytic selection could be an excellent way to improve the selection pressure applied by the plant breeder (Mulcahy et al. 1979; Miller and Mulcahy 1983; Ottaviano and Mulcahy 1989; Landi et al. 1989).

Thus, the challenge is to determine whether or not the gametophytic phase can be used effectivelly in selection methods (Linskens and Campbell 1984; Landi et al. 1989). Here, the evidence for gametophytic selection occurring in natural conditions is reviewed, and its possible use as a tool in plant breeding strategies is discussed.

2. Competition among pollen grains: gametophytic selection in nature?

Is there any evidence of selection in the male gametophyte operating under natural conditions? Competition among pollen grains could lead to pollen selection in vivo (Mulcahy and Mulcahy 1987). The evidence we have so far is indirect, and is based on both unexpected segregation according to Mendelian ratios and correlation between pollen competitive ability and sporophytic vigor.

Competition among pollen grains on the same stigma has long been tacitly assumed (Johnson and Mulcahy 1978; Stephenson and Bertin 1983). However, in the last few years the implications of this competition in determining the characters of the next generation have been reexamined. Under intense pollen competition unexpected segregation for different genetic traits is obtained (Mulcahy and Kaplan 1979; Sari-Gorla and Rovida 1980; Zamir et al. 1982; Wendel et al. 1987; Pedersen 1988; Robert et al. 1989). While this fact has been repeatedly observed, there is no clear explanation of how it is produced. Since there is no male genotype that performs better in all crosses, it may be due to a selective action induced by the pistils, which lead to different kinds of pollen-pistil interactions within the crosses (Pedersen 1988; Waser et al. 1987).

Likewise, under intense pollen competition, a decrease in genetic variability of the offspring occurs, leading to a lower frequency of the extreme genotypes (Willson and Burley 1983; Schlichting et al. 1987). There is substantial information supporting this view in several species: *Gossypium*, *Vigna*, and *Triticum* (Ter-Avanesian 1978); *Dianthus chinensis* (Mc Kenna and Mulcahy 1983); *Raphanus raphanistrum* (Mazer et al. 1986); and *Cucurbita pepo* (Winsor et al. 1987; Davis et al. 1987).

Furthermore, a number of reports have demonstrated a positive correlation between pollen competition ability, evaluated mainly through pollen tube growth rate, and several sporophytic traits such as: seed dry weight, seedling weight, number of seeds per fruit, and root growth (Mulcahy 1971, 1974, 1979; Ottaviano et al. 1980; Snow 1986; Winsor et al. 1987). All these reports have as a common factor the fact that the higher the pollen tube growth rate, the more vigorous the offspring. Thus, genes that affect sporophytic vigor could also be affecting pollen tube growth or, in other words, pollen tube growth and several sporophytic traits would be related genetically.

While it is relatively easy to understand gametophytic selection for a single locus, it is more difficult to do so for quantitative traits under polygenic control. Several reports have suggested that pollen tube growth is under the control of polygenic systems (Mulcahy 1974; Ottaviano et al. 1982; Wendel et al. 1987; Sarr et al. 1988). If the rate of pollen tube growth is controlled by several genes, each with a small individual effect, it would be difficult to find such genes using single genetic markers, unless a great number of descendants are studied (Mulcahy and Kaplan 1979).

Pollen competition could take place at two levels: either through a direct competition among haploid gametophytes (Willson and Burley 1983) or through an interaction between the haploid and diploid genes (Mulcahy 1979). Direct competition between gametophytes could be of two kinds: either a physical competition based on the rate of pollen tube growth (Mulcahy et al. 1983; Lee 1984), or a chemical competition based on pollen inhibition. However, while this allelopathic phenomenon has been recorded on an intergeneric basis in the genus *Parthenium*, where pollen grains contain germination and growth inhibitors for pollen of other genera (Kanchan and Jayachandra 1980), it is unknown whether or not this phenomenon operates on an intraspecific basis.

The idea of an interaction between the male and the female tissue is reasonable. Pollen tube kinetics are affected by the microenvironment generated in the pistil (Herrero and Arbeloa 1989), so that the growth of particular pollen tubes would be favored giving them an advantage in achieving fertilization (Shivanna and Shivanna 1985; Hill and Lord 1987). This could explain the variations found in the progeny in relation to the female parent used (Pedersen 1988; Sarr et al. 1988).

In natural conditions, plants could show a trend towards the enhancement of pollen competition by increasing the style length (Mulcahy 1971; Mulcahy and Mulcahy 1975; Ottaviano et al. 1982, 1988 c), or by increasing the period of time between the arrival of pollen grains on the stigma and fertilization (Frankel and Galun 1977; Willson and Burley 1983; Stephenson and Bertin 1983; Epperson and Clegg 1987; Ottaviano et al. 1988 b). However, the evidence we have so far is mainly indirect and circumstantial, although it may hint that gametophytic selection does actually operate in natural conditions.

3. Requirements for gametophytic selection

The main evidence in favor of gametophytic selection is based on two facts: one, that there is an overlap in genetic expression between the diploid and haploid phases; and two, that there is a correlation in behavior of the haploid and diploid phases in response to different external agents.

3.1 Overlap in the genetic expression between the sporophyte and gametophyte

One of the main requirements for the utilization of gametophytic selection in plant breeding is the necessity of an overlap in the genetic expression between the haploid and diploid generations.

Basically, we can find three kinds of genes, according to their expression in the two phases: (1) genes expressed only in the sporophyte (diploid expression); (2) genes expressed only in the gametophyte (haploid expression); (3) genes expressed in both gametophyte and sporophyte (haplodiploid expression). The third group contains the genes suitable for gametophytic selection. Actually, this could also be extended to the genes expressed only in the sporophyte, but which are closely linked to genes expressed in the gametophyte.

During the last few years, this overlap has been studied at two levels: with isozymes and with nucleic acids.

3.1.1 Isozyme expression. Initial work in this field was carried out by comparing the genetic expression of single enzymes in the two phases of the plant life cycle, but without considering the possibility of a gametophytic selection. One of the first studies pointed out that the activity of alcohol-dehydrogenase in maize (*Zea mays*) is expressed both in the sporophytic and gametophytic phases (Schwartz 1971; Schwartz and Osterman 1976).

Later, studies began to compare the genetic expression of different isozyme groups between the two phases. The initial research in this direction was limited to comparative studies of the expression of specific enzymes of the diploid and the haploid phases; overlap between both phases was found. There is no doubt that the key moment was in 1981 when Tanksley et al. published their work on tomato (*Lycopersicon sculentum*) isozymes. They looked at 9 genes and concluded that 62% of the isozymes studied were expressed in the gametophyte, 58% were expressed in both phases, and 3% only in the pollen grains.

Further work, with different enzymatic groups and different plant species, supports these initial results. The overlap rates seem to be very similar. For example, 72% in *Zea mays* (Sari-Gorla et al. 1986 a, b), 81% in *Prunus* (Weeden 1986), 60% in barley (Pedersen et al. 1987), and between 74 and 80% in *Populus* (Rajora and Zsuffa 1986).

3.1.2 Nucleic acid overlap. Initial work in this field was restricted to RNA studies. It was shown that a part of the pollen mRNAs are presynthesized in the sporophytic phase; these mRNA are useful during the development of the pollen grain and the beginning of the pollen tube growth (Mascarenhas and Bashe 1984).

Later, the hybridization between mRNAs synthesized by the gametophyte and cDNAs from the sporophyte was studied in *Tradescantia paludosa* (Willing and Mascarenhas 1984; Mascarenhas et al. 1986; Stinson et al. 1987). These authors found about 30,000 different mRNAs in the sporophyte tissues and 20,000 in the gametophyte. Hybridization results confirm those of the isozymes: about a 60% of the sequences analyzed were expressed in both gametophytic and sporophytic tissues. However, these studies were done using only shoot tissue from the sporophyte; therefore, by studying additional diploid tissues, the percentage of overlap would presumably be increased. Willing et al. (1988) pursued their studies with pollen and shoot tissues of *Zea mays*, and the results were very similar to those using *Tradescantia* (Mascarenhas et al. 1989; for a review, see also Mascarenhas 1989). An overlap of genetic expression in both gametophytic and sporophytic tissues has also been demonstrated by means of tissue localization of transcripts specified by particular cDNA clones in tomato (Ursin et al. 1989).

Recent evidence indicates that there may also be an overlap in the composition of particular chemicals. This may open the possibility of using pollen selection for lipid quality in seeds based on significant correlation, for linoleic and linolenic acids, found in pollen and seeds of *Brassica* (Evans et al. 1987, 1988). In addition, glucosinolates have been also found in *Brassica* pollen (Dungey et al. 1988). These authors pointed out the possibility of its use for pollen selection, but further studies are necessary to have a clear picture of the correlation between the amounts of glucosinolates in pollen and seeds.

All the above results are supported by the use of mutants. Meinke (1982) studied embryolethal mutants in *Arabidopsis thaliana.* The mutant gene is expressed not only during embryogenesis, but also prior to fertilization: either during pollen grain development or during pollen tube growth. Consequently, the gene is expressed in both gametophytic and sporophytic tissues, and abnormalities during the sporophytic development could be eliminated through gametophytic competition.

In maize (Ottaviano et al. 1988a), a set of 32 endosperm defective mutants (*de* mutants) was analyzed. The mutants represented 32 different genes controlling endosperm development. Segregation frequencies in some F_2 individuals were not as expected, and the authors deduced that 22 (60%) of these genes could affect microspore development or pollen tube growth.

All of these experiments, while having different approaches, appear to indicate that an overlap occurs in the genetic expression of the sporophyte and the gametophyte.

3.2 Correlation between sporophytic and gametophytic responses to external factors

While it is clear that there is a large overlap between the genetic expression at the molecular level in gametophytic and sporophytic tissues, it is necessary to see if this is followed by phenotypic expression in both phases. Indirect evidence is obtained through an analysis of the behavior of the gametophytic and sporophytic phases in relation to different external agents.

3.2.1 Temperature. Early studies in this field were carried out by Zamir et al. in 1981, demonstrating the higher competitive ability of pollen from *Lycopersicon hirsutum*, a plant that lives at high altitudes in the Peruan Andes, compared to that of *Lycopersicon sculentum* grown under cold conditions. However, they were not able to determine at that time whether pollen cold tolerance is dependent on the haploid genome or whether the diploid genome conditions the gametophytic response against cold stress. Further work (Zamir et al. 1982) concluded that the effect was due to the haploid genome, and demonstrated that gametes with a particular chromosomic segment from *L. hirsutum* were more successful in fertilization at low temperatures. Furthermore, they showed that some genes responsible for cold tolerance were located in a small portion of the haploid genome linked to specific isozymes. Moreover, it was demonstrated (Zamir and Vallejos 1983) that the selection pressure was higher during pollen tube growth than during pollen grain formation. Finally, offspring of *Lycopersicon* from crosses carried out at low temperatures showed better cold adaptation, evaluated by root growth, than offspring from crosses at the control temperature.

However, other studies, also using *Lycopersicon*, showed a possible discrepancy (Maisonneuve and Den Nijs 1984; Den Nijs et al. 1986), since there were no differences in response to cold temperatures between gametophytes and sporophytes of *Lycopersicon sculentum.* The difference in these results could lie in the experimental methods used (Zamir and Gadish 1987). A similar situation appears in *Prunus*, where differences in chilling requirements among sporophytes from the same species do not correlate with the behavior of the male gametophytes; however, differences are observed between two different *Prunus* species with different cold requirements: *Prunus dulcis* and *Prunus persica* (Weinbaum et al. 1984).

In spite of those discrepancies, there are several species in which significant correlation between gametophyte and sporophyte behavior, in relation to thermic stress, has been verified. For instance: in *Zea mays* (Frova et al. 1986), *Juglans* (Luza et al. 1987), *Eucalyptus* (Marien 1988), *Pistacia vera* (Polito et al. 1988), *Gossypium hirsutum* (Rodríguez-Garay and Barrow 1988), and *Medicago sativa* (Mulinix and Iezzoni 1987). Barnabás and Kovács (1988) suggested that low temperature storage of maize pollen could be used as a treatment in selecting pollen populations for cold tolerance, since seeds set by treated pollen showed a higher germination rate under low temperatures than seeds set by untreated pollen.

In an attempt to understand the mechanism for an overlap in the expression of heat tolerance, some work has looked at its relationship to heat shock proteins (HSP). The tissues of some plant species respond to high temperatures by synthesizing special proteins (heat shock proteins) that protect the cells against thermic stress. Early studies in this field showed no HSP in pollen from *Tradescantia paludosa* (Mascarenhas and Altschuler 1983), although thermotolerance was achieved by the germinating pollen grains (Xiao and Mascarenhas 1985). Later, HSP synthesis was found in inmature but not in mature pollen from *Zea mays* (Frova et al. 1986, 1989) and in mature pollen from *Nicotiana tabacum* (van Herpen et al. 1988, 1989), *Lilium longiflorum* (van Herpen et al. 1989), and *Sorghum bicolor* (Frova et al. 1991); in *Sorghum*, two kinds of HSPs are expressed: those common to the sporophyte and those expressed only in the gametophyte.

Taken together, these works suggest that to achieve clear results it is probably necessary to have wide genetic variation in the character to be selected. On the other hand, further research is needed to understand the physiological mechanisms underlying the gametophyte-sporophyte behavior at different temperatures.

3.2.2 Salinity and osmotic pressure. Little work has been done in this field. One of the first pieces of indirect evidence on this subject was the work by Eisikowitch and Woodell in 1975 with *Armeria maritima*. In this coastal plant, the pollen grains from a population that lived on a beach were more resistant to sea water than those from another population located farther from the coast.

Later, Sacher et al. (1983) crossed *Lycopersicon sculentum* (salinity nontolerant) with *Solanum penellii* (salinity tolerant). Plants of the F_1 grown under saline conditions showed higher salt concentration in anthers and style than those cultivated under normal conditions. The F_2 plants originated from saline self-pollination of the F_1 had a higher dry weight grown under both saline and normal conditions. The results suggest that fertilization under salt stress can increase not only the resistance to salinity but also the mean vigor of the plants. The authors attributed this to competition among pollen grains, assuming that competition is enhanced under salt conditions and that only the most vigorous gametophytes can fertilize the ovule. With the same two species, but using isozymes as genetic markers, Sari-Gorla et al. (1988) observed that F_2 plants, from plants grown in the presence of salt, showed, if compared with a control F_2 population, a significant deviation in favor of the tolerant parent allele for two studied loci. Later, and also in *Lycopersicon*, studies have been carried out in relation to osmotic stress (Mulcahy et al. 1988); their conclusion is that varieties tolerant to an osmotic stress, studied at the seedling stage, produce pollen that is, in its turn, more tolerant to such stress than pollen from nontolerant varieties.

Although little work has been done so far in this field, it looks to be a very interesting subject for future research from a plant breeding point of view.

3.2.3 Metals. Initial studies consisted of crosses between clones of *Silene dioica* zinc tolerant, *Silene alba* zinc nontolerant, and *Mimulus guttatus* copper tolerant and nontolerant. It was determined that pollen from tolerant plants was able to germinate and grow at metal concentrations that clearly inhibitited the germination and growth of the pollen from nontolerant plants. On the other hand, no differences were found in the behavior between binucleate (*Mimulus*) and trinucleate (*Silene*) pollen in relation to metal tolerance (Searcy and Mulcahy 1985a).

With the same plant material, further studies (Searcy and Mulcahy 1985b) were developed to look at the behavior of pollen at the stigma. Flowers from plants watered with a solution that contained copper or zinc and from plants watered with a control solution were pollinated with pollen from metal tolerant and metal nontolerant plants. The results indicated that, although there was no difference in the pollen tube growth rate, there were significant differences in the number of fertilized ovules and viable seeds. While these authors explained that the lack of difference in pollen tube growth could be due to the small increase in metal concentration in the style, it is still not clear how the differences in number of viable seeds are produced. Later work (Searcy and Mulcahy 1985c) indicated that the metal tolerance in pollen from *Mimulus guttatus* is controlled by the gametophyte genome, and it seems that a small number of genes are involved (Searcy and Mulcahy 1986).

Correlation between sporophyte and gametophyte was also found for aluminum tolerance in *Lycopersicon sculentum* (Searcy and Mulcahy 1990): germination of pollen from aluminum sensitive cultivars is reduced in the presence of the metal but not that of pollen from aluminum resistant cultivars.

Work in gymnosperms, *Pinus silvestris* and *Pinus nigra* (Holub and Zelenakova 1986), further support overlap for metal tolerance. Pollen from plants living in places with high lead concentration germinated better and had higher pollen tube growth on a medium containing lead than pollen from plants living in places with low lead concentration.

3.2.4 Herbicides and other toxic compounds. Interesting studies in relation to gametophytic selection began in 1973, when it was noticed in *Zea mays* that sporophytic susceptibility or resistance to the fungus *Helminthosporium maydis* can be predicted by measuring the pollen response to the toxin produced by the pathogen (Laughnan and Gabay 1973). After several years without progress in this field, studies were carried out with *Brassica* and *Alternaria brassicicola* (Hodgkin and Mc Donald 1986). The authors found that male gametophytes from *Brassica* fungus susceptible plants were also affected by the toxic compounds extracted from a culture of *Alternaria brassicicola*. This work was pursued (Hodgkin 1988, 1990) using pollen from resistant *Brassica* specimens to pollinate a susceptible variety, with both pollen selected and nonselected for resistance to the fungal toxin. Hodgkin concluded that, in the F_1, pollen from plants descended from selected pollen was more resistant to the toxin than pollen from plants descended from nonselected pollen. Bino et al. (1988b) also found that germination of pollen from different *Lycopersicon* species sensitive to *Alternaria alternata* was lower, on a medium with the toxin, than that of pollen from resistant plants, while pollen germination from species not susceptible to the fungus was unaffected by the toxin.

Similar experiments have been carried out with other toxic compounds. Pollen from sugar beet plants (*Beta vulgaris*) resistant to a herbicide (ethofumesate) germinates better on a medium containing the herbicide than pollen from nonresistant plants (Smith and Moser 1985; Smith 1986). With the herbicide chlorsulfuron the results appear similar (Sari-Gorla et al. 1989): when maize pollen is exposed to the herbicide, the resultant progeny exhibit significantly greater tolerance to the herbicide than progeny from untreated pollen.

Another toxic compound studied in this way is ozone. In *Nicotiana tabacum* and in *Petunia hybrida*, it was found (Feder and Sullivan 1969; Feder 1986) that pollen germination and pollen tube growth were lower after exposing the male gametophyte to high ozone concentrations in vitro, but that the response was variable depending on the origin of the pollen grains: pollen from sporophytes susceptible to ozone was more affected than those from less susceptible sporophytes.

Further evidence of this correlation is found in resistance to antibiotics. Bino et al. (1987) studied the influence of kanamycin on pollen germination and pollen tube growth, using tomato plants sensitive to kanamycin and transgenic tomato plants resistent to the antibiotic. Although pollen germination was not affected by kanamycin, pollen from transgenic plants had significantly greater tube growth than that of normal plants. Bino and Stephenson (1988) presented similar results with tomato plants sensible to hygromycin and transgenic tomato plants resistant to that compound.

4. Pollen selection and practical plant breeding

While it is clear that an overlap exists in genetic expression and in the response to different environmental parameters between the sporophyte and the gametophyte, the application of gametophytic selection in plant breeding is still uncommon. One of the reasons is technical difficulty. The use of gametophytic selection as an efficient tool in plant breeding requires that pollen, which has been exposed to a particular treatment, can be effectively recovered and be capable of achieving fertilization.

Recently, several techniques have been used to attempt to recover the selected pollen. Among the most interesting methods are those that separate germinated and ungerminated pollen based on differences in density, since the larger the pollen tube the lower the density, as density gradient in liquid column (Mulcahy et al. 1988) and pollen centrifugation (Hodgkin 1988; Bino et al. 1988a). Flow cytometry (Marien 1988) or nuclear magnetic resonance with ^{31}P (Ladyman and Taylor 1988) may also prove useful in separating these two populations of pollen grains. All these methods are fairly recent and further work needs to be done to make a wider use of these techniques. Separation methods should be innocuous to the male gametophytes, however, a decrease in the in vivo performance is generally observed. To overcome this behavior, it could be useful to achieve fertilization in vitro (Raman et al. 1980) or pollination could be done using cut styles (Hodgkin 1987, 1988). This technique has proved to be valuable when pregerminated pollen, in the presence of fungal toxins, was unable to penetrate the stigma.

Alternatively, instead of applying the selective agent only to the pollen, the whole plant could be grown under selective pressure. While this method appears rigorous it has yielded results. Plants (*Lycopersicon* × *Solanum*) grown in a saline environment have produced plants that have an offspring with a higher dry weight when cultivated in saline conditions (Sacher et al. 1983). Likewise, experiments with *Lycopersicon* (Zamir and Vallejos 1983; Zamir and Gadish 1987) have shown that progeny from crosses made at low temperatures had better cold adaptation than progeny from crosses at normal temperatures.

Selective agents can also be placed on the stigma. While a negative response to fusaric acid was obtained with this technique in *Nicotiana* (Simon and Sandford 1986), the method has proved valuable in maize in selecting against the herbicide chlorsulfuron (Sari-Gorla et al. 1989). A positive response was also observed in the progeny if the pollen developed in tassels that had been submerged in a solution of the herbicide.

While transmission of selected traits from the gametophytic generation has been efficiently demonstrated for salt tolerance (Sacher et al. 1983), cold tolerance (Zamir and Vallejos 1983; Zamir and Gadish 1987), resistance to fungal toxins (Hodgkin 1988), or resistance to herbicides (Sari-Gorla et al. 1989), there is little experimental evidence of the transmission of the selected traits to the next generation, and further work is required to support this idea (Landi et al. 1989). Methods for separation of selected from nonselected pollen and application of selected pollen grains to styles must be optimized (Bino and Stephenson 1988).

5. Synopsis

Twelve years have elapsed since Mulcahy (1979) put forward the theory of gametophytic selection. Since that time information has accumulated supporting this view. While clear results are produced by evaluating sporophytic and gametophytic overlap through the study of isozymes, the definitive point was when Willing and Mascarenhas (1984) identified an approximately 60% overlap by nucleic acid hybridization. This is a solid starting point and molecular genetics should do much to enlighten our understanding of this process.

A wide correspondence has also been found between both phases in the response to a variety of external agents, mainly temperature, salinity, metals, herbicides, fungal toxins, and other toxic compounds. The fact that this external environment may influence selection not only at the sporophytic but also at the gametophytic level may be important for a better understanding of the evolutionary process in higher plants.

The impact of gametophytic selection in plant breeding is still small and this may be partly due to difficulties in the manipulation of the reproductive process. Further research in this field may overcome these difficulties and may also clarify how gametophytic selection operates in vivo. It appears to be a promising and especially valuable field, either where large-scale screening is required or in woody perennial species where selection procedures limit practical plant breeding.

Gametopyhtic selection appears undoubtedly to be an exciting subject, where molecular genetics and a better understanding of the physiological mechanisms that regulate it will open new avenues of research.

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