Theor Appl Genet (1994) 88: 812–817

M. Sari-Gorla · S. Ferrario · E. Frascaroli · C. Frova
P. Landi · M. Villa

Sporophytic response to pollen selection for Alachlor tolerance in maize

Received: 2 November 1993 / Accepted: 10 November 1993

Abstract In order to assess the efficiency of male gametophytic selection (MGS) for crop improvement, pollen selection for tolerance to herbicide was applied in maize. The experiment was designed to test the parallel reactivity to Alachlor of pollen and plants grown in controlled conditions or in the field, the response to pollen selection in the sporophytic progeny, the response to a second cycle of MGS, and the transmission of the selected trait to the following generations. The results demonstrated that pollen assay can be used to predict Alachlor tolerance under field conditions and to monitor the response to selection. A positive response to selection applied to pollen in the sporophytic progeny was obtained in diverse genetic backgrounds, indicating that the technique can be generally included in standard breeding programs; the analysis of the data produced in a second selection cycle indicated that the selected trait is maintained in the next generation.

Key words *Zea mays* L. · Pollen assay
Gametophytic selection · Alachlor · Herbicide tolerance

Introduction

In recent years a large number of reports have been published about the role of selection at the male gametophytic level under natural conditions and its possible use as a tool in plant breeding programs.

The first, and so far most-abundant, findings involve correlations between sporophytic and gametophytic characters. These concern associations between pollen quality and plant fertility and vigour (Mulcahy 1971; Ottaviano et al. 1980; Snow 1986; Winsor et al. 1987), and between pollen and plant responses to different environmental stresses: high (Frova et al. 1991) or low temperatures (Zamir and Vallejos 1983; Kovacs and Barnabas 1992), metals (Searcy and Mulcahy 1985; 1990), pathogens (Laughan and Gabay 1973; Hodgkin and Mac Donald 1986, 1990; Shivanna and Sawhney 1993), and toxic compounds (Feder 1986; Smith 1986; Bino 1987, 1988; Frascaroli et al. 1992). These results are important for the use of pollen as a biological indicator: in comparison with greenhouse- or field-trials, pollen assay allows the evaluation of plant genotypes in a short time, limited space, and at a low cost.

Since a large overlap in the genetic expression between sporophyte and gametophyte has been demonstrated by means of transcripts or gene product analyses (for a review see Ottaviano and Mulcahy 1989), it is reasonable to assume that selection applied to pollen can produce a response in the sporophytic progeny. However, phenotypic association between pollen and plant features does not necessarily mean that the same genes are independently expressed in both of the two generations. The expression (transcription and translation) of the same genes in both generations is clearly demonstrated when a response to selection, applied to pollen of single heterozygous plants, is obtained in the resulting sporophytic progeny. Results of this type have been described in tomato for tolerance to cold (Zamir et al. 1982; Zamir and Gadish 1987) and to salt (Sacher et al. 1983; Sari-Gorla et al. 1988), to high temperature in cotton (Rodriguez-Garay and Barrow 1988), for plant fertility and vigour (Ottaviano et al. 1982, 1988; Landi et al. 1989) and for tolerance to herbicides (Sari-Gorla et al. 1989, 1992) in maize.

Pollen selection can be particularly useful for traits whose genetic control is unknown, or which are due to recessive alleles or rare allelic combinations, as is often the case for tolerance to chemical stresses. The use of herbicides is a well-established necessity in modern agriculture, but frequently herbicides having environmentally-desirable characteristics are not able to control weed species without harming the crop of interest. Thus, it is important to develop genotypes tolerant to specific herbicides (see Shultz et al. 1990 for a review). Alachlor (a chloroacetan-

Communicated by P.L. Pfahler

M. Sari-Gorla (✉) · S. Ferrario · C. Frova · M. Villa
Department of Genetics and Microbiology, University of Milano, Italy

E. Frascaroli · P. Landi
Institute of General Agronomy, University of Bologna, Italy

ilide) is a widely-used herbicide, whose precise mechanism of action is presently unknown. Variability for tolerance, mainly based on ability of plants to detoxify the molecule, has been described in maize (Sari-Gorla et al. 1993). The analysis of segregating generations suggested that the genetic determination of the trait involves a few dominant genes; however, the performance of inbred lines and F_1s revealed that the expression of tolerance is affected by incomplete penetrance and/or variable expressivity; thus the trait appeared particularly appropriate to be manipulated by MGS.

The purpose of the present work was to determine if the gametophytic phase can be efficiently used in selection for Alachlor tolerance. Specific issues include: (1) is pollen assay able to predict plant response in the field? – to our knowledge, the only data concerning the correlation between response of pollen and plant to external agents in field trials are those obtained for tolerance to Glyphosate (Frascaroli et al. 1992); (2) is MGS a generally-applicable technique, over diverse genotypes? – some of the experiments demonstrating MGS have been carried out using interspecific crosses, and thus do not represent the general situation in plant breeding; alternatively, selection has been applied to single F_1 pollen sources, sources perhaps not typical of other genetic combinations; (3) is the selected trait transmitted to the following generations? – since the monitoring of MGS has been generally made on the resultant sporophytic generation, it was necessary to verify that MGS produces stable tolerant genotypes in subsequent generations.

Materials and methods

In-vitro pollen growth and measurement

Pollen from plants grown under normal field conditions, or from tassels undergoing anthesis in a controlled environment, was collected at flowering on 2 days, each representing a replication. For each collection, pollen was divided in two lots that were inoculated in two Petri dishes containing a solid medium (Cheng and Freeling 1976); one was supplemented with the herbicide, (Alachlor, technical preparation 95.6%, kindly provided by Monsanto Europe) at 0.1 g/l, while the other served as the control. All Petri dishes were incubated for 3 h at 27 °C, fixed with Farmer's liquid, stained with aniline blue and used for evaluation of pollen-grain germination (on 500 grains per plate) and pollen-tube length (on 50 tubes per plate).

Pollen tolerance in terms of grain germination was expressed as the ratio of germination percent on medium supplemented with the herbicide to the same trait on control medium; tolerance for tube elongation was expressed as the ratio of mean tube length of pollen grown in the presence to that of pollen grown in the absence of Alachlor.

Seedling treatment and seedling reactivity evaluation

Seeds soaked for 20 h in an Alachlor solution (1 g/l) were sown in pots filled with wet sand and covered with cellophane bags; 12 days after sowing the response to the treatment was assessed by means of visual ratings from 1 (normal plants) to 5 (completely malformed plants). For the data presentation and analyses, the seedlings were classified as normal if they fell into class 1 or 2 (normal or only reduced in growth), or as injured if they fell into classes 3, 4, or 5 (great injury, necrotic areas).

Tolerance was expressed as the proportion of normal plants after treatment with the herbicide; in controlled environments the proportion of normal plants in the absence of herbicide is expected to be 100%.

Field trial: treatment and reactivity evaluation

The field trial was carried out in northern Italy: four inbred lines (B73, K55, H99, Ky226) were evaluated in control conditions and in the presence of 4 kg a.i. ha^{-1}. The experimental design was a complete randomized block with eight replications. Each experimental unit was one row, 3.4 m long and 0.5 m wide, that was split in two equal sub-units, each 1.2 m long, separated by an empty 1-m alley, thereby yielding two strips per replications across genotypes. Fifty seeds were sown per unit.

Immediately after sowing, herbicide was sprayed to one random strip per replication; the untreated strip acted as control. The same day a sprinkler irrigation (150 m^3 ha^{-1} of water) was carried out to promote germination and more uniform distribution of the herbicide.

Eighteen days after sowing the number of emerged and injured plants per plot were scored. The plant reactivity was expressed as the ratio of the proportion of emerged, normal plants in the presence of Alachlor, to that of control plants.

Pollen selection

For treatment during microspore development, tassels were cut about 2 weeks before anthesis, put into an artificial liquid medium (Polowick and Greyson 1982) to which the herbicide was added (1 g/l), and grown in a growth chamber. The same procedure, without Alachlor treatment, was adopted for control plants. Alternatively, the herbicide treatment was applied during pollen function: mature pollen from hybrid plants grown in the field was used to pollinate female plants of the susceptible genotype, the silks of which were sprayed with a solution containing the herbicide (1 g/l). The same pollination procedure, except for the presence of the chemical in the solution, was applied to the control plants.

In order to test the response to pollen selection in the sporophytic progeny, the response to a second selection cycle, and the transmission of the selected trait, the following cross design was adopted.

Two parental lines, one susceptible, H99 or Ky226 inbred lines, and the other tolerant to Alachlor, B73 or K55, were crossed, and the pollen from the resultant F_1 plants was submitted to selective treatment or used as a control. In the case of selection during microspore development, pollen of the herbicide-treated F_1 plants was used to pollinate female plants of the susceptible genotype; in the experiment involving selection during pollen function, pollen of the F_1 plants was used to pollinate herbicide-treated female plants of the susceptible genotype. Thus, in both cases, two backcross (BC) progenies were produced: one by selected pollen, BC(S), and the other by control pollen, BC(C). The comparison of tolerance to the herbicide in the two populations allows the evaluation of the response to pollen selection. Three hybrid combinations and their reciprocal crosses were tested: H99×B73, H99×K55, Ky226×K55.

The BC(S) progenies derived from five of the treated F_1 plants, each represented by seven plants, were submitted to a second cycle of selection and used to pollinate a susceptible inbred female, thus producing a BC(SS) progeny; untreated plants of the same BC(S) generation were used as a control to produce BC(SC) progeny. Finally, plants from a BC(C) population were used, without any treatment, to pollinate female plants of the susceptible genotype, in this way producing a BC(CC), the true control population, never submitted to selection. The comparison between Alachlor tolerance in the BC(SS) and the BC(SC) progenies allows the evaluation of the response to selection in the second cycle, whereas the comparison between BC(SC) and BC(CC), i.e., between the population produced by pollen selected only in the first generation and that from pollen never treated, gives information about the transmission of the selected trait in the next generations, despite the cessation of selection for herbicide tolerance. This second part of the experiment was carried out on the hybrid combination H99×B73 and its reciprocal.

In the Results section the pooled data from the two reciprocal crosses are presented for all the experiments.

Statistical analysis

Correlations between pollen and seedling traits were estimated by the Pearson coefficient of correlation.

The analysis of proportions was performed by a χ^2 test of independence, i.e., testing the hypothesis of equality between the proportions of normal or injured plants in the selected and in the control populations.

Results

In Table 1 the responses of pollen, seedlings in the greenhouse and in the field are illustrated; the results indicated a parallel response to the herbicide at all levels, clearly described by the values of the coefficients of correlation: r=0.77 and 0.57 between pollen reactivity, in terms of germination, and the reactivity of plants grown in a controlled environment and in the field, respectively; r=0.83 and 0.99 between pollen-tube elongation and the same two sporophytic traits. The correlation coefficient between plant reactivity expressed in the greenhouse and in the field was also high, r=0.85. Although these r values are high and statistically significant, we include them only as a general indication of association, since N is too small to allow a meaningful comparison of values. On all aspects, B73 and K55 demonstrated more tolerance, H99 and Ky226 more susceptibility.

In order to obtain a response in the sporophytic progeny to pollen selection, it is necessary that the selective pressure be applied at a stage in which the genes conferring tolerance are expressed. Since this information was not available, the selective treatment was applied either during microspore development or during pollen function. Mature pollen grains are, to a large extent, genetically silent, whereas the expression of a large amount of the genome has been demonstrated both during microspore maturation and during pollen germination and growth (Mascarenhas 1992).

In Fig. 1 the effect of gametophytic selection, according to the gametophytic stage at which the treatment was applied, is illustrated. The experiment was carried out on the H99×B73 genetic combination. The response to selection was evaluated by comparing the proportion of normal or injured plants after herbicide treatment in the sporophytic BC progeny obtained from unselected pollen (A), from pollen selected during its function (B), and from pollen treated during microspore development (C). The data revealed a significant trend in the proportion of normal plants from the control BC progeny to the BC produced by pollen selected during microspore development. On the basis of these results, the selection was applied during microspore development in all further selection experiments.

Table 2 indicates the response to selection obtained in the BC sporophytic progeny after treatment of microspores from three F_1s between tolerant and susceptible parental lines: H99×B73, H99×K55, and Ky226×K55. A positive response was obtained in all three genetic combinations:

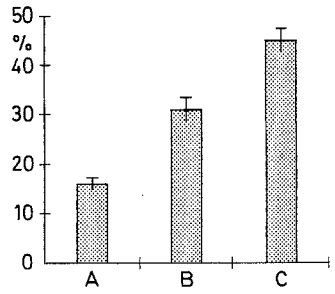

Fig. 1 Response to selection according to the stage of gametophytic phase when treatment was applied. Percentage of normal seedlings produced in the backcross progeny from control pollen (*A*), from pollen selected during its function (*B*) or during microspore development (*C*). The standard error is indicated in each case

Table 1 Reaction to Alachlor, expressed as a percent of the control, of pollen, and of seedlings grown in a controlled environment and in a field trial, using four inbred lines

Lines	Pollen reactivity		Seedlings reactivity in	
	Germination	Tube length	Greenhouse	Field
K55	76.7	62.6	85.2	91.1
B73	126.3	58.1	80.9	81.4
H99	48.3	50.0	10.1	59.6
Ky226	57.6	55.2	2.0	72.7
SE	1.6	3.5	2.1	4.4

Table 2 Response to gametophytic selection in the sporophytic progeny

Backcross	H99×B73		H99×K55		Ky226×K55	
	Percent of normal plants	N	Percent of normal plants	N	Percent of normal plants	N
BC(C)	29.3	526	20.8	183	31.7	240
BC(S)	45.5	747	41.1	937	43.3	120
$\chi^2_{[1]}$	37.4**		26.8**		4.8*	

H99×B73, H99×K55, Ky226×K55: F_1s from which pollen to produce BC progenies was used
N, total number of plants
BC(C), BC progeny from unselected pollen
BC(S), BC progeny from selected pollen
$\chi^2_{[1]}$: χ^2 test of independence, with 1 degree of freedom
**: $P<0.01$; *: $P<0.05$

the proportion of normal plants after herbicide treatment was significantly greater in the selected BC progenies than in the control progeny, as indicated by the resulting χ^2 values. A significant increase in the proportion of normal plants in the population produced by selected pollen, indicates a non-random transmission of male gametes to the progeny, and the expression of the genes conferring tolerance in both the gametophytic and the sporophytic phases.

In order to verify the transmission of the selected trait in the following generations, the BC progeny produced by

Table 3. Transmission of the selected trait and response to the second cycle of selection

Progeny	Percent of normal seedlings	N	$\chi^2_{[1]}$
BC(CC)	29.2	480	9.53**
BC(SC)	39.8	301	0.15^{ns}
BC(SS)	41.0	3300	

BC(CC), Backcross progeny from unselected pollen
BC(SC), Backcross progeny from pollen selected in the first generation only
BC(SS), Backcross progeny from pollen selected in both the first and the second generation
N, total number of plants
$\chi^2_{[1]}$, χ^2 test of independence, with 1 degree of freedom
**: $P<0.01$; ns, not significant

Table 4. Plant and pollen response to Alachlor in BC(SS) progenies derived from five plants (P_1, P_2, ... P_5) submitted to the first selection cycle

Progeny BC(SS)	Percent of normal seedlings	Pollen reaction	
		Germination	Tube length
P_1	43.5	82.3	35.5
P_2	44.2	101.2	55.9
P_3	41.8	100.8	62.4
P_4	38.7	52.5	40.0
P_5	33.6	62.8	36.6
SE	4.8	11.7	6.7

selected pollen and that from unselected pollen were backcrossed again with the susceptible parental line (see Materials and methods section). Tolerance to Alachlor was evaluated in the sporophytic population derived by pollen selected in the first generation, BC(SC), and in that never submitted to selection pressure, BC(CC). In this case, again, the former resulted in more tolerance to the herbicide than did the latter (Table 3), thus indicating that tolerance is maintained also in the next sporophytic generation.

The evaluation of the response to a second selection cycle was made on the basis of the comparison between the performance in the presence of Alachlor of BC(SS) *versus* BC(SC) seedlings. The average data, reported in Table 3 as the percentage of BC(SS) normal seedlings, indicated a slight, but not significant, increase in the proportion of tolerant plants by comparison with the BC(SC) progeny; however, when considering the performance of the progenies produced from five of the plants submitted to the first cycle of MGS, they revealed a non-homogeneous behaviour: 3 out of 5 gave positive results, two did not (Table 4). This could be expected, due to the adopted cross design: from each of the original five F_1 plants, a backcross-segregating population was obtained; thus the plants whose immature tassels were submitted to the second cycle of selection (5–7 plants *per* BC, for a total of 33) were genetically heterogeneous. This is reflected in the rather high values of the mean SE within groups of families, reported in Table 4, indicating a differential behaviour within the BC(SS) progenies.

A parallel response was observed on pollen. Tolerant pollen produced BC seedlings showing better tolerance to the herbicide: the coefficients of correlation, computed on 33 pollen-plant associated data, between the reactivity of pollen in terms of grain germination, in terms of mean tube length and the plant reactivity, revealed a significant association between the two pollen traits (r=0.40*) and between pollen-tube elongation and plant reaction (r=0.43*). The data also revealed a positive, though not significant, association between pollen germinability and seedling behaviour (r=0.29).

Discussion

The data support the utility of MGS application in plant breeding, and also clarify some points essential for combining MGS with traditional selection methods.

The usefulness of pollen assay was demonstrated in predicting plant performance under field conditions and in monitoring the selection response. Furthermore, since the response of pollen from plants produced by selected pollen is indicative of plant performance, pollen performance can be used to choose the plant material to be included in the following generation of selection.

The observation of a positive response to selection applied to pollen from different F_1s, indicates that it can be obtained in diverse genetic backgrounds, provided, of course, that the genes controlling the trait are segregating in the pollen population. Thus, the technique can be easily included in a standard (i.e., sporophytic) breeding scheme. The main advantages of this integrated procedure (selection in both 1N and 2N) would be a greater effectiveness due to the large population size and the haploidy which characterizes the male gametophyte. To some extent, MGS resembles cell selection; MGS, however, does not require plant regeneration. Furthermore, somaclonal variation, sometimes common in regenerative processes and which, in elite material, represents another drawback to cell selection, is avoided in MGS.

MGS has advantages also in regard to other biotechnologies, namely direct gene transfer. MGS is suitable for selecting traits which are controlled by genes not yet isolated, and, in particular, quantitative traits (Vasil 1990).

The main limitation of MGS is that it is applicable only for traits which are expressed at the cellular level. However, many traits of agronomical importance are expressed, at least as a trait component, at cellular and pollen levels, as demonstrated by the large number of cases of gametophytic-sporophytic correlation described in the recent years (for reviews see Hormaza and Herrero 1992; Searcy 1992). Indeed, the evaluation of the response to selection can suffer in these cases from the fact that the selection applied operates on cellular components, whereas its response is evaluated on the plant, where more complex interactions take place at different levels of plant organ-

ization. This problem can be overcome by the use of molecular markers: isozymes were used to monitor pollen segregation after selection for low temperature (Zamir et al. 1982) and salt (Sari-Gorla et al. 1988) tolerance. It is now possible to obtain a much higher resolving power by using polymorphisms at the DNA level, such as those identified using RFLP or RAPD markers.

An aspect that will have to be improved for a better exploitation of MGS concerns the technique of selection application. Theoretically, due to the peculiar features of the pollen population, a very high pressure of selection can be applied, at a relatively low cost. But, since mature pollen grains are frequently not sensitive to selective treatment, it has to be applied either to the plant producing the pollen or to the female plant where the pollen-tube grows. Thus the selection pressure that can actually be applied is limited by the necessity of not injuring the plant. New technologies, however, such as microspore maturation under artificial conditions, allow pollen treatment independent of the mother plant (Stauffer et al. 1991; Barnabas and Kovacs 1992). These, however, will not be routinely available for some time. On the basis of data from the present study, we suggest that a high level of tolerance could be attained by using backcrosses to elite lines.

The last, very important, result of this work is the experimental demonstration that, even in the second generation after MGS, BC(SC), the resultant plants still exhibit a significantly greater tolerance to Alachlor than do the BC(CC) (unselected) plants. This demonstrates that the response to the first selection was a genetic response. Previously, only two studies have examined a second generation: one, on *Petunia hybrida* (Mulcahy et al. 1978), indicated a persistent effect, while the other, on *Cucurbita pepo* (Schlichting et al. 1990) did not. The present study thus suggests the utility of MGS in selecting for resistance in crop species.

Finally, it is worth noting that, in the present work, selection has been applied exclusively at the gametophytic level, since our purpose was to demonstrate that MGS can be effectively used for crop improvement. However, on the basis of earlier data (Ottaviano and Sari-Gorla 1979; Sari-Gorla 1992; Pfahler 1983) and those presented here, we conclude that the maximum effectiveness would be obtained when selection is applied in the same direction in both the gametophytic and the sporophytic generations. An experiment specifically designed to compare the efficiency of MGS and of gametophytic *plus* sporophytic selection is now in progress.

Acknowledgements This work was suppported by Ministero Agricoltura e Foreste, Italy, Special Project: "Sviluppo di Tecnologie Avanzate Applicate alle Piante".

References

Barnabas B, Kovacs G (1992) In-vitro pollen maturation and successful seed production in detached spikelet cultures in wheat (*Triticum aestivum* L.). Sex Plant Reprod 5:286–291

Bino RJ, Hille J, Franken J (1987) Kanamicin resistance during in-vitro development of pollen from transgenic tomato plants. Plant Cell Rep 6:333–336

Bino RJ, Franken J, Witsenboer HMA, Hille J, Dons JJM (1988) Effects of *Alternaria alternata* f. sp. *lycopersici* toxin on pollen. Theor Appl Genet 76:204–208

Cheng DS, Freeling M (1976) Methods of maize pollen germination in-vitro, collection, storage and treatment with toxic chemicals; recovery of resistant mutants. MGC News Lett 50:11–13

Feder WA (1986) Predicting species response to ozone using a pollen screen. In: Mulcahy DL, Mulcahy BG, Ottaviano E (eds) Biotechnology and ecology of pollen. Springer-Verlag, New York Berlin, pp 89–94

Frascaroli E, Landi P, Sari-Gorla M, Ottaviano E (1992) Variability of pollen and plant responses to glyphosate in maize. J Genet Breed 46:49–56

Frova C, Taramino G, Ottaviano E (1991) Sporophytic and gametophytic HSP protein synthesis in *Sorghum bicolor*. Plant Sci 73:35–44

Hodgkin T, MacDonald MV (1986) The effect of a phytotoxin from *Alternaria brassicicola* on *Brassica* pollen. New Phyt. 104:631–636

Hodgkin T (1990) In-vitro pollen selection in *Brassica napus* L. for resistance to phytotoxic compounds from *Alternaria brassicola*. Sex Plant Reprod 3:116–120

Hormaza JI, Herrero M (1992) Pollen selection. Theor Appl Genet 83:663–672

Kovacs G, Barnabas B (1992) Production of highly cold tolerant maize inbred lines by repeated gametophytic selection. In: Ottaviano E, Mulcahy DL, Sari-Gorla M, Bergamini Mulcahy G (eds) Angiosperm pollen and ovules. Springer Verlag, New York Berlin, pp 359–363

Landi P, Frascaroli E, Tuberosa R, Conti S (1989) Comparison between responses to gametophytic and sporophytic recurrent selection in maize (*Zea mays* L). Theor Appl Genet 77:761–767

Laughan JR, Gabay SJ (1973) Reaction of germinating maize pollen to *Helmintosporium maydis* pathotoxins. Crop Sci 13:681–684

Mascarenhas JP (1990) Gene activity during pollen development. Annu Rev Plant Physiol Plant Mol Biol 41:317–338

Mascarenhas JP (1992) Pollen gene expression: molecular evidence. Int Rev Cytol 140:3–18

Mulcahy DL (1971) A correlation between gametophytic and sporophytic characteristics in *Zea mays* L. Science 171:1155–1156

Mulcahy DL, Mulcahy Bergamini G, Ottaviano E (1978) Further evidence that gametophytic selection modifies the genetic quality of the sporophyte. Soc Bot Fr Actualites Bot 125:57–60

Ottaviano E, Sari-Gorla M (1979) Genetic variability of male gametophyte in maize. Pollen genotype and pollen-style interaction. In: Israeli-Italian Joint Meeting on Genetics and Breeding of Crop Plants. Monogr Genet Agraria IV, Rome, pp. 89–106

Ottaviano E, Sari-Gorla M, Mulcahy DL (1980) Pollen tube growth rate in *Zea mays*: implications for genetic improvement of crops. Science 210:437–438

Ottaviano E, Mulcahy DL (1989) Genetics of angiosperm pollen. Adv Genet 26:1–64

Ottaviano E, Sari-Gorla M, Pè ME (1982) Male gametophytic selection in maize. Theor Appl Genet 63:249–254

Ottaviano E, Sari-Gorla M, Villa M (1988) Pollen competitive ability in maize: within-population variability and response to selection. Theor Appl Genet 76:601–608

Pfahler PL (1983) Comparative effectiveness of pollen genotype selection in higher plants In: Mulcahy DL, Ottaviano E (eds) Biotechnology and ecology of pollen. Elsevier Biomedical, New York, pp 361–367

Polowick PL, Greyson RI (1982) Anther development, meiosis and pollen formation in *Zea* tassels cultured in defined liquid medium. Plant Sci Lett 26:139–145

Rodriguez-Garay B, Barrow JR (1988) Pollen selection for heat tolerance in cotton. Crop Sci 28:857–859

Sacher RF, Mulcahy DL, Staples RC (1983) Developmental selection during self-pollination of *Lycopersicon* × *Solanum* F_1 for salt

tolerance of F_2. In: Mulcahy DL, Ottaviano E (eds) Pollen: biology and implications for plant breeding. Elsevier, New York Amsterdam. pp 329–334
Sari-Gorla M (1992) Effects of gametophytic selection on the genetic structure of populations. In: Cresti M, Tiezzi A (eds) Sexual plant reproduction. Springer-Verlag, Berlin, pp 151–159
Sari-Gorla M, Mulcahy DL, Gianfranceschi L, Ottaviano E (1988) Gametophytic selection for salt tolerance. Genet Agrar 42:92–93
Sari-Gorla M, Ottaviano E, Frascaroli E, Landi P (1989) Herbicide-tolerant corn by pollen selection. Sex Plant Reprod 2:65–69
Sari-Gorla M, Ferrario S, Gianfranceschi L, Villa M (1992) Herbicide tolerance in maize. Genetics and pollen selection. In: Ottaviano E, Mulcahy DL, Sari-Gorla M, Bergamini Mulcahy G (eds) Angiosperm pollen and ovules. Springer-Verlag, New York Berlin, pp 364–369
Sari-Gorla M, Ferrario S, Rossini L, Frova C, Villa M (1993) Developmental expression of glutathione S-transferase in maize and its possible connection with herbicide tolerance. Euphytica 67: 221–230
Schlichting CD, Stephenson AG, Small LE (1990) Pollen load and progeny vigor in *Cucurbita pepo*: the next generation. Evolution 44:1358–1372
Searcy KB (1992) Developmental selection in response to environmental stress. Evol Trends Plants 6:21–24
Searcy KB, Mulcahy DL (1985) The parallel expression of metal tolerance in pollen and sporophytes of *Silene dioica* (l.) Clairv., *Silene alba* (Mill.) Krause and *Mimulus guttatus* DC. Theor Appl Genet 69:597–602
Searcy KB, Mulcahy DL (1990) Comparison of the response to aluminium toxicity in gametophyte and sporophyte of four tomato (*Lycopersicon esculentum* Mill) cultivars. Theor Appl Genet 80:289–295
Shivanna KR, Sawhney VK (1993) Pollen selection for *Alternaria* resistance in oilseed brassicas: responses of pollen grains and leaves to a toxin of *A. brassicae*. Theor Appl Genet 86:339–344
Shultz A, Wengermayer F, Goodman HM (1990) Genetic engineering of herbicide resistance in higher plants. Crit Rev Plant Sci 9:1–15
Smith GA (1986) Sporophytic screening and gametophytic verification of phytotoxin tolerance in sugarbeet (*Beta vulgaris* L.). In: Mulcahy DL, Mulcahy Bergamini G, Ottaviano E (eds) Biotechnology and ecology of pollen. Springer-Verlag, New York Berlin, pp 83–88
Snow AA (1986) Pollination dynamics in *Epilobium canum* (Onagraceae): consequences for gametophytic selection. Am J Bot 73:139–151
Stauffer C, Benito-Moreno RM, Heberle-Bors E (1991) Seed set after pollination with in-vitro-matured isolated pollen on *Triticum aestivum*. Theor Appl Genet 81:576–580
Vasil IK (1990) The realities and challenges of plant biotechnology. Bio/Technol 8:296–301
Winsor JA, Davis LE, Stephenson AG (1987) The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigour in *Cucurbita pepo*. Am Nat 129:643–656
Zamir D, Gadish I (1987) Pollen selection for low-temperature adaptation in tomato. Theor Appl Genet 74:545–548
Zamir D, Vallejos EC (1983) Temperature effects on haploid selection of tomato microspores and pollen grain. In: Mulcahy DL, Ottaviano E (eds) Pollen: biology and implications for plant breeding. Elsevier, New York Amsterdam, pp 335–342
Zamir D, Tanksley SD, Jones RA (1982) Haploid selection for low-temperature tolerance of tomato pollen. Genetics 101:129–137