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E. Frascaroli · S. Galletti · P. Landi

Haplo-diploid gene expression and pollen selection for tolerance to acetochlor in maize

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Abstract The objectives of this research were to determine if genes controlling the reaction to the herbicide acetochlor in maize (*Zea mays* L.) are active during both the haploid and the diploid phases of the life cycle and if pollen selection can be utilized for improving sporophytic resistance. Pollen of eight inbred lines, previously characterized through sporophytic analysis for the level of tolerance to acetochlor, showed a differential reaction to the herbicide for *in vitro* tube length; moreover, such pollen reactions proved to be significantly correlated (r=0.786*, df=6) with those of the sporophytes producing the pollen. Pollen analysis of two inbred lines (i.e. Mo17, tolerant, and B79, susceptible) and their single cross showed that the *in vitro* pollen-tube length reaction of the hybrid was intermediate between those of two parents. An experiment on pollen selection was then performed by growing tassels of Mo17×B79 in the presence of the herbicide. Pollen obtained from treated tassels showed a greater tolerance to acetochlor, assessed as *in vitro* tube length reaction, than pollen obtained from control tassels. Moreover, the backcross [B79 (Mo17×B79)] sporophytic population obtained using pollen from the treated tassels was more tolerant (as indicated by the fresh weight of plants grown in the presence of the herbicide) than was the control backcross population. The two populations did not differ when grown without the herbicide. These findings indicate that genes controlling the reaction to acetochlor in maize have haplo-diploid expression; consequently, pollen selection can be applied for improving plant tolerance.

Key words Acetochlor tolerance · Gene expression
Pollen selection · *Zea mays*

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E. Frascaroli · S. Galletti · P. Landi (✉)
Institute of Agronomy,
University of Bologna,
via F. Re 6, I-40126 Bologna,
Italy

Introduction

In the last two decades, increasing attention has been devoted to the investigation of pollen characteristics in higher plants, the main reason being that a large amount of the genome (approximately 60%) has proven to be expressed during both the haploid and diploid phases of the life cycle (see, for a review, Ottaviano and Mulcahy 1989). The haplo-diploid gene expression implies that selection exerted on microspores, pollen grains and/or microgametophytes can lead to changes in allelic frequencies, the effects of which can also be detected on the resultant sporophytic generation. Male gamete selection exhibits several advantages over the conventional selection procedures applied on sporophytes: (1) the unfavorable recessive alleles are not masked by the dominants, (2) the genotypic population size is much smaller (for n segregating loci, the genotypic population size is 2^n instead of 4^n) and (3) very high selection pressures can be applied because large male gamete populations can be easily and inexpensively handled. Selection on the haploid units has been successfully applied for a number of traits (Ottaviano and Mulcahy 1989) including tolerance to herbicides (Sari Gorla et al. 1989; Sari Gorla et al. 1994).

Acetochlor (2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methyl-phenyl)-acetamide) is an herbicide of the chloroacetamide group that controls most weeds competing with maize. This herbicide acts in the area of seed germination and seedling growth of susceptible species (Jaworski 1969), but its crop selectivity is not satisfactory. A previous study (Landi et al. 1990) has shown that there is a large variability in maize with respect to level of tolerance, suggesting that improvement of the trait would be a worthwhile objective. In order to increase breeding effectiveness, conventional sporophytic selection could be integrated with pollen selection (Pfahler 1983), providing that pollen and sporophytic reactions to the herbicide are correlated and that the haplo-diploid gene expression is the main source of this correlation.

This study was carried out in order to: (1) investigate if the variations in reaction to acetochlor are also detectable at the pollen level and determine if such variations are consistent with those previously detected among sporophytes; (2) gather information on the type of gene action affecting the level of pollen tolerance and compare it with the type of gene action affecting the corresponding sporophytic trait and (3) apply pollen selection for the trait of tolerance to acetochlor and evaluate the responses in both the haploid generation and the resultant sporophytes.

Materials and methods

Variation in the pollen reaction to acetochlor

In our previous study (Landi et al. 1990), the reaction of 18 inbred lines to acetochlor was investigated in the field, with the plants being grown at 0, 2.5 and 5.0 kg (active ingredient) ha^{-1} of herbicide. On the basis of the percentage of uninjured plants averaged over the two herbicide rates, 4 inbred lines were identified as being tolerant (Mo17, Va85, Lo876, B84) and 4 as being susceptible (Va26, A634, WF9, B79). Plants of these lines were grown in 1990 adopting the usual field practices; during shedding, pollen was collected from each line on 4 different days, each day representing a replication. Pollen grains from each collection were inoculated onto a solid medium (Cheng and Freeling 1976) containing one of three levels [0 (control), 9 or 18 $\mu l\ l^{-1}$] of acetochlor, incubated for 3 h at 27 °C in the dark, fixed with Farmer's liquid and utilized for measuring pollen-grain germination (on 200 grains) and tube length (on 30 tubes). The effect of acetochlor on both traits was expressed as the ratio (expressed as percentage) between each herbicide treatment and corresponding control in order to overcome possible biases due to scale effects.

Analysis of gene action

One tolerant (Mo17) and one susceptible (B79) inbred line were further investigated together with their hybrid (Mo17×B79). Plants were grown in the greenhouse during the 1990–1991 winter, and pollen was collected from each genotype on 6 different days (replications). For each collection, pollen was evaluated for the *in vitro* reaction to acetochlor at rates of 0 (control), 9, 18, 27, or 36 $\mu l\ l^{-1}$. Measurements of pollen traits and assessment of herbicide reaction were made as previously described.

Pollen selection and evaluation of responses

Selection was applied on the pollen population of the hybrid Mo17×B79 during microspore maturation following the procedure described by Sari Gorla et al. (1989). Plants were field grown in 1991 and tassels were cut about 2 weeks before anthesis and placed into an artificial liquid medium (Polowick and Greyson 1982) supplied with acetochlor at two different concentrations, i.e. 0 and 200 $\mu l\ l^{-1}$. This procedure was adopted since chloroacetamides are translocated within the plant through the apoplastic system following the same pathway as water (Obrigawitch et al. 1980). The 200 $\mu l\ l^{-1}$ concentration was chosen on the basis of preliminary experiments in which we compared the effects on the *in vitro* reaction of pollen produced by tassels treated with different herbicide concentrations. Hereafter, the two pollen populations obtained from tassels grown either in the presence or absence of acetochlor will be indicated as non-selected and selected pollen populations, respectively.

A portion of these two populations was utilized for studying the response of pollen to this selection. Pollen samples were collected from treated and untreated tassels for 12 days (replications). On each date, pollen grains from each treatment were inoculated on a solid medium containing one of five concentrations [0 (control), 18, 27, 36 and 45 $\mu l\ l^{-1}$] of acetochlor. The measurements of pollen traits and assessment of the reactions were made as previously described.

In addition, pollen populations from each of the two treatments were used for making backcrosses [B79(Mo17×B79)] to the susceptible parent. Hereafter, the backcrosses obtained by the non-selected and selected pollen populations will be indicated as NBC (non-selected backcross) and as SBC (selected backcross). To evaluate the response to pollen selection at the sporophytic level, NBC and SBC were compared in the greenhouse in the presence and absence of the herbicide (at a rate corresponding to 5 kg a.i. ha^{-1}). Pregerminated seeds were sown in trays containing sand, and plants were grown with 15 h light at 25±2 °C and 9 h dark at 13±1 °C. A randomized complete block design with 32 replications (trays) was used for both trials. Each experimental unit consisted of 6 plants. Twenty-one days after sowing plants were cut at the soil level and shoot fresh weight was measured.

Results and discussion

Variation in the pollen reaction to acetochlor

The analyses of variance (not shown) indicated that the difference between the two acetochlor concentrations was significant ($P \leq 0.05$) for tube length only and not for germination. For tube length the reaction was positive at 9 $\mu l\ l^{-1}$ (107%) and negative at 18 $\mu l\ l^{-1}$ (97%). No significant interaction acetochlor rates × inbred lines was detected for either traits: therefore, only the mean reaction of each inbred over both acetochlor rates is given in Table 1.

With germination, differences among inbred lines within tolerant and susceptible groups were significant ($P \leq 0.05$). A negligible difference was found between the two groups. Furthermore, no significant relationship was noted between the reaction for this trait and the reaction assessed at the sporophytic level, as percentage of uninjured plants, in our previous study (Landi et al. 1990).

Table 1 Pollen reaction to acetochlor of 8 maize inbred lines previously characterized for tolerance and susceptibility at the sporophytic level. Reaction is expressed as percentage of control and as the mean of 9 and 18 $\mu l\ l^{-1}$ of acetochlor in the *in vitro* medium (*NS* not significant)

Tolerance level	Inbred line	Germination (%)	Tube length (%)
Tolerant	B84	107	124
	Mo17	106	120
	Lo876	94	107
	Va85	140	102
	Mean	112	113
Susceptible	Va26	103	97
	A634	136	95
	B79	89	91
	WF9	92	79
	Mean	108	90
LSD (0.05) [a] 1		31	NS
2		NS	11

[a] LSD for comparison among lines within each group (1) and between tolerant and susceptible groups (2)

A highly significant difference was found between tolerant and susceptible groups for the reaction concerning tube length (113% vs. 90%), while the differences among lines within each group were non-significant. Moreover, the relationship between this reaction and the one previously assessed at the sporophytic level proved to be significant (r=0.786*, df=6). This finding is noteworthy because it indicates that more than 60% of the plant variation for reaction to acetochlor in the field could be explained by pollen-tube length analysis.

A good accordance between pollen and plant performances was also found in previous studies on the reaction to such toxic substances as salt (Sacher et al. 1983), heavy metals (Searcy and Mulcahy 1985 a, b, 1990), pathotoxins (Bino et al. 1988; Laughnan and Gabay 1973; Hodgkin 1990; Shivanna and Sawhuey 1993) and herbicides (Smith 1986; Frascaroli et al. 1992; Richter and Powles 1993). Such an accordance, however, does not necessarily imply that the involved genes are active in both haplo-diploid phases. This relation could also be due to genes active only in the diploid phase and able to extend their influence into the haploid. Because only homozygous genotypes were tested, the present analysis did not allow us to assess which of the two causes was responsible for the detected relationship. Indeed, the ability to distinguish between these two causes is of great importance because only in the case of haplo-diploid gene action can the selection among haploid units (pollen grains and/or microgametophytes) produced by the same heterozygous plant influence the resulting sporophytic generation (Mulcahy and Mulcahy 1975).

Analysis of gene action

Acetochlor exerted a negative effect (significant at $P \leq 0.05$) on *in vitro* pollen-grain germination in the two parental lines and their hybrid, and no genotype × rate interaction was found (data not shown). For tube length, in contrast, a highly significant effect of the herbicide and a highly significant genotype × rate interaction were detected (Fig. 1). Consistent with the findings of the previous analysis, Mo17 proved to be tolerant, given its moderate reaction at increasing herbicide concentrations, and B79 proved to be rather susceptible, showing a marked decline in pollen-tube length, especially at higher herbicide concentrations. The gametophytic reaction of the F_1 was very close to the parental mean at all concentrations tested. This finding is in clear contrast with that of the previous study performed at the sporophytic level (Landi et al. 1990), where the investigated hybrids largely exceeded their parental means for tolerance to acetochlor. A possible cause accounting for this discrepancy is that acetochlor reaction is controlled by genes with important dominance effects and active in both phases. In fact, the intra-locus interaction can not occur in the haploid units, so that the gametophytic performance of the F_1 is expected to be intermediate between those of the two parents, unless the trait is also controlled by complex loci interactions (epistasis).

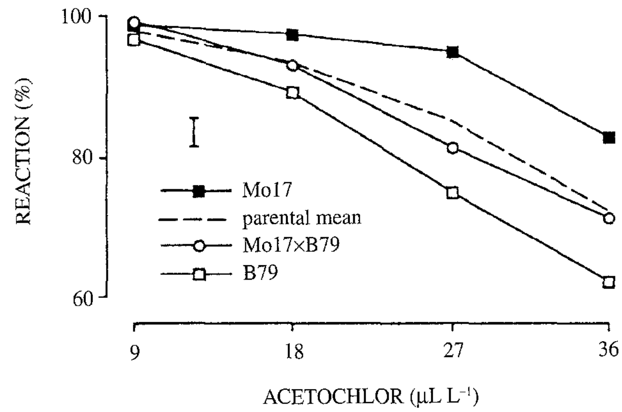


Fig. 1 Pollen-tube length reaction, as % of control, of Mo17, B79 and their F_1 at increasing acetochlor concentrations. The *vertical bar* indicates standard error of the mean

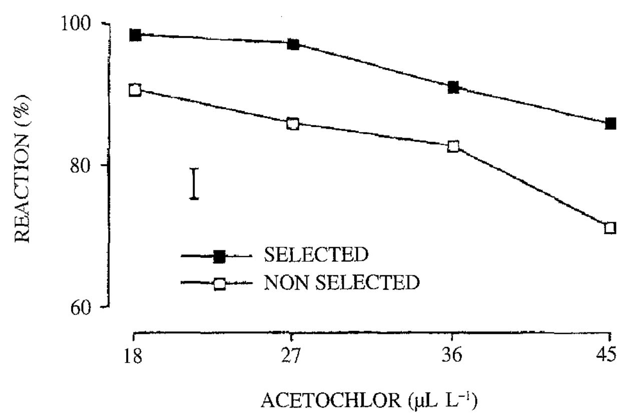


Fig. 2 Pollen-tube length reaction, as % of control, of selected and non-selected pollen populations at increasing acetochlor concentrations. The *vertical bar* indicates standard error of the mean

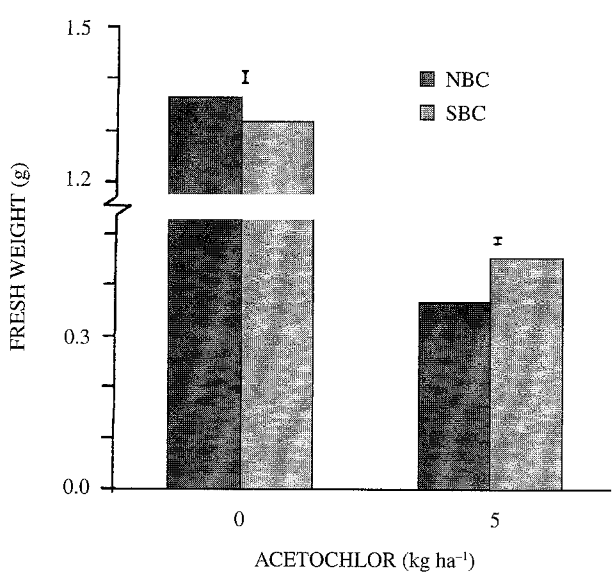
Pollen selection and evaluation of responses

The effects of acetochlor on the pollen-grain germination were quite similar for the two non-selected and selected pollen populations, being rather mild at the lower concentrations and markedly negative at the higher ones (data not shown). The two populations showed different performances with respect to the herbicide reaction for pollen-tube length (Fig. 2). In fact, the selected population exhibited a higher degree of tolerance than the non-selected one at all concentrations tested. The interaction of population × herbicide concentrations was not significant, indicating that the differences among populations for the tolerance level did not appreciably change from one concentration to another. The superior performance for tube length of the selected pollen population throughout the concentrations tested suggests that pollen selection was able to increase the frequencies of alleles conferring tolerance to the herbicide at the gametophytic level. In fact, our selection was carried out on the haploid units produced by single heterozygous plants all having the same genotype (single cross of two inbred lines), so that the observed gametophytic response cannot be related to differences among the genotypes of the pollen mother plants. However, the herbicide could have induced an accomodation by activating genes in the sporophyte (tassel), whose products could then have moved to the pollen grains. In fact, chloroacetamides can stimulate the synthesis of enzymes involved in the detoxification of such herbicides (Shimabukuro 1985). This is the same effect as that shown by certain safeners that have structural similarity to chloroacetamides (Yenne and Hatzios 1990 a, b) and which act inducing the enhancement of detoxification in safened plants (Hatzios 1989).

In order to gain a better insight into the effectiveness of pollen selection and into the possible haplo-diploid expression of the genes involved, NBC and SBC populations were evaluated at the sporophytic level. The comparison between the two populations provided different results depending on whether the plants were grown in the presence or absence of the herbicide (Fig. 3). In the absence of the herbicide the mean values of the two populations were very close to one another, the SBC being just 3.3% smaller than the NBC (the difference was non-significant). On the contrary, when plants were grown in the presence of acetochlor, the mean value of the SBC population largely exceeded (20.5%) that of the NBC, and the difference was highly significant.

As demonstrated by Sacher et al. (1983), pollen selection for tolerance to a toxic substance can lead to changes in allelic frequencies at loci that control the vigor per se, so that the resultant selected sporophytes exceed the non-selected ones irrespective of the fact of whether they are grown in the presence or absence of that factor. This possible bias, however, can be dismissed from our data, given the close similarity between NBC and SBC performances in the absence of acetochlor. Moreover, even in case an accomodation occurred and some products moved to the pollen grains, it is reasonable to assume that they could not affect the performance of the subsequent diploid generation. Therefore, the greater tolerance to acetochlor of SBC, as compared with NBC, can be accounted for only by assuming that the herbicide treatment applied during microspore maturation was effective in changing allelic frequencies at loci that are expressed not only in the haploid phase but also in the diploid. This hypothesis can also account for the gametophytic-sporophytic relationship observed with the 8 inbred lines as well for the lack of heterosis showed by the Mo17×B79 gametophytic reaction.

Fig. 3 Shoot fresh weight (21 days after sowing) of the non-selected (*NBC*) and selected (*SBC*) backross populations in the presence and absence of acetochlor. The two *vertical bars* indicate the standard error of the mean of the two populations in the presence and absence of acetochlor



The ability of the plant to metabolize chloroacetamide herbicides at a rate sufficient to prevent irreversible damage has been proposed as an important mechanism determining the level of tolerance (Shimabukuro 1985). Leavitt and Penner (1978) reported that these herbicides may form glutathione (GSH) conjugates, and the role of GSH as the major initial metabolite of acetochlor has been demonstrated by Breaux (1986). The conjugation of acetamide herbicides can be catalyzed by glutathione-*S*-transferase (GST), which was found to have a higher activity in the tolerant genotypes than in the susceptible ones (Cottingam and Hatzios 1992). GSTs capable of catalyzing the reaction have been reported in etiolated maize tissues (Mozer et al. 1983) and in maize cell cultures (Edwards and Owen 1986; Gronwald 1989). Moreover, Sari Gorla et al. (1993) have demonstrated that genes coding for GST are active in the haploid phase. Therefore, all such findings do suggest that our pollen selection may have been effective in changing the allelic frequencies at loci controlling the GST metabolism.

In conclusion, our data indicate that at least a portion of the genes controlling the reaction to acetochlor is expressed in both the haploid and diploid phases and that pollen selection can be utilized for improving the trait of the resultant sporophytes. In a breeding program, pollen selection can be effectively applied when selfing heterozygous plants or making backcrosses to the recurrent susceptible parent; moreover, to increase efficiency, a pollen bioassay could be adopted for preliminary and rapid screening of the resulting progenies. The possibility of integrating pollen and sporophytic selection is very desirable for a trait like herbicide tolerance, which can have quite a low heritability because of the complex interaction among genotypes, herbicide rates and environments that can be often found in the field investigations (Wych and Schoper 1987).

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