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The extent of gametophytic-sporophytic gene expression in maize

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Summary. To determine the extent of gametophytic gene expression and the type of transcription, haploid or haplo-diploid, of the genes, isozymes were used as genetic markers. Fifteen enzymatic systems, including thirty-four isozymes, were studied. The determination of the type of expression of genes coding for multimeric enzymes was based on the comparison of electrophoretic patterns of pollen and of sporophytic tissues from plants heterozygous for electrophoretic mobility: if gene expression in pollen is of a gametophytic (haploid) origin, pollen, unlike the sporophyte, would reveal only the parental homomultimeric bands. The enzymes analyzed can be grouped in three categories according to type of gene expression: i) enzymes present in both pollen and sporophyte, coded by the same gene with haplo-diploid expression; ii) enzymes controlling analogous functions in pollen and sporophyte, coded by different genes, expressed in only one of the two phases; iii) enzymes present in two or more forms in the sporophyte and only in one form in the gametophyte. The data allow the proportion of haplo-diploid gene expression in the loci examined to be estimated at 0.72; 0.22 and 0.06 being the proportions attributable to the sporophytic and gametophytic domains, respectively.

Key words: Maize pollen – Gene expression – Isozymes – Gametophytic-sporophytic genetic overlap

Introduction

The many metabolic activities that have been detected in the male gametophyte of higher plants (Mascarenhas 1975; Brewbaker 1971; Loewus and Labarca 1973; Mascarenhas et al. 1984) indicate that this stage of the life cycle involves complex functions which are not confined to the transmission of male gametes to the embryo sac. In fact, if a significant part of the physiological processes controlling pollen development, pollen germination and pollen tube elongation are under the control of the haploid gametophytic genome, it is to be expected that the genetic variability expressed in the pollen population of a single heterozygous plant would lead to important selection effects due to pollen competition (Pfahler 1975, 1983). This expectation is based on the assumption that not only the genetic variability expressed by the male gametophytic population is of haploid origin, but also that several genes expressed in the gametophytic phase are equally expressed in the sporophyte ("haplo-diploid" gene expression or gametophytic-sporophytic genetic overlap).

Clear indications that this phenomenon exists and is not restricted to a small number of genes are obtained when complex traits, representing gametophytic and sporophytic vigour, are considered. The variability found in the pollen population of F_1 and of inbred generations is that expected in the case of gametophytic gene expression (Sari Gorla et al. 1975; Johnson et al. 1976); pollen competitive ability, mainly due to pollen tube growth rate, is correlated with sporophytic traits (Mulcahy 1971; Ottaviano et al. 1980); gametophytic selection affects the sporophytic generation (Ottaviano et al. 1982; Zamir et al. 1982; Sacher et al. 1983).

However, to estimate the extent of gametophytic gene expression and of the haplo-diploid overlap, precise genetical tests must be applied to discriminate between pollen traits controlled by the haploid genome of the pollen itself and those due to the sporophyte. The importance of such tests is stressed by the fact that in maize several recessive chromosomal male sterile mutants have been detected which act at different stages of pollen development (Albertsen and Phillips 1981), but are under sporophytic control (Majodelo et al. 1966).

Direct evidence of gametophytic gene expression of single genes affecting sporophytic traits has been obtained in maize in waxy (Nelson 1962), *adh-1* (Freeling 1976) and *ae* (Moore

and Creech 1972) and in *Brassica campestris* for *β-gal* (Singh et al. 1982) on the basis of specific pollen staining techniques. However, only by using isoenzymes as genetic markers has it been possible to carry out comprehensive studies based on large numbers of genes. The first account based on this approach was given by Tanksley et al. (1981) in tomato.

In this paper, the data obtained from the analysis of haplo-diploid gene expression of genes controlling a large number of isoenzymes in maize are reported. The results are discussed with respect to the evolutionary significance of the variability expressed in the male gametophytic genome.

Materials and methods

The type of expression, haploid or diploid, of genes coding for multimeric enzymes was established by comparing the electrophoretic pattern of gametophytic and of sporophytic tissues from plants heterozygous for electrophoretic mobility, following a method first proposed by Brewbaker (1971) and Mascarenhas (1975). For example, in a dimer, if the expression is haplo-diploid, the sporophyte reveals three bands, two homodimers and one heterodimer, while pollen is expected to produce only the two homodimeric parental bands since the transcription occurred after meiosis when the single grains contained information for only one of the two monomeric forms. The reliability of the method was confirmed using partially diploid pollen for ADH-1 and GOT-1 (Frova et al. 1983) and for PHI (this laboratory, unpublished data). When the expression is only gametophytic, the absence of the corresponding band in the sporophytic zymogram, or the presence of a different activity region, without mobility concordance between sporophytic and gametophytic variants, is to be expected. Thus, for each enzyme considered, the analysis included the parental inbred lines with different mobility and the corresponding F_1s; a sporophytic tissue and pollen of each genotype were tested.

Fifteen enzymatic systems were analyzed. They included 34 isozymes (see Table 1): ACP (Acid Phosphatase), ADH (Alcohol Dehydrogenase), CAT (Catalase), ENP (Endopeptidase), β-GLU (β-Glucosidase), GOT (Glutamate-Oxalacetate-Transaminase), GDH (Glutamate Dehydrogenase), IDH (Isocitrate Dehydrogenase), MDH (Malate Dehydrogenase), PGM (Phosphoglucomutase), 6-PGD (6-Phosphogluconate Dehydrogenase), PHI (Phosphohexose Isomerase), SOD (Superoxide Dismutase), UDPGpp (UDPG Pirophosphorilase), INV (Invertase).

The choice of the parental lines, carrying electrophoretic variants for the different enzymes, was made in most cases on the basis of information from Stuber and Goodman (1982). The rare variants of ACP-2, CAT-1, CAT-2, GDH, SOD, UDPGpp and INV were searched for by screening our collection lines.

Scutellum and coleoptile were used as sporophytic sources of enzymes. For gametophyte analysis, about 100 mg of pollen (fresh or frozen at −80 °C) was homogenized in 1 ml of buffer and sand, to help break down the pollen wall.

The extraction buffers were: 1. Tris-HCl 0.05 M or 0.01 M pH 8.0, DTT 10 mM, EDTA 0.2 M for ADH, β-GLU, IDH, PGM, 6-PGD, PHI, UDPGpp; 2. Glycylglycyne 0.025 M pH 7.4 for ENP, MDH, CAT, SOD; 3. Phosphate 0.1 M pH 7.0, DTT 10 mM, EDTA 0.2 M for GDH and INV; 4. Distilled water for ACP-2, GOT.

Homogenates were centrifuged at 12,000 rpm for 15 min at 4 °C and the supernatant was used. For ACP, β-GLU, IDH, 6-PGD, and PHI, the extract was directly absorbed onto Whatman 3 MM paper wicks for horizontal gel electrophoresis in a L-hystidine-citrate buffer pH 6.5 starch gel system (Goodman et al. 1980). ADH, CAT, ENP, β-GLU, GOT, GDH, PGM, 6-PGD, SOD, UDPGpp and INV were analyzed by means of Disc-PAGE 7.5% in a Tris-glycyne, Tris-HCl buffer system (Andrews 1981). MDH was subjected to isoelectric focusing, pH 3.5–9.5 (Andrews 1981).

After being allowed to run to completion, the gels were incubated in their staining solution at 37 °C for at least 1 h. The staining procedure used for each enzyme was one of those already published, mainly by Scandalios (1969); Shaw and Prasad (1970), and Goodman et al. (1980). The type of expression of *bz*, which codes for UDPG-flavonoid-glucosyltransferase (Larson and Coe 1976), was verified by means of in situ staining of single grains. Since quercetin, which is fluorescent, is transformed into isoquercetin, non-fluorescent, by the enzyme, observation by UV microscope allows the presence or absence of enzymatic activity to be revealed.

Results

As to the type of gene expression, the enzymes analyzed fall into three main categories.

The first includes enzymes which are present in both pollen and sporophyte, coded by the same gene with haplo-diploid expression. For dimeric enzymes the observed pattern is exemplified in Fig. 1: PHI forms three bands in the sporophyte; only two in pollen from F/S plants. The same type of data was obtained for ADH-1, GOT-1, GOT-2, IDH, 6-PGD-2 and one MDH isozyme. For a tetramer such as CAT, a large band in an intermediate position relative to the F and S positions was observed in F/S sporophytes, according to the expected five bands with a 1 : 4 : 6 : 4 : 1 ratio for the different tetrameric types. Pollen from the same genotype showed only two bands. In maize at least three catalases have been described (Tsaftaris and Scandalios 1983), expressed in different tissues and developmental stages. Only one form is present in pollen, probably CAT-1. In fact, mobility concordance between pollen and CAT-1 variants was consistently

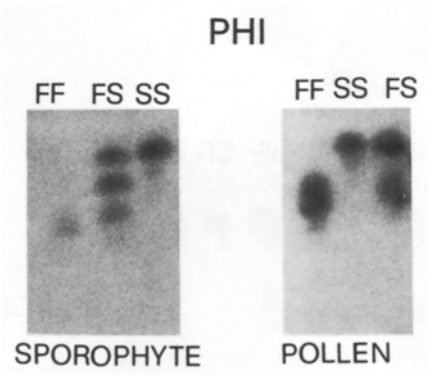

Fig. 1. Zymograms of PHI extracted from scutellum and pollen. Plant genotype is indicated

observed; only CAT-1 F/S genotypes showed two bands in pollen (Fig. 2), while CAT-2 and CAT-3 heterozygotes (but homozygous for CAT-1) revealed a single band in pollen. In the case of GDH-1, the active molecule is probably an examer (Pryor 1974). The hybrid sporophyte shows a large intermediate band, while pollen from the same genotype shows a simple overlapping of the two parental patterns, as expected for haplo-diploid transcription. For ACP-2 a single band was observed in parental sporophytic genotypes and two in the hybrids, thus suggesting a monomeric form of the enzyme. Pollen produced two and four bands respectively, however parental and hybrid bands in pollen reflected the mobility of the sporophytic pattern. This mobility concordance was verified in twenty inbred lines of different genetic origin. The appearance of four bands in pollen from hybrid plants (which showed two bands in the sporophyte) was always revealed. Therefore, it seems that the same gene is involved while the type of expression (haploid or diploid) has yet to be defined (Fig. 3).

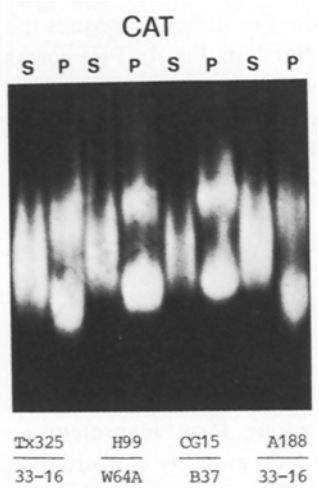

Fig. 2. CAT pattern in one-day-old scutellum (*S*) and pollen (*P*) extracts of different hybrid F/S plants. Plant genotype is indicated

The second category includes enzymes controlling analogous functions in pollen and sporophyte but coded by different genes, each expressed in only one of the two phases. Only β-GLU (Sari Gorla et al. 1983) and probably INV were found to fall into this category.

Finally, as to 6-PGD, at least two forms are active at the same sporophytic stage, while pollen displays only one (6-PGD-2 in this specific case).

The results obtained are summarized in Table 1, where the presence (+) or absence (–) of isozyme activity in the sporophytic tissues analyzed and in pollen is reported; the result of the test for haploid expression is also indicated.

Cases are indicated as "not detected" for different reasons. Some relate to monomeric forms (PGM, ENP, ACP-2), and detection of the relative type of expression if still in progress by means of a more complex procedure than the one here described. In other cases the enzyme proved to be invariant: about 200 genotypes from different sources were investigated in search of UDPGpp variants, without success.

The type of expression of Bronze was determined by direct observation of the grains under UV light. Pollen from *BzBz* plants was non-fluorescent, from *bzbz* plants strongly fluorescent, and pollen from *Bzbz* plants did not segregate, but was homogeneously weakly fluorescent. Thus the enzyme is considered to be of sporophytic origin.

Discussion

The estimate of the amount of gametophytic-sporophytic genetic overlap can be based on the group of isozymes present in pollen and tested for haploid expression. Thirteen enzymes can be included in this group (Table 1). As all these clearly revealed haploid expression and eleven out of thirteen were also found in the sporophyte, it follows that a proportion of 0.846 of the enzymes found in the pollen revealed haplo-diploid gene expression. Considering that a proportion of 0.853, i.e. 29 out of 34, of the enzymes are found

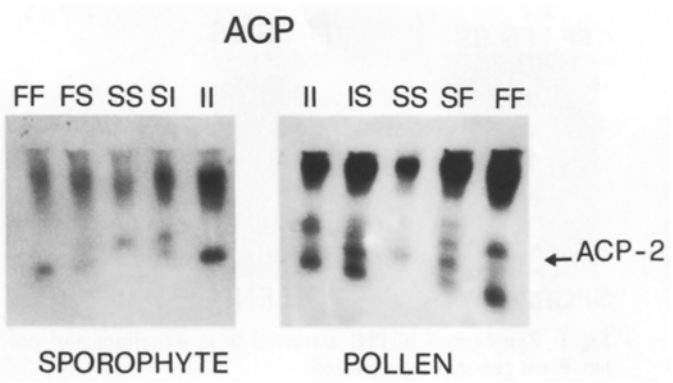

Fig. 3. Zymograms of ACP-2 in scutellum and pollen. Plant genotype is indicated: *F*=fast, *S*=slow, *I*=intermediate mobility

Table 1. Expression of genes controlling enzymatic proteins in maize

Enzyme	Sporophyte	Gametophyte	Haploid expression
ACP-1	+	+	+
ACP-2	+	+	nd
ACP-3†	+	+	nd
ADH-1	+	+	+
ADH-2†	+	+	nd
CAT-1	+	+	+
CAT-2	+	–	
CAT-3	+	–	
ENP	+	+	nd
βGLU-1	+	–	
βGLU-2	–	+	+
GOT-1	+	+	+
GOT-2	+	+	+
GOT-3	+	+	nd
GDH-1	+	+	+
IDH-1	+	+	+
IDH-2	+	+	+
MDH-1	+	+	nd
MDH-2	+	+	nd
MDH-3	+	+	nd
MDH-4	+	+	+
MDH-5	+	+	nd
PGM-1	+	+	nd
PGM-2	+	+	nd
6-PGD-1	+	–	
6-PGD-2	+	+	+
PHI	+	+	+
SOD-1	+	+	nd
SOD-2	+	+	nd
SOD-3	+	+	nd
SOD-4	+	+	nd
UDPGpp	+	+	nd
INV-1	+	–	
INV-2	–	+	+

nd: not detected
+ : data from Chandlee and Scandalios 1982

both in the sporophyte and in the gametophyte, the extent of haplo-diploid gene expression can be estimated at $0.853 \times 0.846 = 0.72$, while 0.22 and 0.06 are the proportions attributable to the sporophytic and gametophytic domains, respectively.

There is a remarkable correspondence between these data, those obtained using the same approach in tomato (Tanksley et al. 1981), and the recent results obtained by Willing and Mascarenhas (1984) and Willing et al. (1984), who compared mRNA of pollen grains with that of vegetative tissues. This analysis revealed that in *Tradescantia paludosa* pollen about 20,000 sequences are transcribed, and that about 64% of these are also found in sporophytic tissues. In maize this proportion was close to 85%.

Pollen isozyme specificity was found in only two cases, i.e. β-GLU-2 and INV-2. However, not all sporophytic tissues were explored and consequently haplo-diploid expression for these enzymes also cannot be ruled out. On the other hand, as pointed out by Heslop-Harrison (1979), despite expectation of the evolution of specific gametophytic functions in the Angiosperms, this specificity is rarely confirmed by experimental findings. In maize, several mutant genes affect the male gametophytic generation (chromosomal male sterility, gametophytic factors, cytoplasmic male sterility restorers, starch metabolism genes), but only one, *RF3*, seems to be specifc for the gametophytic generation (Laughnam and Gabay 1978).

Only in one case (UDP-flavonoid-glucosyltransferase) was a pollen trait controlled by the sporophyte. However, the enzyme is concerned with exine traits, which are determined by diploid maternal tissue.

It is important to note that the majority of the enzymes considered in this work are involved in the control of basic physiological processes. At this moment information is lacking concerning the comparison of entire metabolic pathways, showing that all steps of a given physiological function are controlled by genes having haplo-diploid expression. It is likely that in the sporophyte, where adjustment to different physiological processes is required, specific genes are involved in the control of functions found also in the gametophyte. For instance, in maize, if one considers endosperm starch mutant genes well characterized as to their enzymatic base, such as *waxy, amilose extender, brittle-2, shrunken-1* and *-2,* only two out of the five reveal haploid expression (Nelson 1978). Similar indications have been found in *Arabidopsis* for defective kernel mutants (Meinke 1982).

In conclusion, even if the type of approach used in this work permits exploration of only a part of the existing genetic variability – since it does not reveal differences in enzyme activity or differences in proteins other than enzymatic – the amount of data available points to a considerable independence of the gametophytic genome and seems to be sufficient to support the theory that gametophytic selection plays a major role in the evolution of Angiosperms (Mulcahy 1979). In fact, considering the large amount of genetic variability due to genes with haplo-diploid expression, the haploid state and the size of the gametophytic population (much larger than the sporophytic one), gametophytic selection is a mechanism by which:

a) a considerable part of the genetic load produced by recombination can be eliminated at a cost which is not incompatible with the biological feature of the species.
b) a high evolutionary rate (allelic substitution in a unit of time) can be attained without a drastic reduction of sporophytic fitness.

However, several aspects of the problem remain to be clarified, such as the real amount of gametophytic selection in nature, and the role of pollen-type inter-

action. As to the latter, in maize, pollen tube and style show physiological complementation (Linskens and Pfahler 1977) and, on the average, self pollen behaves better than cross pollen (Ottaviano et al. 1983). Further analysis in this direction should lead to a more complete evaluation of the biological significance of the phenomenon, which, artificially controlled, could be a very efficient tool for the manipulation of the genome of crop plants (Zamir 1983).

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