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Overlap of gametophytic and sporophytic gene expression in barley

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Summary. The overlap of gametophytic and sporophytic gene expression in barley was studied by means of enzyme electrophoresis. Of the isozymes found, 60% were expressed in both gametophyte and sporophyte, 30% were sporophyte specific, and 10% were gametophyte specific. A considerable amount of the barley genome is thus potentially amenable to gametophytic selection. The estimated sizes of the common, sporophytic and gametophytic domains in barley gene expression correspond with the estimates obtained in other plant species.

Key words: Barley – Pollen – Isozymes – Gametophytic-sporophytic gene expression

Introduction

The issue of gene expression in pollen is important in determining the extent to which selection on the gametophytic generation can influence the sporophyte. If the genes expressed in the gametophyte are completely unrelated to sporophytic gene expression, competition between gametophytes will only be expected to have transient, linkage-mediated effects on the sporophyte (Hartl 1970; Mulcahy and Mulcahy 1975). Gametophytic selection will cause gene frequency changes but these changes will be uncorrelated with the quality of the resultant sporophyte (Mulcahy and Mulcahy 1975). If, however, a significant overlap of gametophytic and sporophytic gene expression exists, there will be potential for selection on gametophytic characters to have positive correlated effects on the sporophyte (Mulcahy 1971, 1979).

Evidence for gametophytic gene expression has been obtained from studies of the waxy gene, alcohol dehydrogenase and glutamate-oxaloacetate transaminase in maize (Demerec 1924; Schwartz 1971; Frova et al. 1983), β-galactosidase in oilseed rape (Singh and Knox 1984), phosphoglucose isomerase in *Clarkia* (Weeden and Gottlieb 1979), mRNA synthesis in *Tradescantia* (Willing and Mascarenhas 1984) and from electrophoresis of single pollen grains (Mulcahy et al. 1979; Mulcahy et al. 1981). Other examples are provided by gametophytic self-incompatibility (de Nettancourt 1977), gametophytic factors (Bianchi and Lorenzoni 1975) and embryo-lethal mutants (Meinke 1982).

In recent years electrophoretic studies of isozymes have demonstrated an extensive overlap of gametophytic and sporophytic gene expression in several plant species (Tanksley et al. 1981; Sari Gorla et al. 1986; Rajora and Zsuffa 1986; Mulcahy and Mulcahy 1987). Moreover, positively correlated effects of pollen competition with sporophytic characters such as seed and seedling weight, seedling germination time and metal tolerance have been detected (Mulcahy 1971, 1974; Mulcahy and Mulcahy 1975; Searcy and Mulcahy 1985).

Since the genetic basis and tissue distribution of many isozymes in the sporophyte have previously been determined (Brown 1983; Pedersen and Simonsen 1987), barley is well suited for a study of the overlap between gametophytic and sporophytic gene expression. Several studies have revealed the existence of gametophytic selection in barley (Kahler et al. 1975; Clegg et al. 1978; Wagner and Allard 1985), but it remains to be established the extent to which the selected genetic variation is a product of gametophytic gene expression. The results will be compared to the estimated overlaps obtained in other species.

Materials and methods

Plant material

The following cultivars (sources named in brackets) of *Hordeum vulgare* L. were used: Brita (W. Weibull AB, Sweden), Claret (Nickerson RPB, England), Gula (Abed, Denmark), and Gunhild (Pajbjergfonden, Denmark). In addition, plants of *Hordeum bulbosum* L. from three different geographical locations in Greece were used (Scandinavian Hordeum Accession numbers): 50 km SW of Patrai (630), Lake Ioannina (427), and 5 km S of Ioannina (391).

Sample preparation

Seeds were germinated on moist filter paper for five days and planted in soil. The plants were grown in a greenhouse and fertilized with commercial fertilizer (Hornum).

Germinating endosperm, seedling roots, the first leaf, the flag leaf, pollen, anthers (containing residual pollen) and pistils were sampled for electrophoresis. The sample buffer composition was: 0.033 M maleic acid, 0.053 M NaOH, 0.033 M Tris, 0.004 M Titriplex II, 0.003 M $MgCl_2$–6 H_2O, and pH 7.4. 50 µl sample buffer was used for all tissues.

The pollen samples were prepared as follows. The anthers from one ear were removed and placed in Eppendorf tubes containing 50 µl of sample buffer. The pollen grains were squeezed out by pressing the anthers gently with a preparation needle. The partly empty anthers were then transferred to a second Eppendorf tube. All tissue samples were frozen at −60 °C for later use.

Electrophoresis

The following enzymes were assayed: Aspartate aminotransferase (AAT, GOT, E.C.2.6.1.1), acid phosphatase (ACP, E.C. 3. 1. 3. 2), aconitase (ACO, E.C.4.2.1.3), alcohol dehydrogenase (ADH, E.C.1.1.1.1), aminopeptidase (AMP, E.C.3.4.11.1), endopeptidase (ENP, E.C.3.4.22.?), esterase (EST, E.C.3.1.1.?), fructose-1,6-diphosphatase (F-1,6-DP, E.C.3.1.3.11), glucose-phosphate isomerase (GPI, E.C.5.3.1.9), hexokinase (HK, E.C. 2.7.1.1), isocitrate dehydrogenase (IDH, E.C.1.1.1.42), malate dehydrogenase (MDH, E.C.1.1.1.37), NADH dehydrogenase (NDH, E.C.1.6.99.3), peroxidase (PRX, E.C.1.11.1.7), phosphogluconate dehydrogenase (PGD, E.C.1.1.1.43), phosphoglucomutase (PGM, E.C.2.7.5.1), shikimate dehydrogenase (SDH, E.C.1.1.1.25), triosephosphate isomerase (TPI, E.C. 5.3.1.1).

Electrophoresis was performed in starch gels (12% Connaught) using four different buffer systems: (1) Histidine buffer pH 8.0 (Brown 1983) (ACO, ACP, ADH, F-1,6-DP, GPI, HK, IDH, MDH, PGD, PGM); (2) Borate buffer (Brown 1983) (ACP, AMP, ENP, EST, NDH, PRX); (3) Histidine buffer pH 7.0 (modified after Fildes and Harris 1966) (SDH, TPI); and (4) TEB buffer (Markert and Faulhaber 1965) (AAT).

The gels were run at 100 mA for three hours (buffer systems 1, 3 and 4) and at 50–100 mA for four hours (buffer system 2).

Assay references are given in Pedersen and Simonsen (1987). Additional assay references are given for ADH (Brown et al. 1978), AMP and ENP (Shaw and Prasad 1970), HK (Jelnes 1971), F-1,6-DP (Soltis et al. 1983), PRX (Nielsen and Johansen 1986).

Detection of postmeiotic gene expression

Direct evidence for postmeiotic gene expression can be obtained by electrophoresis of pollen from plants heterozygous for dimeric enzymes (Weeden and Gottlieb 1979). If a dimeric enzyme is the result of sporophytic (diploid) gene expression, three bands, two homodimers, and a hybrid heterodimer will appear after electrophoresis. With haploid gene expression, since each pollen grain contains only one allele, no heterodimer is formed and thus the hybrid band is absent after electrophoresis. The *H. vulgare* cultivars were chosen on the basis of a survey of electrophoretic variants in 66 barley cultivars from "The Danish List of Varieties of Agricultural Crops 1983/84" (Nielsen and Johansen 1986) in order to select variants in dimeric enzymes. Variants in dimeric enzymes were found only in Gpi 1 and Pgd 2. Crosses Gula × Gunhild and Brita × Claret were made to produce heterozygotes in Gpi 1 and Pgd 2, respectively. Likewise, as an outcrossing species, *H. bulbosum* was tested for heterozygotes in dimeric enzymes.

Results

Table 1 compares the expression of 50 isozymes in various representative sporophyte tissues, pollen and anthers containing residual pollen. Thirty isozymes are expressed in both pollen and sporophyte. These include the majority of the assayed isozymes with known metabolic functions.

Fifteen isozymes were found to be sporophyte specific. Apart from ADH 2 and F-1,6-DP, all sporophyte specific isozymes are substrate unspecific esterases and peroxidases. The anodal peroxidases are arbitrarily numbered 'APX 1' to '7' with decreasing anodal migration (Fig. 1). They are not well characterized and may not be the same as the peroxidases revealed using other substrates. The genetic basis of the cathodal peroxidases (PRX 1 to 4) has, however, been established (Felder 1976; Brown 1983; Nielsen and Johansen 1986).

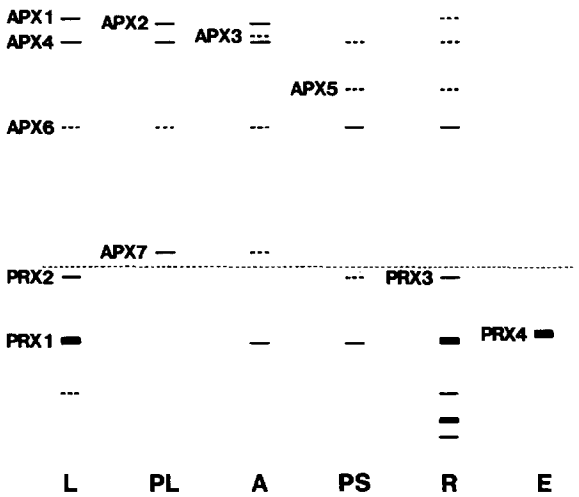

Fig. 1. Peroxidase zymograms in leaves (L), pollen (PL), anthers (A), pistils (PS), roots (R) and endosperm (E). Designations of isozyme bands are indicated

Table 1. Expression of isozymes in sporophyte tissues, pollen and anthers containing residual pollen. MDH 2″ designates the changed migration of MDH 2 in pollen. +: present, (+): weakly expressed, –: absent, nt: not tested

	Sporophyte					Gameto-phyte Pollen	Sporophyte + Gametophyte Anthers	Sporo-phyte specific	Gameto-phyte specific	Over-lap	
	1st leaf	Flag leaf	Roots	Endo-sperm	Pistils						
ACO 1	+	+	(+)	(+)	+	+	+			+	ACO 1
ACO 2	+	+	(+)	(+)	+	+	+			+	ACO 2
ACP 1	–	(+)	–	+	+	+	+			+	ACP 1
ACP 2	+	+	+	+	(+)	+	+			+	ACP 2
ACP 3	+	(+)	–	–	+	+	+			+	ACP 3
'ACP 5'	–	–	–	–	–	+	+		+		'ACP 5'
ADH 1	–	–	+	+	+	+	+			+	ADH 1
ADH 2	–	–	+	–	–	–	–	+			ADH 2
AMP 1	+	+	+	–	+	+	+			+	AMP 1
'AMP 2'	–	–	–	–	–	+	+		+		'AMP 2'
ENP	+	+	–	–	+	+	+			+	ENP
EST 1	+	+	(+)	+	+	(+)	+			+	EST 1
EST 2	+	+	–	+	(+)	–	–	+			EST 2
EST 3	–	–	–	+	–	–	–	+			EST 3
EST 4	+	–	–	–	–	–	–	+			EST 4
EST 5	+	+	–	–	+	+	+			+	EST 5
EST 6	–	–	+	–	–	–	–	+			EST 6
EST 7	+	+	–	–	+	+	+			+	EST 7
EST 9	+	+	–	–	–	–	–	+			EST 9
EST 10	–	–	–	+	–	–	–	+			EST 10
'EST 11'	–	–	–	–	–	+	+		+		'EST 11'
F-1,6-DP	+	+	–	–	–	–	–	+			F-1,6-DP
GOT 1	+	+	+	–	+	(+)	(+)			+	GOT 1
GOT 2	+	+	+	–	+	+	+			+	GOT 2
GOT 3	+	+	+	+	+	+	+			+	GOT 3
GPI 1	+	+	+	–	+	+	+			+	GPI 1
HK	+	+	–	–	+	+	+			+	HK
IDH 1	+	+	+	–	+	+	+			+	IDH 1
IDH 2	+	+	(+)	nt	+	+	+			+	IDH 2
MDH 1	+	+	+	+	+	+	+			+	MDH 1
MDH 2	+	+	+	+	+					+	MDH 2
MDH 2″						+	+				MDH 2″
NDH 1	+	+	(+)	–	+	+	+			+	NDH 1
NDH 2	+	+	+	–	+	+	+			+	NDH 2
'APX 1'	(+)	+	(+)	–	–	–	–	+			'APX 1'
'APX 2'	–	–	–	–	–	+	+		+		'APX 2'
'APX 3'	–	–	–	–	–	–	+	+			'APX 3'
'APX 4'	+	+	(+)	–	(+)	+	+			+	'APX 4'
'APX 5'	–	–	(+)	–	(+)	–	–	+			'APX 5'
'APX 6'	+	(+)	+	–	+	(+)	(+)			+	'APX 6'
'APX 7'	–	–	–	–	–	+	(+)		+		'APX 7'
PRX 1	+	+	+	–	+	–	+	+			PRX 1
PRX 2	+	+	–	–	(+)	–	–	+			PRX 2
PRX 3	–	–	+	–	–	–	–	+			PRX 3
PRX 4	–	–	–	+	–	–	–	+			PRX 4
PGD 1	+	+	+	–	+	+	+			+	PGD 1
PGD 2	+	+	–	–	+	+	+			+	PGD 2
PGM	+	+	–	–	+	+	+			+	PGM
SDH	+	+	–	–	+	+	+			+	SDH
TPI 1	+	+	(+)	–	+	+	+			+	TPI 1
TPI 2	+	+	(+)	–	+	+	+			+	TPI 2

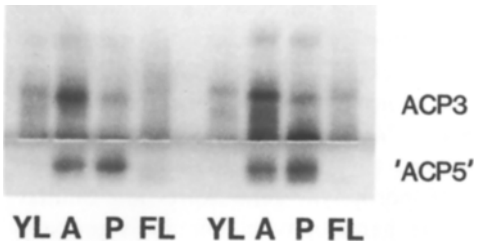

Fig. 2. ACP zymograms in young leaves (YL), anthers (A), pollen (P) and flag leaves (FL) of two F_1-plants from the cross Gula × Gunhild

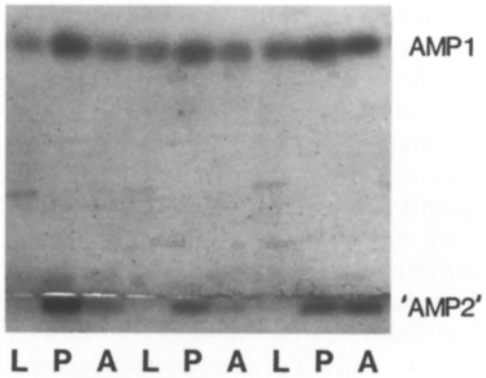

Fig. 3. AMP zymograms in leaves (L), pollen (P) and anthers (A) of three *H. bulbosum* plants

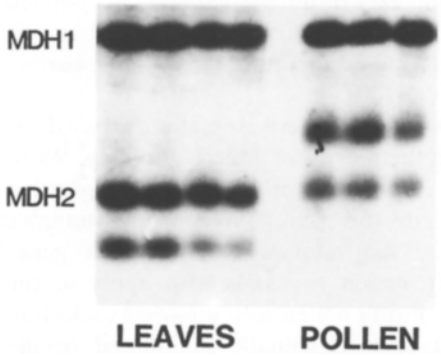

Fig. 4. MDH zymogram showing increased anodal migration of MDH 2 in pollen of the cultivar Gunhild

Five pollen specific isozymes were found: One cathodal acid phosphatase ('ACP5', Fig. 2), one cathodal esterase ('EST 11'), one cathodal aminopeptidase ('AMP 2'; Fig. 3) and two anodal peroxidases ('APX 2' and 'APX 7'; Fig. 1). These isozymes are put in inverted commas since their genetic basis has not been characterized. However, there were indications of variants in the pollen specific ACP and EST isozyme bands of *H. bulbosum,* but this material was too small to give conclusive data. In addition, a marked pollen specific migration change in the anodal direction of the gel was detected for the MDH 2 isozyme (Fig. 4).

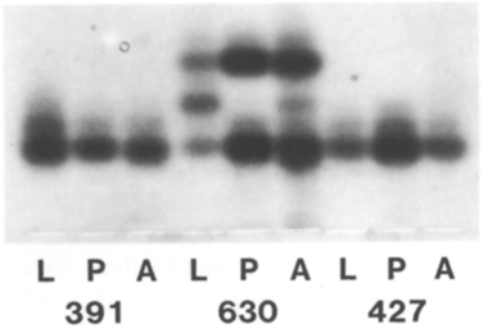

Fig. 5. GPI zymograms in leaves (L), pollen (P) and anthers (A) of three *H. bulbosum* plants (391, 630 and 427). The 630 plant is heterozygous for GPI 1

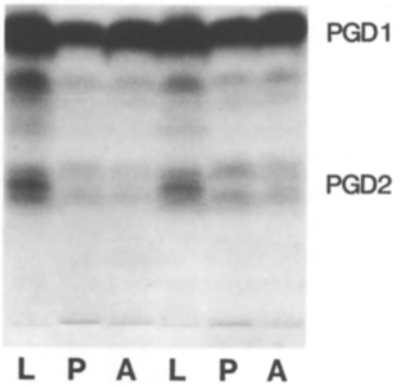

Fig. 6. PGD zymograms in leaves (L), pollen (P) and anthers (A) of two F_1-plants from the cross Brita × Claret

Due to lack of variation between dimeric isozymes in the available material only two dimeric isozymes, GPI 1 and PGD 2, were amenable to a direct test for postmeiotic gene expression. In both cases postmeiotic expression was confirmed as only the two homodimers were expressed in pollen (Figs. 5 and 6). In *H. bulbosum* a weak heterodimeric band was observed in GPI from the anthers, probably reflecting expression of Gpi 1 in the diploid anther wall (Fig. 5).

Assuming that all the expressed isozymes have a genetic basis, the estimated overlap between gametophytic and sporophytic gene expression is 60%, whereas 30% of the gene expression is sporophyte specific and 10% is pollen specific.

Discussion

A striking result of this study is that thirteen out of the fifteen sporophyte specific and all five gametophyte specific isozymes are substrate unspecific enzymes, whereas nearly all the assayed substrate specific enzymes are found to be expressed by both the sporophyte and gametophyte. However, since the majority of the substrate specific enzymes are part of essential

metabolic pathways, they would be expected to be found in both stages of the life cycle. This is also true when different sporophytic tissues are compared (Table 1; Pedersen and Simonsen 1987). On the other hand, substrate unspecific enzymes may participate in the differentiation of tissues, e.g. the pollen grain. For example, an esterase has been suggested to be involved in the formation of the pore in the barley pollen wall where an acid phosphatase is also localized (Ahokas 1975). The finding of several pollen specific isozymes is thus not surprising, considering the very specialized structure and functions of the pollen grain, e.g. formation of the pollen wall, sperm development, germination, and growth of the pollen tube.

The possible causes of the observed migration change of the MDH 2 isozyme will be discussed in a later publication.

Although only two isozymes (GPI 1 and PGD 2) were amenable to direct testing for postmeiotic gene expression, it seems likely that the other isozymes found in pollen are the result of gametophytic gene expression. This is suggested by the fact that a process of extensive elimination and later restoration of ribosomes takes place during microsporogenesis (Dickinson and Heslop-Harrison 1977). The elimination process has been proposed to be a way of inactivating persistent diplophase information, i.e. mRNA, in the transition from the sporophytic to the gametophytic stage of the life cycle (Heslop-Harrison 1971, 1980). This would imply that, unless some pollen enzymes are presynthesized by the sporophyte and persist throughout meiosis and subsequent pollen development, the mRNA responsible for synthesis of the isozymes found in pollen is coded by the haploid genome. Willing and Mascarenhas (1984) have found that 20,000 different mRNA sequences are expressed during pollen development in *Tradescantia paludosa.*

The estimates of the sizes of the common, sporophytic and gametophytic domains in barley gene expression correspond very well with the figures found in similar studies of other species (Table 2). In the poplar study of Rajora and Zsuffa (1986) the size of the sporophytic domain is probably underestimated since only one sporophytic tissue, i.e. roots, was tested. Because differences exist in the expression of isozymes between various sporophyte tissues in plants (see e.g. Scandalios 1964, 1974; Pedersen and Simonsen 1987), several tissues need to be tested to give an accurate representation of sporophytic gene expression. Similarly, the size of the gametophytic domain is probably overestimated: no less than four Adh and three G-6-pdh loci were found to be pollen specific. It seems unlikely that none of these loci is expressed in the sporophyte. ADH is normally inducable in roots when they are subjected to anaerobic conditions, and is otherwise often present in seeds (e.g. Brown 1980; Freeling and Bennett 1985; Tanksley and Jones 1981).

Sari Gorla et al. (1986), in their study of maize, concentrated on multimeric enzymes in order to test for postmeiotic gene expression. By omitting many of the substrate-unspecific enzymes, the estimated size of the common domain becomes relatively high as the esterases and peroxidases are known to be highly tissue specific in their distribution (Macdonald and Brewbaker 1975; Brewbaker and Hasegawa 1975). Based on a literature survey which includes aminopeptidases, esterases, and peroxidases, the estimated overlap is lowered from 72% to 62% (Table 2).

It seems that the best correspondence between the estimated domain sizes of different species is obtained when esterases and peroxidases are included in the data (i.e. tomato, maize literature survey and barley; Table 2).

The enzymes on which these estimates are based are expressed during the process of pollen formation. When we consider the impact of overlapping gene expression between sporophyte and gametophyte on selection, we must ensure that the estimates obtained for genes expressed during pollen *formation* also apply to the genes expressed during pollen *tube growth,* as selection in this latter stage is potentially manifested in the subsequent sporophytic generation.

In this connection the situation could be different in species with bi- and trinucleate pollen. In trinucleate

Table 2. Estimated sizes of the common, sporophytic and gametophytic domains of gene expression in different plant species

Plant (species)	Common domain (%)	Sporophytic domain (%)	Gametophytic domain (%)	Reference
Tomato (*Lycopersicon esculentum*)	60	37	3	Tanksley et al. 1981
Poplar (*Populus spp.*)	74–80	9–10	11–17	Rajora and Zsuffa 1986
Maize (*Zea mays*)	72	22	6	Sari Gorla et al. 1986
–	62	32	6	Literature survey[a]
Barley (*Hordeum spp.*)	60	30	10	This study

[a] References: Brewbaker and Hasegawa 1975; Chao and Scandalios 1975; Goodman and Stuber 1983; Macdonald and Brewbaker 1975; Sari Gorla et al. 1986; Scandalios 1964; Stuber and Goodman 1984; Vodkin and Scandalios 1979

species, such as barley and maize, the synthesis of RNA and proteins needed for pollen tube growth are generally completed prior to dehiscence (Hoekstra and Bruinsma 1979). The mature, ungerminated pollen grains of maize contain a store of presynthesized mRNA (Mascarenhas et al. 1984). Accordingly, in trinucleate species estimates apply to both the stages of pollen formation and pollen function. In contrast, gene expression occurs during the stage of pollen function in binucleate species (Mascarenhas 1975), where the generative cell does not divide until during pollen tube growth. In the binucleate species tomato, fewer isozymes were detected during pollen tube growth than in the mature, ungerminated pollen (Tanksley et al. 1981), indicating that the majority of gametophytic gene expression occurs during pollen formation. The binucleate species *Tradescantia paludosa* is somewhat intermediate to typical bi- and trinucleate species in that pollen tube germination and growth is very rapid (Hoekstra and Bruinsma 1979), and the pollen contains a store of presynthesized mRNA (Frankis and Mascarenhas 1980). Mascarenhas and Mermelstein (1981) have demonstrated that in this species the genes expressed during at least the latter part of pollen maturation appear to be identical to the genes expressed during pollen germination and tube growth.

In conclusion, the results obtained in this study, which demonstrate a significant overlap of gametophytic and sporophytic gene expression in barley, suggest the potential for pollen competition to have positively correlated effects on the sporophyte of this plant species. The genetic variation contained in the estimated 70% of the barley genome which is expressed in pollen is potentially amenable to gametophytic selection.

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