COLUMBIA, MISSOURI
USDA-ARS and University of Missouri
ATHENS, GEORGIA
USDA-ARS
TIFTON, GEORGIA
USDA-ARS

Metabolic channeling suggested by QTL analysis of silk maysin and apimaysin concentration

--Byrne, PF; McMullen, MD; Snook, ME; Musket, TA; Xu, G; Lee, EA; Flint, S; Widstrom, NW; Wiseman, BR; Coe, EH

How does a cell maintain separate control over the synthesis of structurally similar compounds formed from common precursors? If enzymes and chemical precursors and intermediates were randomly diffused through the cytoplasm, such discrete control is difficult to envision. In contrast, the concept of metabolic channeling through membrane-bound multienzyme complexes (as presented by H. A. Stafford, Flavonoid Metabolism, CRC Press, Inc., Boca Raton, FL, 1990) accounts for the presence in a cell of an array of similar end products, exemplified by flavonoid compounds. In Stafford's model, flavonoid pathway enzymes are aligned on the endoplasmic reticulum, facilitating the transfer of metabolites from one enzyme to the next. The hypothesized final enzyme in the complex controls the transport of flavonoid glycosides into the vacuole or into vesicles that fuse with the vacuole, similar to the function of the glutathione-S-transferase encoded by *bz2* (Marrs et al., Nature 375:397-400, 1995). Once sequestered in a specific complex, the flavonoid precursors and intermediates are unavailable to the action of enzymes in complexes with other end product specificities.

Results of our quantitative trait locus (QTL) study of silk maysin and apimaysin concentrations are consistent with the concept of metabolic channeling. Maysin and apimaysin are C-glycosylflavones that inhibit the growth of corn earworm larvae, *Helicoverpa zea* (Boddie). They differ by a single hydroxyl group at the 3'-position of the flavonoid B ring. Because the maize *pr1* locus encodes a flavonoid-3'-hydroxylase that adds a hydroxyl group at this same position on anthocyanin molecules, we hypothesized that the *pr1* locus also controls the 3'-hydroxylation of flavones, and therefore, the relative concentrations of maysin and apimaysin.

To test this hypothesis, we developed an F2 population from the cross of inbred line GT114 (high maysin, low apimaysin) by line NC7A (high maysin, high apimaysin). Testcrosses had indicated functional *Pr1* in GT114 (purple aleurone in crosses to a recessive *pr1* tester), and a nonfunctional or reduced function *pr1* allele in NC7A (red aleurone in crosses with the *pr1* tester). In the summer of 1996 we grew 312 F2 plants, from which we collected leaves for RFLP analysis and silks for chemical evaluation via HPLC (see Byrne et al., Proc. Natl. Acad. Sci. USA 93:8820-8825 1996, for details of methodology). To date we have genotyped RFLPs at 53 loci distributed on 18 of 20 chromosome arms.

The F2 population displayed a broad distribution of values for both maysin and apimaysin concentrations (Table 1). Mean apimaysin concentration was about one-third that of maysin, but this ratio varied from near 0 to over 5. Surprisingly, maysin and apimaysin concentrations were not significantly correlated (r = 0.03).

Single-factor analysis of variance detected a major QTL for apimaysin concentration in the *pr1* region of chromosome 5L; the peak R2 value (percent phenotypic variance explained) was 36.7% and occurred at locus *bnl5.71* (Table 2). As predicted, plants homozygous for the NC7A allele at *bnl5.71* had apimaysin concentrations much higher than GT114 homozygotes or heterozygotes (0.300% fresh silk weight vs. 0.054% and 0.061%, respectively). Gene action was dominant for low apimaysin, consistent with *pr1* gene action in the anthocyanin pathway. Analysis of maysin concentration revealed only minor effects (maximum R2 = 3.2%) on chromosome 5 (Table 2).

The only other region with major effects was on chromosome 9S, where results for the two traits were opposite those on 5L: a large effect on maysin concentration (peak R2 = 21.8%), but no significant effect on apimaysin level (Table 2). We have detected QTLs for maysin concentration in this same region of chromosome 9S, between *bz1* and *wx1*, in three other populations (see following article).

Taken together, our results on chromosomes 5 and 9 suggest largely separate mechanisms for the synthesis and accumulation of maysin and apimaysin, such that one is not formed at the expense of the other. These results contrast with QTL analyses of compounds formed one from another along a linear pathway; Tanhuanpaa et al. (Theor. Appl. Genet. 91:477-480, 1995) found that the same chromosome region of spring turnip rape (*Brassica rapa* ssp. *oleifera*) increased seed oil content of palmitic acid and reduced oleic acid content, consistent with a single role for the detected QTL between the two compounds along the fatty acid biosynthetic pathway. <[> Some plants homozygous recessive for *pr1* had appreciable amounts of maysin, as did the parent NC7A (Table 1). This implies either that there is another 3'-hydroxylase gene (if the parental lines are not polymorphic at that locus, it would not be detected in our QTL analysis); or that the *pr1-NC7A* allele is capable of partial function. Specifications of that allele may affect 3'-hydroxylase abundance, conformation, or stability such that its function is reduced but not eliminated, and both maysin and apimaysin could be produced in individuals homozygous for that allele.

To interpret our results in terms of the multienzyme complex, one can envision the formation of two types of complex, one with and one without a 3'-hydroxylase. The relative frequency of the two complexes may be a function of 3'-hydroxylase concentration, physical characteristics that affect membrane binding, or variation in other enzymes of the complex that influences the rate at which the hydroxylase is recruited into the complex.

Stafford proposed that competition for precursors for different flavonoid end products occurs at the level of chalcone synthase, the first enzyme of flavonoid synthesis, or even earlier in the general phenylpropanoid pathway. Our unpublished results for another

population, (GE37 x FF8) F2:3, show intriguing results for *c2* and *whp1*, both of which encode chalcone synthase. The *whp1* region had large effects on the concentration of maysin and on the sum of apimaysin and methoxymaysin (another maysin analog). However, the *c2* region had a large effect on apimaysin + methoxymaysin, but no detectable effect on maysin. This suggests that the enzymes encoded by the two chalcone synthase loci have different affinities for flavonoid precursors with different B-ring substitution patterns, or for the enzymes that catalyze those reactions.

Our data at first seemed at odds with previous observations on the relationship of maysin and apimaysin concentrations. Widstrom and Snook (unpublished) and Byrne et al. (unpublished) have found that over a broad range of germplasm, apimaysin concentrations are typically from 3 to 10% of maysin concentrations, and that the two compounds are highly correlated. This seems to be the standard situation, suggesting that even when functional *Pr1* is present, as in most inbred lines and populations, some of the time the hydroxylase enzyme does not become incorporated into the multienzyme complex. In contrast, the (GT114 x NC7A) F2 population has segregating *pr1* alleles, and apimaysin values much higher than normally encountered. It is in this situation that the inheritance of the two compounds appears largely independent.

Table 1. Mean and range of silk maysin and apimaysin concentrations of 312 (GT114 x NC7A) F2 plants and mean values for the parental lines and their F1 hybrid.

	% fresh silk wt	
Entry	Maysin	Apimaysin
F2 plants (mean)	0.359 ± 0.020[a]	0.125 ± 0.010
F2 plants (range)	0.003 - 1.376	0.000 - 1.272
GT114	0.274 ± 0.019	0.013 ± 0.002
NC7A	0.487 ± 0.067	0.131 ± 0.029
(GT114 x NC7A) F1	0.326 ± 0.031	0.034 ± 0.017

[a]Mean ± standard error

Table 2. Significant ($P < 0.01$) chromosome regions in the single-factor analysis of variance of silk maysin and apimaysin concentration in the population (GT114 x NC7A) F2.

		% Maysin		% Apimaysin	
Chrom.	Locus	P-value	R^{2Y}	P-value	R^2
1L	umc128	**	3.0		
5S	umc90			***	5.2

5S	umc107b			*****	8.1
5S	tub4			*****	12.8
5S	bnl4.36	**	3.2	*****	17.6
5L	bnl5.71 (near pr1)			*****	36.7
5L	umc126 (near pr1)			*****	34.9
5L	bnl5.24			**	5.6
6L	npi393			**	3.0
9S	umc109	*****	10.9		
9S	bz1	*****	21.8		
9S	wx1	*****	21.2		
9L	umc95	****	6.0		

, *, ****, ***** Significant at the 0.01, 0.001, 0.0001, and 0.00001 probability levels, respectively.
[Ý]Percent phenotypic variance explained.
