9-1998

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Keywords
NCRPIS, Isozyme, Segregation ratios, Starch gel electrophoresis

Comments
This article is from *Journal of the Society for Horticultural Science* 123 (1998): 868–874.

J. Amer. Soc. Hort. Sci. 123(5):868–874. 1998.

Allozyme Inheritance in Anise Hyssop [*Agastache foeniculum* (Pursh) Kuntze] (Lamiaceae)

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Additional index words. isozyme, segregation ratios, starch gel electrophoresis

Abstract. **The inheritance of five allozymes was studied in anise hyssop (*Agastache foeniculum*) by analyzing the progeny of controlled crosses. The loci studied [*Cat-1*, *Got-2*, *Pgm-2*, *Tpi-1*, and *Tpi-2*] were scored by using starch gel electrophoresis. Segregation analyses of families polymorphic at each of these loci support the following hypotheses: *Cat-1* is controlled by a single gene with codominant alleles; *Got-2* is controlled by a single gene with codominant alleles coding for dimeric protein products; *Pgm-2* is controlled by a single gene with codominant alleles coding for monomeric proteins; and *Tpi-1* and *Tpi-2* are each controlled by a single gene with codominant alleles coding dimeric protein products. Distorted segregation ratios were observed in some families segregating for *Got-2* and *Pgm-2*. No linkages were detected among any of the cosegregating loci.**

For more than three decades, enzyme electrophoresis has provided valuable data to study patterns of genetic and biosystematic variation in plants (Kephart, 1990). Numerous reports document how variability in enzymes resolved by starch gel electrophoresis has deepened our understanding of the breeding behavior of plant populations (Brown, 1979; Hamrick and Godt, 1991) and of the heterotic response of crops based on parental isozymic differences for various hybrid combinations (Sekhon and Gupta, 1995). Polymorphic enzymes (isozymes) have also been used as genetic markers in genetic linkage and mapping studies (Arus et al., 1994; Goodman et al., 1980) as well as for determining ploidy levels (Gottlieb, 1982; Rieseberg and Soltis, 1989). Other applications of isozyme markers include acceleration of gene introgression, evaluation of wild populations, and gene tagging (Cousineau and Donnelly, 1992). More generally, isozymes have also been reported as appropriate genetic markers for many aspects of plant germplasm management (Bretting and Widrlechner, 1995).

Anise hyssop (*Agastache foeniculum*) is a perennial plant native to north central North America. This species is a candidate for large scale domestic cultivation as a source of nectar for honey bees (Ayers and Widrlechner, 1994) and as an ornamental and aromatic plant, with wide variation in the composition and content of its essential oils which are used in foods, drugs, and perfumes. Fuentes-Granados et al. (1998) recently assembled an extensive review of scientific literature related to the systematics, biochemistry, cultivation, and uses of anise hyssop and related species of *Agastache*.

Allozyme variability has been used to elucidate patterns of genetic diversity within and among populations of *Agastache* (Vogelmann, 1983; Fuentes-Granados and Widrlechner, 1995). A locus controlling malate dehydrogenase banding patterns in *A. rugosa* (Fish. & Meyer) Kuntze was recently described (Fuentes-Granados and Widrlechner, 1997). The present study was conducted to determine the mode of inheritance of five isozymes reported to be polymorphic in *A. foeniculum* (Vogelmann, 1983; Fuentes-Granados and Widrlechner, 1995), to support inferences regarding their quaternary structures, and to test for possible linkages among those loci.

Materials and Methods

Plant materials. Five populations of *A. foeniculum* (Table 1) were obtained from the North Central Regional Plant Introduction Station, Ames, Iowa. These populations were screened for isozyme variability with four staining systems known to detect polymorphic loci in *Agastache* (Fuentes-Granados and Widrlechner, 1995). Parental plants differing in banding patterns at one or more putative loci were selected for controlled hybridizations (Table 2). F_1 plants were either sib-crossed or were self-pollinated to generate F_2 families (Table 3).

F_1 and F_2 plants were grown from seeds that received a moist, chilling treatment (4 °C for 1 week) to improve germination. Seeds were germinated in a growth chamber with temperatures alternating between 20 °C and 30 °C with a 16-h photoperiod. Two-week old seedlings were transplanted from the germination chamber into the greenhouse.

Sample preparation. At least 1 cm^2 of leaf tissue was harvested from the youngest leaves of each seedling and was brought to the laboratory in a crisper with crushed ice. Leaf material (20 mg) was ground with 100 µL of a modification of the extraction buffer reported by Hashemi et al. (1991). For all staining systems except for catalase, the extraction buffer consisted of 0.1 mol·L^{-1} Tris-HCl (pH = 8.0), 0.02 mol·L^{-1} Dithiothreitol, 2 mL·L^{-1} 2-mercaptoethanol, and 0.2 g·L^{-1} polyvinylpyrrolidone (PVP) (Sigma PVP-40T, St. Louis). The extraction buffer for catalase was the same except that the pH of the buffer was 7.0. The samples were ground for 1 min in an electric grinder (model 4555-30; Cole Parmer, Chicago). After grinding, the samples were speed microcentrifuged (Beckman

Table 1. Origin of the accessions of *Agastache foeniculum* used in the study.

Accession no.	Origin
PI-561057	Wild: Barnes Co., ND
PI-561058	Wild: Cass Co., MN
PI-561059	Wild: Hennepin Co., MN
PI-561061	Wild: Las Animas Co., CO
PI-561063	Wild: Manitoba

Received for publication 21 Oct. 1997. Accepted for publication 21 Apr. 1998. Iowa Agriculture and Home Economics Experiment Station journal paper J-17594, project no. 1018 and supported by Hatch Act and State of Iowa funds and in part by a Minigrant from Sigma Xi. We thank Randy Shoemaker, Basil Nikolau, Edward Pollak, and Peter Bretting for useful comments and contributions to the preparation of this paper. Mention of commercial brand-named products does not constitute an endorsement of any product by the USDA Agricultural Research Service or cooperating agencies. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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Table 2. Isozyme genotypes of five polymorphic loci for *Agastache foeniculum* parents and their F_1 offspring.[z]

Individual	*Cat-1*	*Got-2*	*Pgm-2*	*Tpi-1*	*Tpi-2*
PI-561059	5/5	6/6	5/5	5/5	5/6
PI-561061	3/3	5/5	5/5	5/5	5/5
F_1 hybrids	3/5	5/6	5/5	5/5	5/5–5/6
PI-561057	5/5	5/5	4/5	5/6	5/5
PI-561063	5/5	3/3	5/5	5/5	5/5
F_1 hybrids	5/5	3/5	4/5–5/5	5/5–5/6	5/5
PI-561058	5/5	5/5	5/5	5/5	5/5
PI-561059	5/5	6/6	5/5	5/6	5/6
F_1 hybrids	5/5	5/6	5/5	5/5–5/6	5/5–5/6
PI-561057	5/5	5/5	4/5	5/6	5/5
PI-561058	5/5	5/5	5/5	5/5	5/5
F_1 hybrids	5/5	5/5	4/5–5/5	5/5–5/6	5/5

[z]Terminology follows Fuentes-Granados and Widrlechner (1995).

Table 3. Parental origin of the F_2 families of *Agastache foeniculum*.

F_2 family	Parentage
1–3	PI-561061 x PI-561059 F_1 full-sib
4	PI-561063 x PI-561057 F_1 full-sib
5–9	PI-561059 x PI-561058 F_1 full-sib
10–12	PI-561061 x PI-561059 F_1 selfed
13	PI-561063 x PI-561057 F_1 selfed
14	PI-561057 x PI-561058 F_1 selfed

Microfuge E, Palo Alto, Calif.) for 90 s at a relative centrifugal force of 12,100 g_n, and then stored in a freezer at –62 °C for 24 h.

Enzyme electrophoresis. Two gel-buffer systems were used for electrophoresis of the enzymes (Stuber et al., 1988): system C for catalase (CAT, EC 1.11.1.6), glutamate-oxaloacetate transaminase (GOT, EC 2.6.1.1), and triose phosphate isomerase (TPI, EC 5.3.11); and system D for phosphoglucomutase (PGM, EC 2.7.5.1). The electrode buffer for system C consisted of 0.19 mol·L^{-1} boric acid and 0.04 mol·L^{-1} lithium hydroxide. The gel for system C was prepared with 129.6 g·L^{-1} starch in a buffer of nine parts of Tris-citrate [0.05 mol·L^{-1} Trizma base and 0.007 mol·L^{-1} citric acid, pH 8.3] with one part of C-electrode buffer. The electrode buffer for system D contained 0.05 mol·L^{-1} L-histidine and 0.007 mol·L^{-1} citric acid. The gel for system D consisted of 129.6 g·L^{-1} starch in a 1:3 aqueous dilution of the electrode buffer.

After pouring, gels were left overnight at room temperature and covered with polyvinylidene chloride to avoid dehydration. Prior to loading, gels were cooled to 4 °C for 15 min. Frozen ground-leaf samples were allowed to thaw and adsorbed from the supernatant on 1.5 × 12mm Whatman no. 1 paper wicks. Twenty samples were inserted in each gel. Once the samples were inserted, the gels were placed in electrode trays containing fresh electrode buffer. A bag of cool water (4 °C) was placed on the surface of each gel to avoid warming and protein denaturation. Lithium borate gels were run for 6 h at a constant power of 12 W (model EC504; E C Apparatus Corp. St. Petersburg, Fla.); whereas, histidine-citric acid gels were run on the same apparatus for 6.5 h at 16 W of constant power. Stain recipes and procedures were taken from Stuber et al. (1988) for CAT, GOT, and PGM and from Wendel and Weeden (1989) for TPI.

Loci and alleles are designated following nomenclature established by Fuentes-Granados and Widrlechner (1995). Loci are numbered to correspond with zones of staining activity where 1 designates the most anodal zone, 2 the next most anodal zone, etc. The most common allele among 11 populations is numbered 5, and other alleles are designated in relation to allele 5, with lower numbers assigned to alleles producing progressively slower migration rates and higher numbers to those with progressively faster migration rates.

Statistical analysis. Segregation ratios obtained from F_2 families were tested by χ^2 goodness of fit to expected Mendelian ratios. Additivity of χ^2 tests were conducted according to Steel and Torrie (1980). Tests for independent segregation between pairs of loci cosegregating in the same families were conducted to determine if any linkage existed between those loci. When appropriate, the computer program MAPMAKER v. 3.0 (Lander et al., 1987) was used to test for independent segregation of the loci under study.

Results and Discussion

Table 3 summarizes the genotypes of parents and F_1 hybrids for each of the five loci studied. There was no single cross in which both parents differed genotypically at all loci. A summary of the phenotypic classes observed in nine segregating full-sib families and five segregating selfed families for each of the five loci under investigation is presented in Table 4. The results provide evidence for the genetic basis of the inheritance of *Cat-1*, *Got-2*, *Pgm-2*, *Tpi-1*, and *Tpi-2* in *A. foeniculum*. The data support the hypotheses that each of these five putative loci is controlled by a single gene with codominant alleles. In 23 of the 26 tests, each of the segregating loci displayed the segregant classes in the progenies evaluated that would be expected in a simple single-gene, codominant allele model. A discussion of individual loci is presented below.

Catalase. A schematic drawing and photo of the phenotypes of individuals with different genotypes of *Cat-1* are presented in Fig. 1. Parental plants were either homozygous for the 3 or 5 alleles.

We analyzed three families obtained from self pollination of heterozygous individuals for the two most common alleles reported for *Cat-1* in *Agastache*. Nonsignificant χ^2 values calculated indicated that observed segregation ratios fit the expected segregation ratio of 1:2:1, indicating that in *Agastache* CAT is controlled by a single locus with codominant alleles (Table 4). Additive χ^2 test including the three families segregating for *Cat-1* also supports a 1:2:1 segregation (Table 4). Heterozygotes produced phenotypes with a smeared banding pattern, and, thus, quaternary structure could not be confirmed from this study. Werner (1992) reported that in peach [*Prunus persica* (L.) Batsch], heterozygous individuals for catalase usually showed a streaked band intermediate between the homozygotes.

Catalase is a ubiquitous hemeprotein that catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen (Stryer, 1988). Studies of catalase demonstrate different

Table 4. Observed F_2 segregation ratios and χ^2 statistics for the segregating families of *Agastache foeniculum*.

Locus	Family	F_2 segregation	χ^2 for 1:2:1 segregation	*P*
		3/3 : 3/5 : 5/5		
Cat-1	10	12 : 26 : 12	0.08	0.95–0.975
Cat-1	11	15 : 22 : 7	2.90	0.1–0.25
Cat-1	12	13 : 23 : 14	0.36	0.75–0.90
Total[z] χ^2			3.34	0.75–0.90
		5/5 : 5/6 : 6/6		
Got-2	1	5 : 16 : 17	8.52	0.01–0.025*
Got-2	2	7 : 16 : 5	0.85	0.95–0.975
Got-2	3	1 : 7 : 7	4.87	0.05–0.1
Got-2	5	7 : 9 : 4	1.1	0.5–0.75
Got-2	6	11 : 19 : 6	1.5	0.25–0.5
Got-2	7	6 : 22 : 8	2.0	0.25–0.5
Got-2	8	10 : 17 : 6	1.0	0.5–0.75
Got-2	9	2 : 9 : 6	1.94	0.25–0.5
Got-2	10	13 : 24 : 13	0.08	0.95–0.975
Got-2	11	10 : 17 : 12	0.85	0.5–0.75
Got-2	12	9 : 22 : 10	0.18	0.9–0.95
Total χ^2			22.89	0.25–0.50
		3/3 : 3/5 : 5/5		
Got-2	4	9 : 17 : 1	6.56	0.025-0.05*
Got-2	13	4 : 15 : 3	3.0	0.1–0.25
Total χ^2			9.56	0.025–0.05*
		4/4 : 4/5 : 5/5		
Pgm-2	4	5 : 10 : 20	19.28	0.001–0.005**
Pgm-2	14	8 : 9 : 2	3.84	0.1–0.25
Total χ^2			23.12	0.001–0.005**
		5/5 : 5/6 : 6/6		
Tpi-1	5	4 : 11 : 5	0.3	0.75–0.90
Tpi-1	14	6 : 8 : 5	0.58	0.5–0.75
Total χ^2			0.88	0.90–0.95
		5/5 : 5/6 : 6/6		
Tpi-2	5	6 : 9 : 5	0.3	0.75–0.90
Tpi-2	8	7 : 19 : 7	0.76	0.5–0.75
Tpi-2	9	6 : 9 : 2	1.94	0.25–0.50
Tpi-2	10	8 : 31 : 11	3.24	0.1–0.25
Tpi-2	12	16 : 19 : 15	2.71	0.1–0.25
Tpi-2	13	5 : 12 : 5	0.19	0.9–0.95
Total χ^2			9.14	0.25–0.50

[z]Computed following additivity of χ^2 by Steel and Torrie (1980).
*,**Significant at $P = 0.05$ or 0.01, respectively.

metabolic activities in this enzyme among species (Havir and McHale, 1989). Gastony and Gottlieb (1982) reported a single catalase band in the fern, *Pallaea andromedifolia* (Kaulf.) Fée. In asparagus (*Asparagus officinalis* L.) and peach, Maestri et al. (1991) and Werner (1992), respectively, reported that catalase was controlled by a single locus with two alleles. In tetraploid tobacco (*Nicotiana tabacum* L.) and, the ancient tetraploid, maize (*Zea mays* L.), three CAT isozymes have been reported to be expressed at different stages of plant development and with different enzymatic activity (Acevedo and Scandalios, 1990; Havir and McHale, 1989). Up to five tetrameric catalase isozymes were reported in tetraploid cotton (*Gossypium hirsutum* L.) by Ni and Trelease (1991).

Glutamate-oxaloacetate transaminase. A schematic drawing and photo of phenotypes of individuals with different genotypes for *Got-2* are presented in Fig. 2. In accordance with Vogelmann (1983) and Fuentes-Granados and Widrlechner (1995), two zones of activity were identified for GOT. Only *Got-2* was evaluated in the present study. There were three alleles segregating in the F_2 families, alleles 3, 5, and 6 (Table 3). Those three alleles were the only alleles of *Got-2* encountered in populations of *A. foeniculum* (Fuentes-Granados and Widrlechner, 1995).

We evaluated nine families resulting from crossing two heterozygous individuals and four families resulting from selfing heterozygotes. Significant variation from expected segregation ratios occurred in two families (1 and 4) originating by crossing heterozygous individuals. Family 1 had an excess 6/6 vs. 5/5 individuals and family 4 had an excess of 3/3 vs. 5/5 individuals (Table 4), with χ^2 values for an expected 1:2:1 ratio of 8.52 and 6.56, respectively. Each of the three alleles tested encodes the production of dimeric isozymes, which can be inferred from the consistent three-banded phenotypes of heterozygotes. Additive χ^2 tests for all the segregating families support a 1:2:1 segregation for the families segregating for alleles 5 and 6 but not for the families segregating for alleles 3 and 5 (Table 4).

Distorted segregation ratios for *Got-2* have been reported for

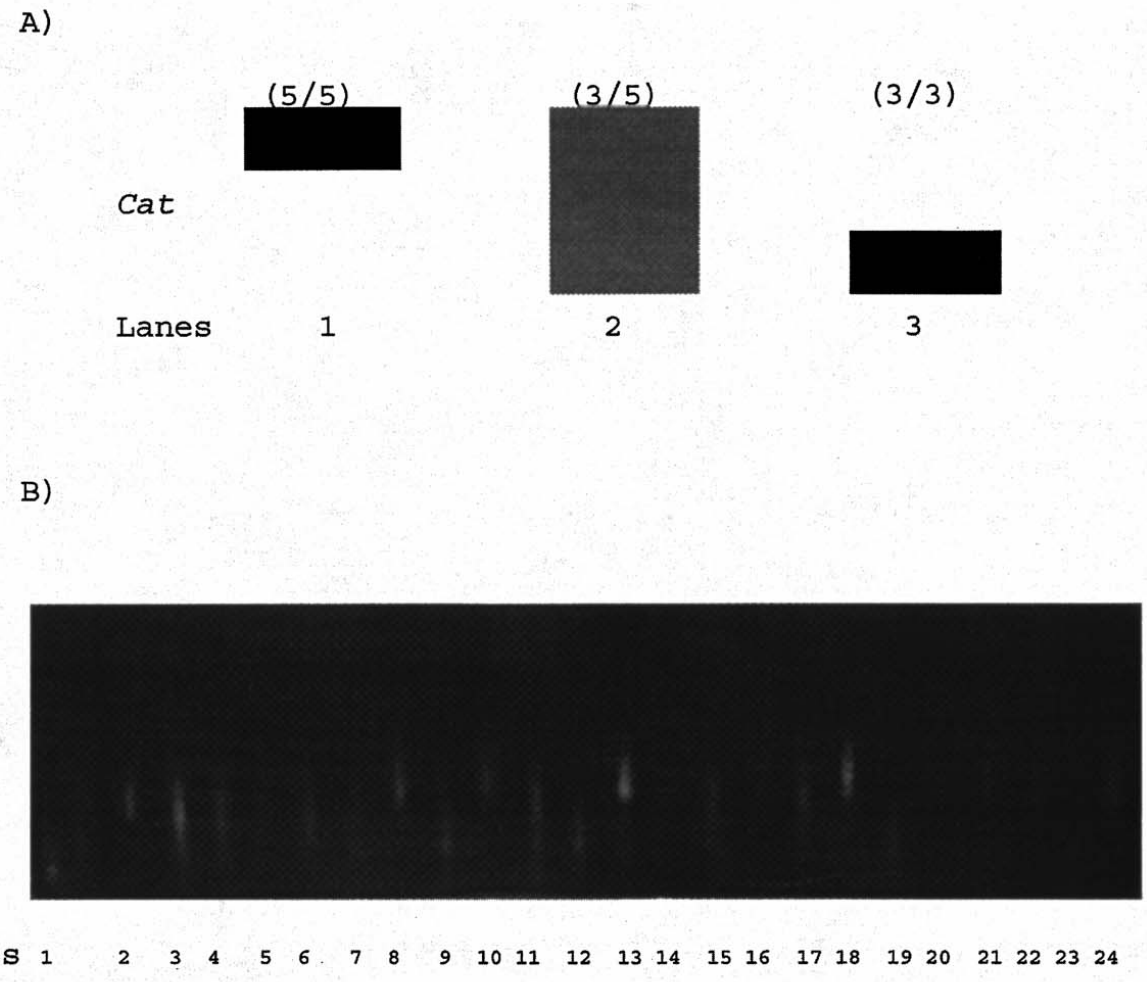

Fig. 1. (**A**) Schematic drawing of putative phenotypes of *Agastache foeniculum* individuals with different genotypes for *Cat-1*. Lanes 1 and 3 represent homozygous individuals for alleles 5 and 3, respectively. Lane 2 illustrates the phenotype of heterozygous individuals. (**B**) Scanned photograph of a catalase gel. Lanes 10, 11, and 12 show the phenotypes of individuals with *Cat-1* genotypes (5/5), (3/5), and (3/3), respectively.

hazelnut (*Corylus*) (Rovira et al., 1993) and apple (*Malus*) (Weeden and Lamb, 1987). This could be explained by linkage of the marker locus to deleterious or highly favorable genes or by some direct effect of the allele itself (Rovira et al., 1993; Weeden and Lamb, 1987; Marshall et al., 1973). Distorted ratios could also have origins in what Kidwell and Kidwell (1976) defined as hybrid disgenesis, "a syndrome of correlated genetic traits that is spontaneously induced in hybrids between certain mutually interacting strains of *Drosophila* usually in one direction only" because the distorted ratios did not appear in selfed families. Another possible explanation for at least one of two significant χ^2 values might be that, with so many tests, the probability of at least one χ^2 value being significant at the 0.05 level is high: p(that at least one among 13 values would be significant) = 1 – p(all 13 not significant) = 1 – $(0.95)^{13}$ = 1 – 0.513 = 0.487. The χ^2 tests for the other families resulting from crossing heterozygous individuals all fit the expected segregation values for a 1:2:1 segregation ratio, $P > 0.05$. Chi-square tests for the three families originating from selfed heterozygotes also supported hypotheses of 1:2:1 segregation ratios, $P > 0.1$.

Glutamate-oxaloacetate transaminase (GOT), also known as aspartate aminotransferase (AAT), catalyzes the reversible conversion of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate using pyridoxal phosphate as a coenzyme (Scandalios et al., 1975). GOT plays an important role in transamination reactions, in supplying keto acids for the Krebs' cycle and gluconeogenesis, and in maintaining the amino acid balance during growth and development of plant tissues (Scandalios et al., 1975).

Studies of GOT have reported this enzyme as being dimeric (Diaz and Jouve, 1986; Gottlieb, 1973; Scandalios et al., 1975), with as many as four loci reported to control GOT in plants. Gottlieb (1982) reported that certain GOT isozymes are compartmentalized within organelles, whereas others are sequestered in the cytosol. Vogelmann (1983) reported two *Got* loci in *Agastache*, *Got-1* and *Got-2*, and concluded that *Got-1* is a chloroplastic isozyme and that *Got-2* is likely cytosolic.

Phosphoglucomutase. Two zones of activity for PGM, representing expression of *Pgm-2* alleles 4 and 5, were identified in our study as illustrated in Fig. 3.

Our analysis of progenies segregating for *Pgm-2* supported a model of control by a single locus with codominant alleles (Table 4). However, we observed distorted segregation in one of the two families evaluated ($\chi^2 = 19.28$, $P < 0.005$). That family presented an excess of homozygous individuals for *Pgm-2*(5/5). Distorted ratios for PGM isozymes have also been reported to occur in apple and other plant species (Rovira et al., 1993; Weeden and Lamb, 1987). Linkage of allele 5 to a highly favorable, recessive factor or hybrid disgenesis are possible explanations for the distorted ratio. Analysis of the other family fits the hypothesis of *Pgm-2* being controlled by one gene with codominant alleles. Each of these

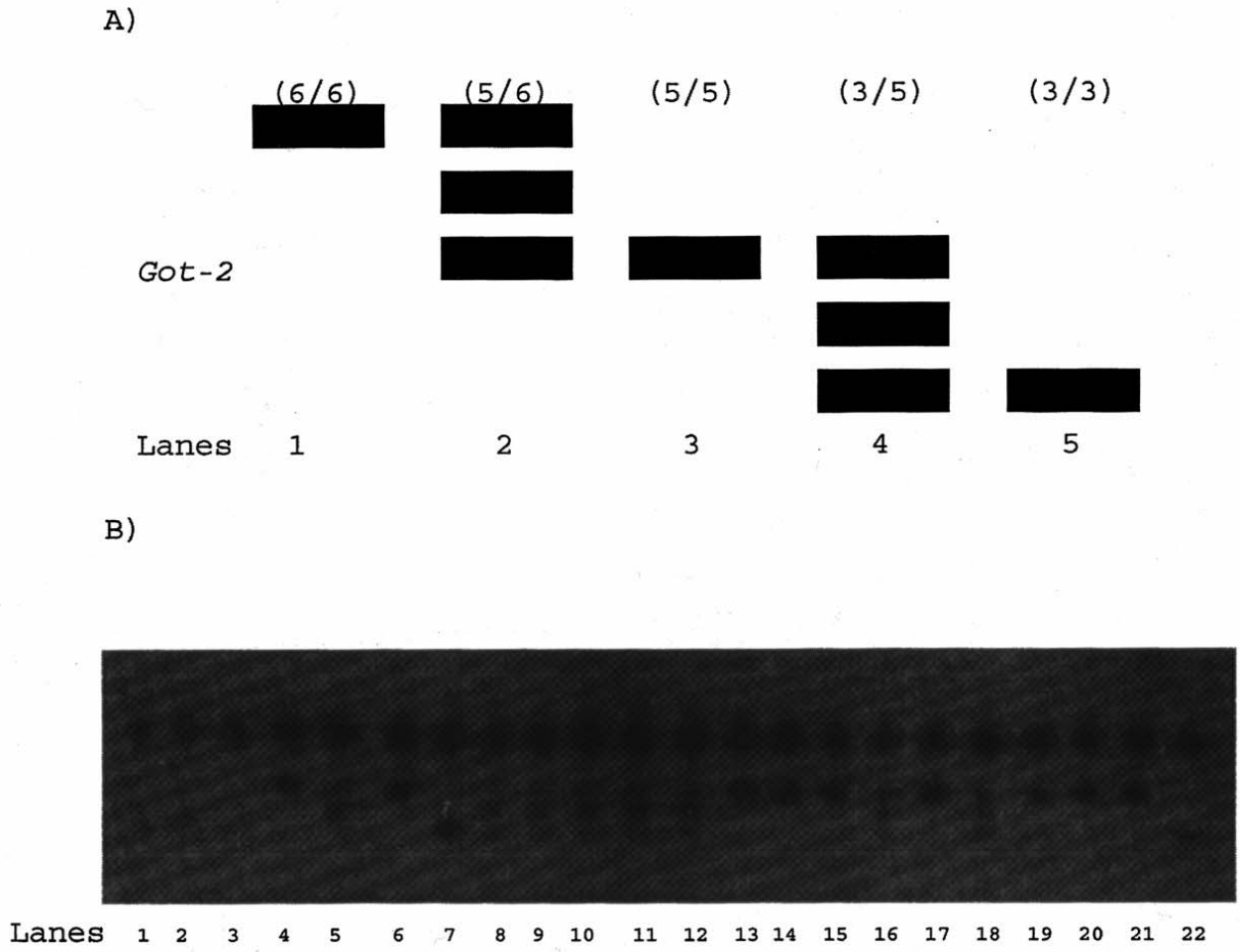

Fig. 2. (**A**) Schematic drawing of phenotypes of individuals of *Agastache foeniculum* with different genotypes for *Got-2*. Lanes 1, 3, and 5 represent homozygous individuals for alleles 6, 5, and 3, respectively. Lanes 2 and 4 represent heterozygous individuals 5/6 and 3/5, respectively. (**B**) Scanned photograph of a *Got* gel. The top band illustrates the phenotypes of individuals homozygous for *Got-1* (5/5), not discussed in this study. Lanes 7, 10, and 13 in the lower zone represent the phenotypes of individuals with *Got-2* genotypes (5/5), (5/6), and (6/6), respectively.

alleles encodes the production of monomeric isozymes, as inferred from the consistent two-banded phenotypes of the heterozygous individuals (Fig. 3). Because of the large deviation from a 1:2:1 ratio exhibited by family 4 and its direct weight on the total (additive) test, the additive χ^2 test including the two segregating families does not support a 1:2:1 segregation for *Pgm-2* (Table 4).

Phosphoglucomutase (PGM) catalyzes the isomerization of glucose-1-phosphate to glucose-6-phosphate by means of phosphorolytic cleavage (Stryer, 1988). PGM isozymes had been reported to be monomeric with one isozyme located in the chloroplast and another in the cytosol (Gastony and Gottlieb, 1982). Even though most plants have been reported to contain two PGM isozymes, some studies have reported up to four PGM isozymes in Asteraceae. Hashemi and Estilai (1992) reported four PGM isozymes in diploid guayule (*Parthenium argentatum* A. Gray). In two other genera, *Helianthus* and *Heliomeris*, Rieseberg and Soltis (1989) also reported the existence of four PGM isozymes and concluded that this was evidence of gene duplication. In *Agastache*, Vogelmann (1983) reported two PGM isozymes, PGM-1 and PGM-2. He noted that the isozymes are monomers and that PGM-1, the most anodal isozyme, is chloroplastic, whereas PGM-2 is cytosolic.

Triose phosphate isomerase. Two zones of activity were observed for *Tpi,* controlled by *Tpi-1* and *Tpi-2,* as illustrated in Fig. 4. In accordance with Fuentes-Granados and Widrlechner (1995), *Tpi-1* was used to designate the locus coding for the isozymes within the more anodal zone of activity and *Tpi-2* was used to designate the locus coding for the isozymes within the more cathodal zone of activity.

Segregation analysis of families segregating for *Tpi-1* and *Tpi-2* supported a model of control by two independent loci with codominant alleles. For both loci, we tested the two most common alleles (5 and 6).

Neither of the two families segregating for *Tpi-1*, nor any of the six families segregating for *Tpi-2*, presented evidence of distorted segregation ratios. Levels of significance for the tests analyzing all of those families were >0.1. Each allele encodes the production of dimeric isozymes, as inferred from the consistent three-banded phenotypes of the heterozygote individuals (Fig. 4). Additive χ^2 tests conducted in the families segregating for *Tpi-1* and *Tpi-2* support a 1:2:1 segregation for each of the two loci (Table 4).

Triose phosphate isomerase (TPI) catalyzes the isomerization of dehydroxyacetone phosphate and glyceraldehyde-3-phosphate, two three-carbon phosphorylated sugars that are intermediate and necessary products in glycolysis, gluconeogenesis, and photosynthetic carbon dioxide formation (Gottlieb 1982; Stryer, 1988). Two isozymes of this enzyme have been reported in species of *Aster*, *Lycopersicon*, and *Salicornia* (Gottlieb, 1982). The enzyme has been reported to be a dimer. Senechal (1990) used TPI for his studies on

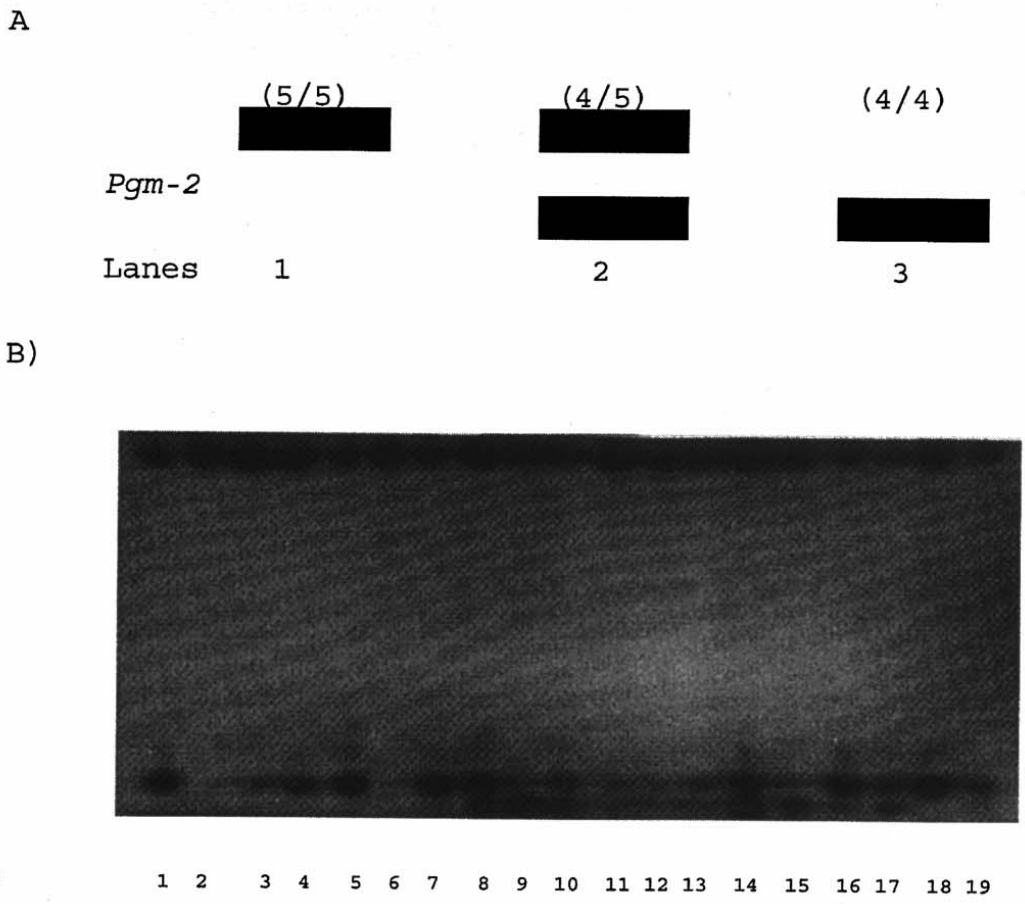

Fig. 3. (**A**) Schematic drawing of phenotypes of individuals of *Agastache foeniculum* with different genotypes for *Pgm-2*. Lanes 1 and 3 represent homozygous individuals for alleles 5 and 4, respectively. Lane 2 represents a heterozygous individual. (**B**) Scanned photograph of a *Pgm* gel. The top band illustrates the phenotypes of individuals homozygous for *Pgm-1* (5/5), not discussed in this study. Lanes 7, 8, and 9 of the bottom rows represent the phenotypes of individuals with *Pgm-2* genotypes (5/5), (4/5), and (4/4), respectively.

Agastache to determine the parentage of interspecific hybrids.

Our results support the hypotheses that each of the five loci studied is controlled by a single gene with codominant alleles, in most cases following Mendelian segregation patterns. Tests for independent segregation of *Cat-1, Got-2, Pgm- 2, Tpi-1,* and *Tpi-2* in cosegregating families revealed that no linkage is present among the loci evaluated. With the exception of catalase, quaternary structures for the isozymes encoded by *Got-2*, *Pgm-2*, *Tpi-1*, and *Tpi-2* were supported. More generally, our results also confirmed the genetic behavior of *A. foeniculum*, which had been reported (Vogelmann, 1985) to be that of functional diploid.

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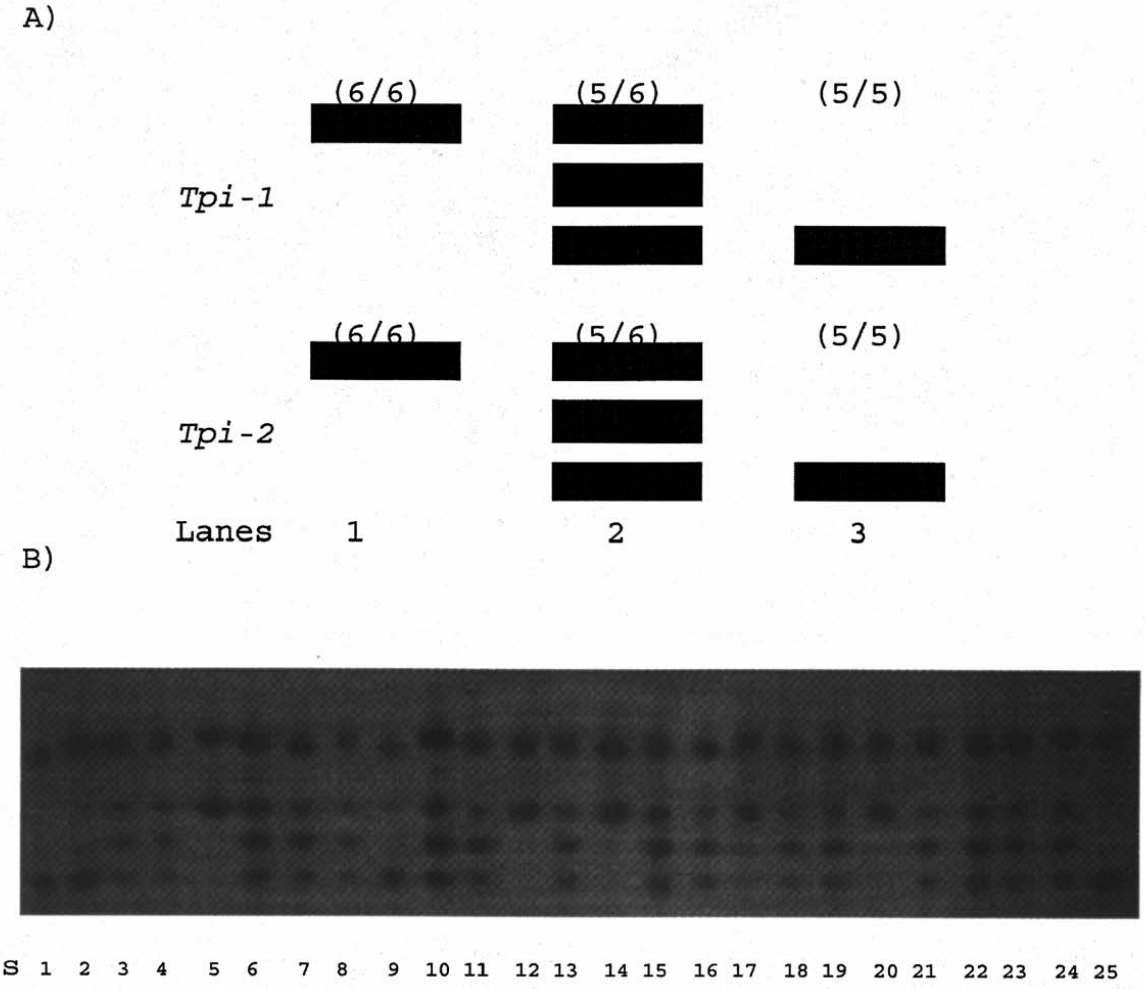

Fig. 4. (**A**) Schematic drawing of observed phenotypes of individuals of *Agastache foeniculum* with different genotypes for *Tpi-1* and *Tpi-2*. Lanes 1 and 3 represent homozygous individuals for alleles 6 and 5, respectively, at both loci. Lane 2 represents heterozygous 5/6 individuals at the two loci. (**B**) Scanned photograph of a *Tpi* gel. The top band illustrates the phenotypes of homozygous individuals for *Tpi-1* (5/5). Lanes 9, 10, and 12 in the lower zone illustrate the phenotypes for individuals with *Tpi-2* genotypes (5/5), (5/6), and (6/6), respectively.

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