Inheritance of Aconitase, Shikimate Dehydrogenase, and Phosphoglucose Isomerase in Guayule

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Abstract. Starch gel electrophoresis was carried out to investigate inheritance of aconitase (ACO), shikimate dehydrogenase (SKDH), and phosphoglucose isomerase (PGI) in guayule (*Parthenium argentatum* Gray). Self-incompatibility of diploid guayule ($2n = 36$), prevented production of F_2 generations. A series of crosses was made for segregation analysis. The enzyme ACO showed two zones of activity—one monomorphic and the other polymorphic, representing one locus with two alleles. SKDH was found to be coded by two loci with three alleles at each locus. Four zones of activity were found for PGI. PGI-1, the fastest zone, was poorly resolved and appeared variable. Segregation data indicated that PGI-2 is monogenic and controlled by five codominant alleles. Poor resolution of PGI-4 made it impossible to determine whether PGI-3 was the product of a separate locus or resulted from intergenic cross dimerization between PGI-2 and PGI-4. The dimeric characteristic of PGI-2 and the monomeric structure of SKDH and ACO-1 in guayule agreed with the protein structures previously reported for these enzymes in other plant species. The isozyme variation of this investigation maybe used in a breeding program to identify sexual and maternal type progenies of facultative apomictic guayule plants at the early seedling stage.

Guayule, a rubber-producing shrub of the Chihuahuan desert, is a potential industrial crop for arid and semiarid regions. In addition to rubber, guayule produces significant amounts of resin, a by-product that can be used as a wood preservative, a fuel source, and a rubber additive (Schloman, 1986). Due to low rubber yield, guayule has not yet been commercialized. However, the high rubber-yielding selections developed by breeding programs (Estilai, 1986; Thompson and Ray, 1989) may bring about commercialization in the near future.

Guayule plants have 36, 54, 72, and higher chromosome numbers. Diploid plants ($2n = 36$) are sexual and self-incompatible (Gerstel, 1950; Gerstel and Mishanec, 1950; Powers, 1945). Polyploids, however, reproduce by facultative apomixis (Esau, 1944), producing progenies derived from either maternal tissue or fertilized eggs. Morphological and biochemical markers are needed to identify the two progeny types at the seedling stage. So far, only one morphological marker (purple flower), which can be observed only at flowering, has been identified and genetically analyzed (Estilai, 1984). Potential linkage between biochemical markers and desirable traits may allow early screening of many seedlings, as has been demonstrated in pea (Weeden et al., 1984) and tomato (Tanksley et al., 1982). Isozyme research in guayule is limited to two studies. Zaiger et al. (1984) used isozymes to demonstrate variation in a sexual diploid germplasm population. Estilai et al. (1990) showed a uniform electrophoretic pattern in two polyploid apomictic germplasms. To the best of our knowledge, no isozyme inheritance study has been reported in guayule.

We report the inheritance of aconitase (EC 4.2.1.3; ACO), shikimate dehydrogenase (EC 1.1.1.25; SKDH), and phosphoglucose isomerase (EC 5.3.1.9; PGI) in diploid guayule.

Materials and Methods

A total of 50 glasshouse-grown, diploid guayule plants, derived from the only available diploid germplasm, Cal-3 (Tysdal et al., 1983), were initially assayed for isocitric dehydrogenase (EC 1.1.1.42; IDH), malate dehydrogenase (EC 1.1.1.37; MDH), SKDH, PGI, triosephosphate isomerase (EC 5.3.1.1; TPI), and ACO. IDH, MDH, and TPI were eliminated from further study because they showed monomorphic patterns. Hybrid progenies between enzyme variants were generated by controlled crosses (Table 1) following the procedures described by Hashemi et al. (1986). Young leaf samples were ground in Plexiglass wells with a glass rod using 0.1 ml of refrigerated (4°C) extraction buffer. The extraction buffer consisted of 30 mg DL-dithiothreitol (DTT), 3 g polyvinylpyrrolidone (PVP-40), and four drops of 2-mercaptoethanol to which 20 ml 0.1 M Tris-HCl (pH 8.0) was added. The electrophoretic buffer and the staining recipes for IDH, MDH, SKDH, PGI, and TPI have been described. (Estilai et al., 1990); the recipe for ACO staining was reported in Garvin (1987). The gel used for ACO, PGI, and SKDH consisted of 9.6% starch and 0.009 M L-histidine, titrated to pH 6.0 with citric acid. The electrode buffer was 0.065 M L-histidine, titrated to pH 6.0 with citric acid (Ellstrand, 1984). The extracts were absorbed onto filter paper wicks that were loaded into a transverse slice cut in the starch gels. Electrophoresis was conducted at 4°C, 150 V and 25 MA. After 6 h, gels were sliced and stained.

Designation of allozymes was based on the migration distance of the most common allele at each locus, which was assigned the number 100. Other alleles were identified numerically as to the percentage of migration compared with the 100 allele.

Inheritance analyses were carried out using a χ^2 goodness-of-fit test assuming monogenic and codominant expression of alleles at a single locus (Table 1).

Results and Discussion

Enzymes ACO, SKDH, and PGI showed allozyme variation at four loci, the inheritance of which are discussed below.

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Aconitase. ACO showed two zones of activity, polymorphic at the fast zone, ACO-1, and monomorphic at the slow zone, ACO-2. At ACO-1, two bands with different mobility (representing alleles 100 and 82) were identified (Figs. 1A and 2). Hybrid progenies between ACO-1 variants showed the two parental bands with no intermediate heterodimeric band (Table 1). Segregation analyses of ACO-1 confirmed the hypothesis that one locus controls the fast zone. In guayule, ACO-1 allozymes are monomeric, similar to those observed for other plant spe-

ties, such as *Festuca ovina* (Weibull et al., 1988) and *Phaseolus acutifolius* (Garvin et al., 1989).

Shikimate dehydrogenase. SKDH showed two zones of activity, designated SKDH-1 and SKDH-2, for the fast- and the slow-migrating zones, respectively (Figs. 1 and 2). At zone 1, three bands representing three alleles, fast (allele 105), intermediate (allele 100), and slow (allele 94), were recognized. Three alleles (100, 88, and 76) were also found for the SKDH-2 locus. Crosses between homozygous plants with 105/105 and

Table 1. Segregation of three isozymes in diploid guayule

Isozyme (locus)	Parental genotypes	Frequencies of observed genotypes	Total	Expected ratio	χ^2	P
ACO-1	$\frac{82}{82} \times \frac{100}{82}$	26 $\frac{100}{82}$: 19 $\frac{82}{82}$	45	1:1	1.09	0.5 > P > 0.1
SKDH-1	$\frac{100}{94} \times \frac{100}{100}$	27 $\frac{100}{100}$: 34 $\frac{100}{94}$	61	1:1	0.803	0.5 > P > 0.1
SKDH-2	$\frac{100}{100} \times \frac{100}{76}$	20 $\frac{100}{100}$: 24 $\frac{100}{76}$	44	1:1	0.364	0.9 > P > 0.5
PGI-2	$\frac{121}{114} \times \frac{106}{100}$	15 $\frac{121}{106}$: 18 $\frac{121}{100}$: 15 $\frac{114}{106}$: 19 $\frac{114}{100}$	64	1:1:1:1	0.761	0.9 > P > 0.5
	$\frac{121}{106} \times \frac{114}{106}$	20 $\frac{121}{114}$: 15 $\frac{121}{106}$: 11 $\frac{114}{106}$: 10 $\frac{106}{106}$	56	1:1:1:1	4.427	0.5 > P > 0.1

Fig. 1. Expression of guayule isozymes. (A) ACO variants and their hybrid (left). (B) SKDH, homozygous parents (far left and far right) and their hybrids (middle) at the fast and slow zones. (C) Segregating progenies at SKDH-2, putative genotypes, from left to right, Parent (100/76), progenies (100/100, 100/100, 100/100, 100/76, 100/76, 100/76, 100/76, 100/76), and parent (100/100). (D, E) PGI-2 segregation. (D) From left to right, parents (121/106 and 114/106) and their hybrids (121/114, 114/106, 121/106, 121/114, 121/114, 121/106, 114/106, and 106/106). (E) Parents (106/100 and 121/114) and their hybrids (114/100, 114/106, 121/106, 121/100, 121/106, and 114/100).

ACO-1	SKDH-1	SKDH-1	PGI-2	PGI-2
82 100 100 82 82 100	105 105 100 105 100 100	105 105 105 105 105 105 105 105	121 114 121 121 114 106 106 114 106 106	121 121 121 114 114 106 114 106 100 106 100 100
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-- --	-- --	-- -- -- --	-- -- -- --	-- -- -- --
100 100 100 100 100 100	76 100 100 76 76 100	100 100 100 100 76 100 76 100		
ACO-2	SKDH-2	SKDH-2		

Fig. 2. Schematic illustration showing isozyme patterns for ACO-1, ACO-2; SKDH-1, SKDH-2, and PGI-2 in guayule.

100/100 genotypes at the SKDH-1 zone produced 20 progenies, all of which showed the hybrid pattern with two bands (105/100). Hybrid progenies obtained from crosses between two homozygous 100/100 and 76/76 plants at SKDH-2, also produced two bands (100/76) indicating monomeric structure (Fig. 1 B, C). The monomeric behavior of SKDH allozymes in guayule conformed with that found in other species (Garvin et al., 1989; Tanksley and Rick, 1980). Since self-incompatibility prevented production of F₂ generations through selfing, segregation analyses were based on the results from two crosses shown in Table 1. No significant differences were found between the observed and expected ratios, which indicated that SKDH at each zone is controlled by one locus.

Phosphoglucose isomerase. PGI showed four zones of activity designated PGI-1, PGI-2, PGI-3, and PGI-4. Allozymes of PGI-1 appeared to be variable. However, the genetic basis of PGI-1 was not addressed due to poor resolution of the allozymes. As in other species, PGI-1 allozymes maybe localized in plastids (Weeden, 1983; Weeden and Gottlieb, 1979). Five bands (representing alleles 121, 114, 106, 100, and 95) were observed at zone PGI-2 in different individuals. Hybrid individuals at PGI-2 showed a three-banded pattern, which suggests that PGI-2 enzymes are active as dimers (Figs. 1 and 2). The dimeric structure of PGI has also been demonstrated in tomato (*Lycopersicon esculentum*; Tanksley, 1980) and tepary bean (*Phaseolus acutifolius*; Garvin et al., 1989). Inheritance analyses indicated that the PGI-2 zone is coded by one locus (Table 1). Plants showed one, two, or three bands at PGI-3 zone. PGI-4 zone was observed in some plants, but it was of a poor staining quality. Consequently, we did not characterize those two zones. The question remains whether the bands observed at PGI-3 are the product of intergenic cross dimerization or products of a separate locus. More research is needed to improve staining intensity of the PGI-4 zone before the inheritance of PGI at these two zones can be elucidated.

The genetic control of the isozyme variation that resulted from this study is valuable for identification of the zygotic and asexual progenies produced by facultative apomictic polyploid guayule plants. These markers may also be used to determine the degree of apomixis in different guayule genotypes. In addition, the potential association of these isozymes with traits of interest, such as rubber content and rubber quality, could provide for early screening of many plants at seedling stage, thus simplifying and speeding up the guayule improvement program.

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