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Duplicated phosphoglucose isomerase genes in avocado

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Summary. Avocado (*Persea americana*) cultivars were assayed for phosphoglucose isomerase (PGI) isozymes using starch gel electrophoresis. Three PGI genes were identified: one monomorphic locus, *Pgi-1,* coding for the plastid isozyme and two independently assorting loci, *Pgi-2* and *Pgi-3,* coding for the cytosolic isozymes. The genetic analysis was based on comparisons of PGI zymograms from somatic and pollen tissue and on Mendelian analysis of progeny from selfed trees. The isozymic variability for PGI can be used for cultivar identification and for differentiating between hybrid and selfed progeny in avocado breeding.

Key words: Gene duplication – Phosphoglucose isomerase isozymes – *Persea americana* – Breeding

Introduction

Duplication of genes that code for dimeric enzymes can be verified by progeny testing (Gottlieb 1977) and by comparing the banding patterns obtained from extracts of somatic and pollen tissue (Weeden and Gottlieb 1979). A diploid plant heterozygous for duplicated dimeric enzymic genes shows the heterodimeric allozymes (intragenic heterodimers) which are absent in pollen (haploid) zymograms. The isozymic heterodimers (intergenic heterodimers) are present both in pollen and somatic extracts.

Avocado (*Persea americana* Mill.) is an insect pollinated crop of synchronous dichogamy which is the subject of intensive breeding work (Bergh 1975). Since hand pollinations result in very low fruit set, the common procedure used for hybridization is to cage the desired parents in a screenhouse with a beehive. The fruit collected from the caged trees can be either the result of an outcrossing event or selfing. As previously demonstrated, identification of the pollen parent can be achieved by enzyme electrophoresis providing there is known isozymic variability between the parents (Degani and Gazit 1984).

Zymograms of the dimeric enzyme phosphoglucose isomerase (PGI; EC 5.3.1.9) in avocado show complex patterns characteristic of duplicated enzymic genes. In this study we present evidence for PGI gene duplication in avocado.

Materials and methods

The following avocado cultivars were examined in this study: 1) 'Fuerte', 2) 'Hass', 3) 'Nabal', 4) 'Horshim', 5) 'Ettinger', 6) 'Tova', 7) 'Seedling A' (Breeding material), 8) 'Shomrat', 9) 'Benik', 10) 'Anaheim', 11) 'Seedling B', 12) 'Sharwill', 13) 'Regina', 14) 'Irwing', and 15) 'Pinkerton'. The cultivars 'Hass' (2) and 'Anaheim' (10) were assayed by comparing PGI zymograms from leaf and pollen tissue and also by progeny testing of embryos of selfed fruitlets (2) or leaves of selfed seedlings (10). Cultivars No. 4, 5, 6, 9 and 13 were compared for leaf and pollen isozymes while the remaining eight cultivars were characterized only by their sporophytic PGI pattern.

Extracts for electrophoresis were obtained from leaves, pollen and embryos. Fully expanded leaf sections of 6 mm^2 were ground in 100 μl extraction buffer containing 0.1 M potassium phosphate (pH 7.5), 0.1 M 2-mercaptoethanol, and 12% soluble PVP. Extracts were absorbed onto 4×6 mm Whatman No. 3 paper wicks. Pollen grains were collected by shaking 10 stamens, harvested at anthesis, in 100 μl of extraction buffer; the enzyme extracts were obtained by crushing the pollen in the same buffer. Embryo enzyme were extracted from thin slices (200 mg) of tissue previously frozen in liquid nitrogen by grinding the embryos into a fine powder and mixing them with the buffer (Torres 1984).

The starch gel buffer was 0.03 M Tris Citrate (pH 8.0) and the electrode buffer was 0.4 M Boric-NaOH (pH 8.7). The gels

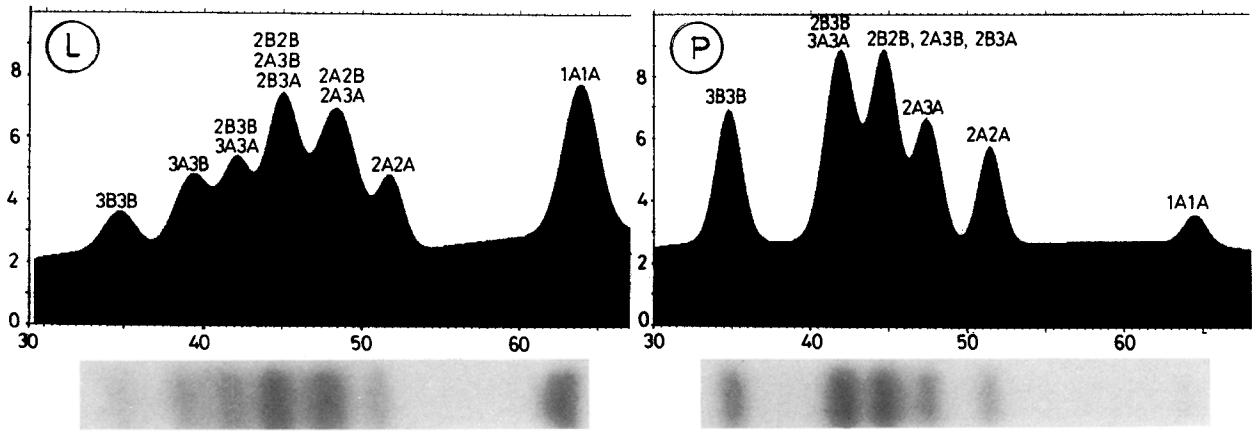

Fig. 1. Electrophoretic patterns and subunit compositions of PGI isozymes from leaf (*L*) and pollen (*P*) of the avocado cultivar 'Hass' (2). The photographs and the densitometric scans of the gels show the migration distances (mm) of the bands from the origin

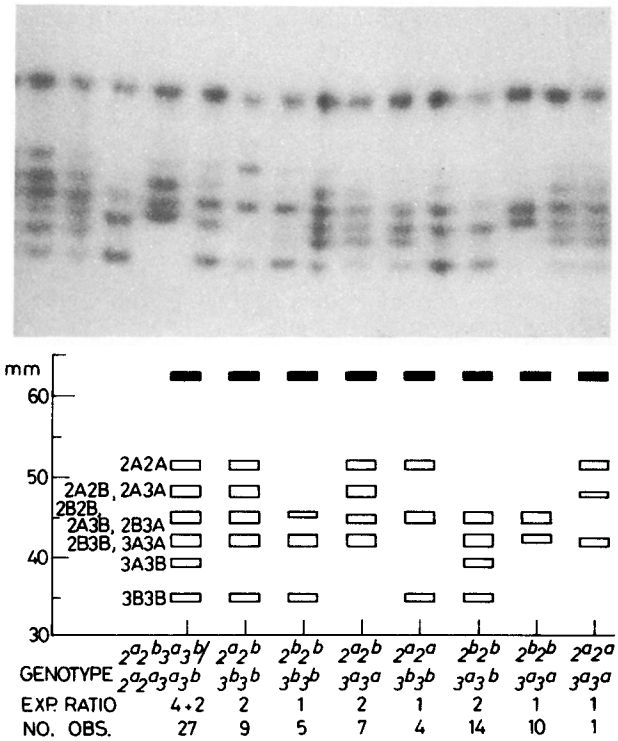

Fig. 2. Representation of segregation at *Pgi-2* and *Pgi-3* in progeny of self-pollinated 'Hass' (2). The photograph shows seven different patterns of the possible eight (*2A2A3A3A* is not shown). Diagramatic representation of the eight patterns, their subunit composition and coding genotypes are indicated along with the observed number of plants and their expected Mendelian ratios ($\chi^2 = 11.6$, $0.20 > P > 0.10$)

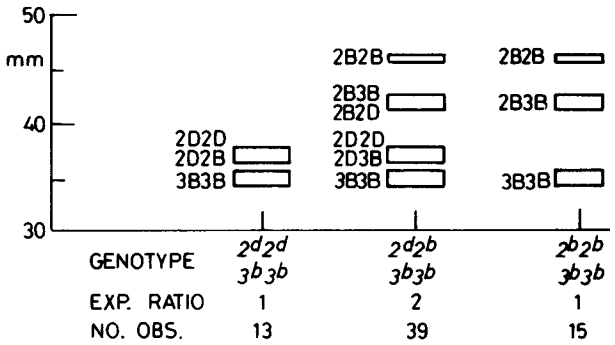

Fig. 3. Diagramatic electrophoretic patterns, genotypes and subunit composition of progeny from self-pollinated 'Anaheim' (10) segregating for *Pgi-2*. The expected Mendelian ratios and the observed number of plants is also indicated ($\chi^2 = 1.9$, $0.50 > P > 0.30$)

were run for 3 h at 3.8 mA/cm² gel cross section. The staining mixture for phosphoglucose isomerase (PGI) contained 20 mg fructose 6-phosphate, 12.5 mg NADP, 15 mg MTT, 5 mg PMS, 35 ml 0.2 M Tris-HCl buffer (pH 8.0), 5 ml 0.1 M $MgCl_2$ and 50 units of glucose 6-phosphate dehydrogenase. Gels stained for PGI from somatic and pollen tissue of the cultivar 'Hass' were scanned using a laser densitometer (LKB 2202) with an absorbance range of 1.0.

The system of nomenclature designated the most anodal isozyme as PGI-1 and its coding gene *Pgi-1*. The second and third isozymes from the anodal end were designated as PGI-2 and PGI-3 and their coding genes *Pgi-2* and *Pgi-3*. The subunits composing the isozymes were designated with capital letters and the alleles of the PGI loci received small letter superscripts. For example *Pgi-2*^{a} is the allele coding for the enzyme subunit PGI-2A.

Results and discussion

Genetic analysis of PGI in the cultivar 'Hass' was conducted by comparing zymograms of leaf and pollen tissue and by assaying selfed embryos resulting from bee pollinations of a caged tree. The PGI zymograms of

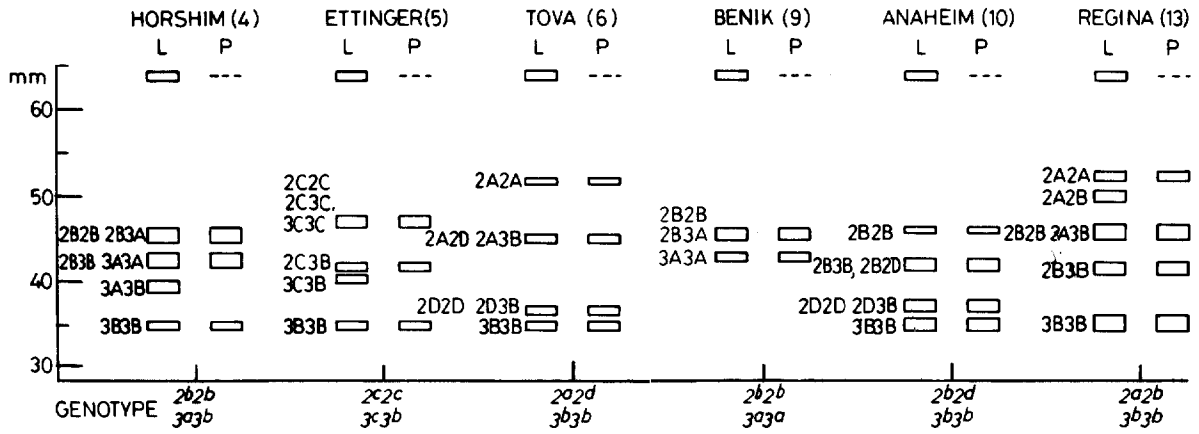

Fig. 4. Diagramatic representation of electrophoretic patterns of PGI from leaf (*L*) and pollen (*P*) extracts of six avocado cultivars. Possible genotypes and subunit composition are given for each cultivar

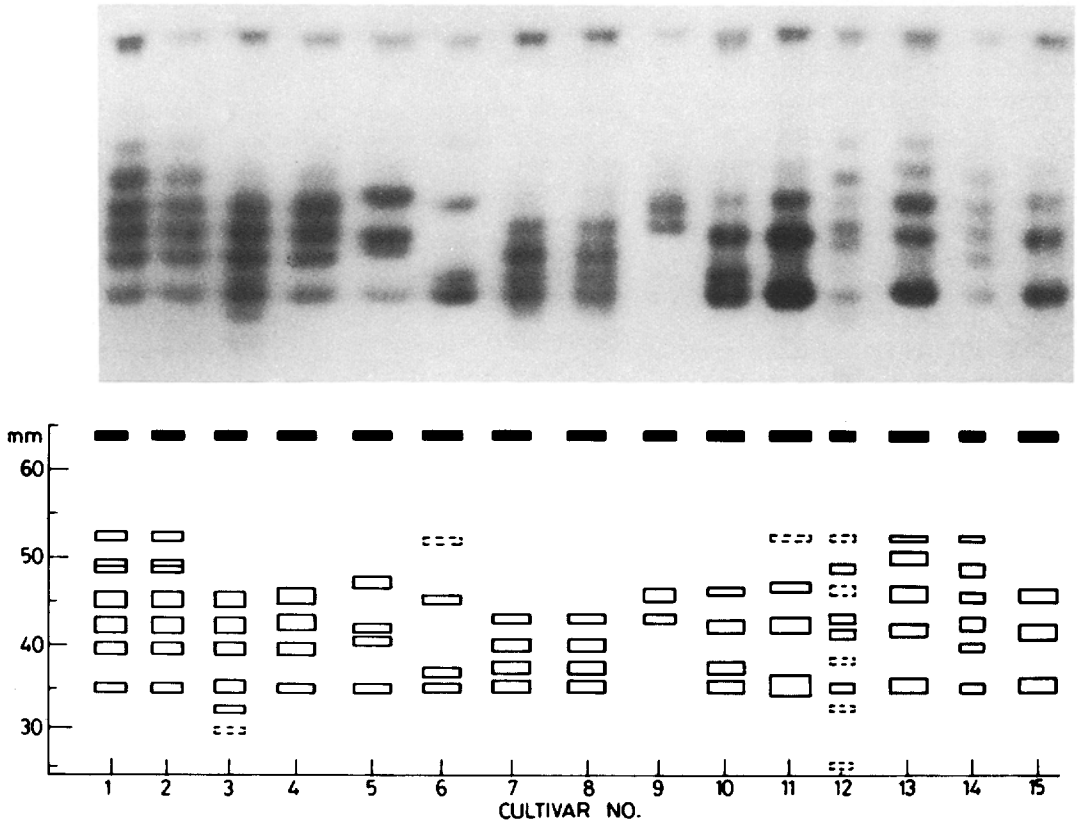

Fig. 5. Electrophoretic patterns of PGI extracted from leaves of avocado cultivars (see "Materials and methods" for cultivars' name). The isozymes located in the plastids are shown as solid boxes; the open boxes are the cytosolic isozymes and the open boxes with dotted margins indicate weakly stained cytosolic isozymes

'Hass' leaf and pollen and their densitometric scans are presented in Fig. 1. The patterns are distinguished by the very low activity of the most anodal migrating isozyme (64 mm) in crushed pollen compared to leaves. This differential activity of the anodal PGI suggests that this is the plastid isozyme (Weeden and Gottlieb 1980); this PGI was monomorphic in all the cultivars and therefore not included in our following analysis. The five isozymes common to the haploid and diploid tissues had the following migration distances from the origin: 52, 47, 45, 42 and 35 mm. The pollen pattern was distinguished by the absence of two isozymes

present in leaves: 49 and 39 mm. The more anodal of the two is the intragenic heterodimer of the first locus, *Pgi-2*, (subunit composition – 2A2B) while the band migrating 39 mm is the intragenic heterodimer of the second locus, *Pgi-3*, (3A3B). The two homodimers of *Pgi-2* are the bands migrating 52 mm (2A2A) and 45 mm (2B2B) and those of *Pgi-3* are 42 mm (3A3A) and 35 mm (3B3B). The pattern of such a double heterozygote is expected to exhibit 10 isozymes; however, due to an overlap in the electrophoretic mobilities of the isozymes we detected only seven distinct bands. The four intergenic heterodimers (heterodimeric isozymes) are: 2A3A at 47 mm, 2A3B and 2B3A both at 45 mm and 2B3B at 42 mm. The densitometric scans of the sporophytic and gametophytic zymograms show that the bands which according to our analysis are composed of more than one isozymic form have higher intensities than those composed of a single isozyme.

Our interpretation of the leaf versus pollen zymograms is strengthened by the progeny test. Selfing of a double heterozygote is expected to yield nine different genotypes. A total of 77 selfed 'Hass' embryos were assayed electrophoretically displaying 8 different patterns (Fig. 2); the two genotypes $2^a2^b3^a3^b$ and $2^a2^a3^a3^b$ produced the same electrophoretic patterns. The Mendelian ratio indicated that the duplicated loci assort independently in the same manner described for the cytosolic PGI in *Clarkia* (Gottlieb 1982).

The cultivar 'Anaheim' (10) has two alleles in common with 'Hass' (2): $Pgi\text{-}2^b$ and $Pgi\text{-}3^b$ (Fig. 5). Both leaf and pollen zymograms displayed a 4 banded pattern which suggested that the tree was heterozygous for *Pgi-2* and homozygous for *Pgi-3* (Fig. 4). The Mendelian ratios obtained from seedlings resulting from self pollination of 'Anaheim' were in agreement with the expected ratio of 1 : 2 : 1 (Fig. 3).

The proposed subunit composition of the PGI isozymes and the genotypes of the cultivars analysed by comparing zymograms of leaves and pollen is presented in Fig. 4.

The PGI patterns from leaf extracts of the 15 avocado varieties show no variability in the plastid isozyme, PGI-1, and high variation for the cytosolic isozymes (Fig. 5). Each variety had a unique PGI pattern except for the pairs 'Fuerte'-'Hass' (1, 2) and 'Seedling A'-'Shomrat' (7, 8). This variability can be used for cultivar identification and for the determination of the pollen parent in avocado crosses.

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