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Inheritance and linkage relationships of glutamate oxaloacetate transaminase isoenzymes in apple

2. The genes *GOT-2* and *GOT-4**

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Summary. Independent dimeric genes *GOT-2* and *GOT-4* determining activity for glutamate oxaloacetate transaminase (E.C.2.6.1.1; GOT) in zones GOT-II and GOT-IV respectively were identified. Three alleles were found for *GOT-2* and two for *GOT-4*, including a null allele for *GOT-2* which produced detectable heterodimeric bands but not homodimeric bands. Linkage studies with leucine aminopeptidase (E.C.3.4.11.1; LAP) genes suggested linkage of *GOT-2* with *LAP-2* ($r=0.13\pm0.23$) and *GOT-4* with *LAP-1* ($r=0.10\pm0.40$).

Key words: *Malus pumila* Mill – Glutamate oxaloacetate transaminase – Genes *GOT-2, GOT-4, LAP-1, LAP-2,* – Linkage

Introduction

Glutamate oxaloacetate transaminase (E.C.2.6.1.1; GOT) catalyses the reversible conversion of glutamate and oxaloacetate to α-ketoglutarate and aspartate. This enzyme, the most widely studied transaminase in plants, has been found to be polymorphic in several crops. Four isoenzymes of GOT associated respectively with the cytosol, chloroplasts, mitochondria and peroxisomes have been isolated from spinach leaves (Huang et al. 1976).

The genetic control and degree of polymorphism of GOT isoenzymes has been examined in many crops notably maize (Scandalios et al. 1975) and tomato (Tanksley and Rick 1980). The distribution of GOT in apple tissues and its seasonal variation has been examined (Cooper and Hill-Cottingham 1974); polymorphism has been reported amongst apple cultivars, which included three bands, a, b and c in the GOT-II zone (Weeden and Lamb 1985).

Four zones of enzymatic activity are known for GOT in apple tissues. These zones, GOT-I, GOT-II, GOT-III, and GOT-IV showed distinct banding patterns according to the allelic constituents of the genotypes. The hypothetical positions of bands representing null alleles for GOT-I and GOT-II were also determined (Manganaris and Alston 1987). Genetic studies of GOT-I have revealed a *GOT-1* locus, with six alleles, which is closely linked to an isocitrate dehydrogenase (E.C.1.1.1.42; IDH) gene, *IDH-1* and an *S* incompatibility locus (Manganaris and Alston 1987). The inheritance of electrophoretic variants in the GOT-II and GOT-IV zones is described in this paper.

Materials and methods

Plant material and sample preparation

Seedling progenies from controlled crosses were studied. Cotyledons, old leaves, flower buds, bark, young roots, pollen and seeds (mature and immature) were examined in addition to young leaves. Samples from plants of different ages were used throughout the year. Sample preparation from leaves and young roots followed methods previously described (Manganaris and Alston 1987).

The external scales of flower buds were removed before crushing since GOT activity was inhibited if they were included. It was found important to cover bark scraped from annual shoots immediately with the extraction solution to avoid discolouration.

Seed testae were removed since they were found to inhibit GOT activity; the remaining tissue (30–40 mg) was crushed with 0.5 ml extraction solution (0.05 M Na-phosphate buffer pH 7.1, 0.2 M sucrose, 10% (W/V) insoluble PVP, 14 mM mercaptoethanol). Anthers were collected from flowers at balloon stage and stored at −20 °C after drying. A quick rinse with pentane

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separated pollen from anthers after storage (Chevreau et al. 1985). Extracts were prepared by homogenisation of 100 mg pure pollen in 0.5 ml Tris-KCl 0.1 M pH = 7, followed by centrifugation.

Electrophoretic procedures

Previous procedures (Manganaris and Alston 1987) were followed. Where improved definition was required in the GOT-II zone, 0.12 M Tris-HCl was incorporated into the 'running' gel in place of Tris-glycine. The same electrophoretic technique was used for leucine aminopeptidase where gels were stained with L-leucine-β-napthylamide HCl (Arus et al. 1982).

Statistical analysis

The program Linkage-1 (Suiter et al. 1983) was used in gene and linkage analyses.

Results

Description of electrophoretic patterns

Bands for GOT-II occurred in either of two positions or both bands were present together with a third intermediate band. The two principal bands are designated a and b. All tissues examined showed activity in this zone; pollen and seeds showed greater activity than roots and bark. Pollen of diploid plants did not show the "hybrid" band.

Separation in the GOT-IV zone varied according to the tissue used producing five (leaves, bark) or eight (seeds, pollen) bands (Fig. 1). The three slower bands showed polymorphism producing three different phenotypes (Fig. 2) while it was difficult to define the faster bands. Of the three phenotypes, A had only the fast band a while B and C each had three bands, two differing quantitatively. The b band was intensively stained in the B phenotype, the remaining two bands being faint. In the C phenotype the centre ("hybrid") band was most intensively stained. These quantitative differences were not observed in all tissues or with each electrophoretic technique. Seeds and flower buds gave the clearest separation in this zone. Young leaves showed low activity. The electrophoretic system using tris-glycine pH 8.7, in the 'running' gels, gave the best resolution for GOT-IV.

Genetic control of GOT-II and GOT-IV electrophoretic variants

Crosses between parents with different single-banded GOT-II phenotypes gave only one type of plant with three bands in this zone; the two parental bands and an additional "hybrid" band of twice the intensity between the two parental bands. In a cross between a parent with three bands and a single-banded parent two classes of seedlings, single-band and three-bands, were produced in approximately equal frequency.

In no case was intergenic dimerization observed. The results in Table 1 can be explained on the basis of segre-

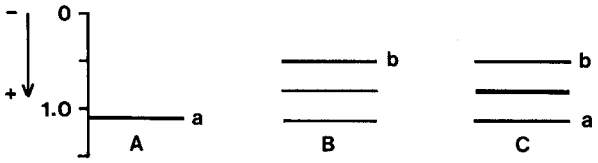

Fig. 2. Schematic representation showing polymorphism producing three different phenotypes for GOT-IV with migration distances (cm) of the electrophoretic bands

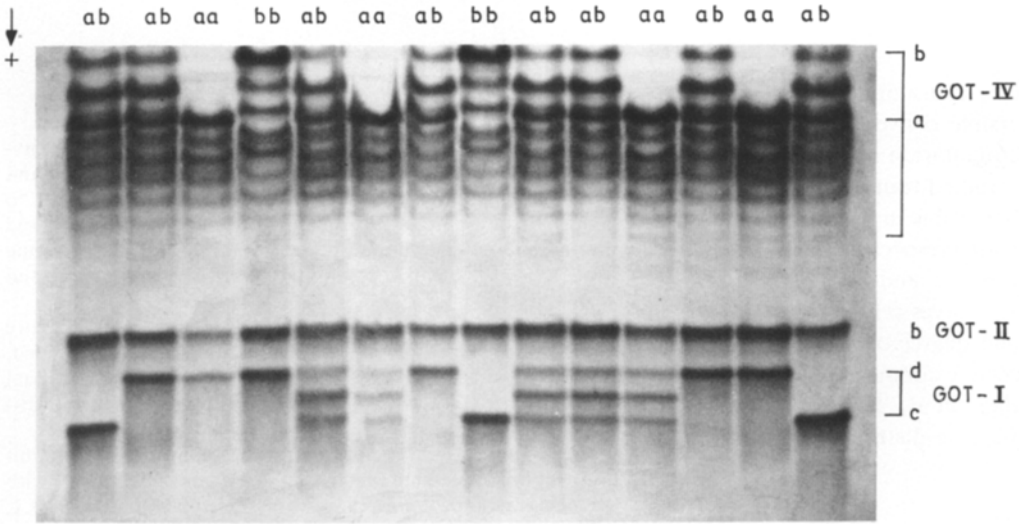

Fig. 1. Glutamate oxaloacetate transaminase zymograms from seed extracts of progeny F106:T30-9 (*GOT-4 ab*) × T31-12 (*GOT-4 ab*) showing genotypes at the *GOT-4* locus after prolonged "running"; anode is towards the bottom of the figure

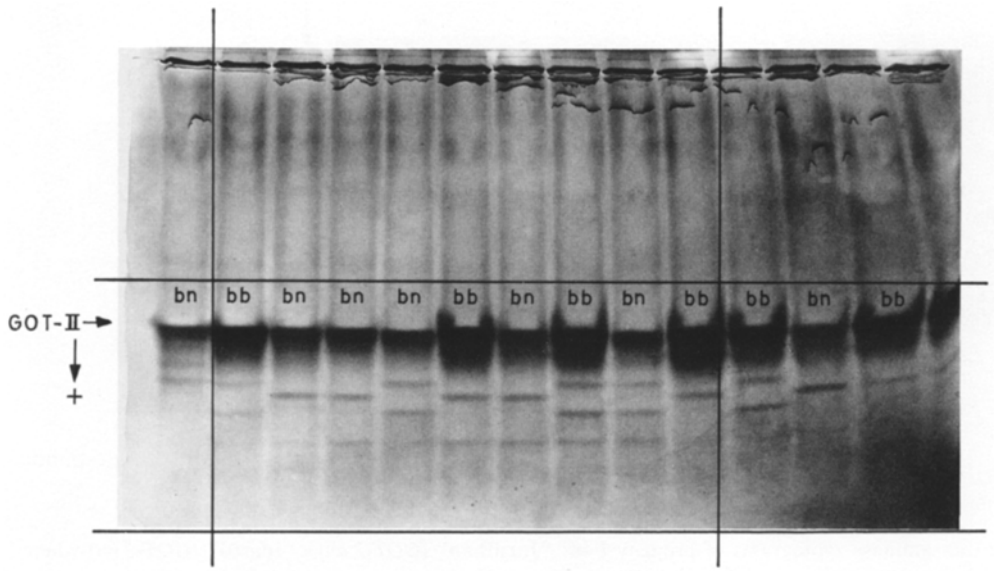

Fig. 3. Glutamate oxaloacetate transaminase zymograms of progeny F6:T31-12 (*GOT-2 bb*) × "Idared" (*GOT-2 bn*); genotypes *bb* and *bn* were distinguishable with the incorporation of Tris-HCl in the "running" gel

Table 1. Segregation for *GOT-2*

Family	Parental phenotypes	Progeny phenotypes				Expected ratio	χ^2	*P*
F48 ("Jonathan" × "Idared")	an × bn	16ab	11an	10bn	9nn	1:1:1:1	2.52	0.47
F5 & 6 ("Idared" × T31-12[a])	bn × bb	31bb	25bn			1:1	0.89	0.33
F9 & 10 ("Golden Delicious" × A463-70[b])	bn × bb	20bb	20bn			1:1	0.00	1.00
F11 & 21 ("Idared" × "Fiesta")	bn × bb	18bb	27bn			1:1	1.80	0.18
F34 ("Idared" × "Spartan")	bn × bb	23bb	23bn			1:1	0.00	1.00
F23 ("Vista Bella" × "Katja")	ab × bb	10ab	6bb			1:1	1.00	0.32
F24 ("Golden Delicious" × "Golden Hornet")	bn × bb	12bb	12bn			1:1	0.00	1.00
F25 ("Gloster 69" × "Golden Hornet")	ab × bb	11ab	16bb			1:1	0.92	0.34
F27 & 29 ("Delprim" × "Katja")	bn × bb	16bb	17bn			1:1	0.03	0.86
F30 ("Idared" × "Golden Hornet")	bn × bb	7bb	7bn			1:1	0.00	1.00
F93 ("Jonathan" × A849-7[c])	an × bb	18ab	16bn			1:1	0.12	0.73
F98 (A722-6[d] × "Cox")	bn × bb	6bb	6bn			1:1	0.00	1.00
F99 (A723-14[e] × "Jester")	bb × ab	10ab	8bb			1:1	0.22	0.64
F100 (A723-5[e] × "Jester")	bb × ab	18ab	16bb			1:1	0.12	0.73
F102 ("N. Spy" × "Worcester")	bb × ab	7ab	15bb			1:1	2.91	0.09
F104 (A721-19[f] × "Cox")	an × bb	8ab	8bn			1:1	0.00	1.00
F115 ("Kent" × "Fiesta")	ab × bb	15ab	12bb			1:1	0.33	0.56
F135 ("Idared" × A679-12[g])	bn × bb	47bb	38bn			1:1	0.95	0.33
F140 ("Glengyle Red" × 3762[h])	an × bb	19ab	24bn			1:1	0.58	0.44

[a] "Cox" × "Idared"
[b] "Granny Smith" × "Boutiful" ("Cox" × ?)
[c] "Edward VII" × A423-2
[d] "Worcester" × ORI8T26
[e] "Worcester" × PI172623
[f] "Worcester" × OBIR6T25
[g] "Worcester" × A363-38
[h] *M. robusta* o.p.

gation for a single gene (*GOT-2*) with two alleles (*a* and *b*) controlling bands a and b respectively. The data from reciprocal crosses (F5 and 6, F9 and 10, F11 and 21, F27 and 29) were pooled since no differences between them were observed. The presence of the "hybrid" band denotes the dimeric structure of this enzyme.

A near-null allele (*n*) was found for *GOT-2*, which had no detectable activity but was deduced from the position of very weak "hybrid" bands (Fig. 3). Similar alleles have been reported in maize (Goodman et al. 1981) and in apples for *GOT-1* (Manganaris and Alston 1987). Identification difficulties arose when the *n* allele

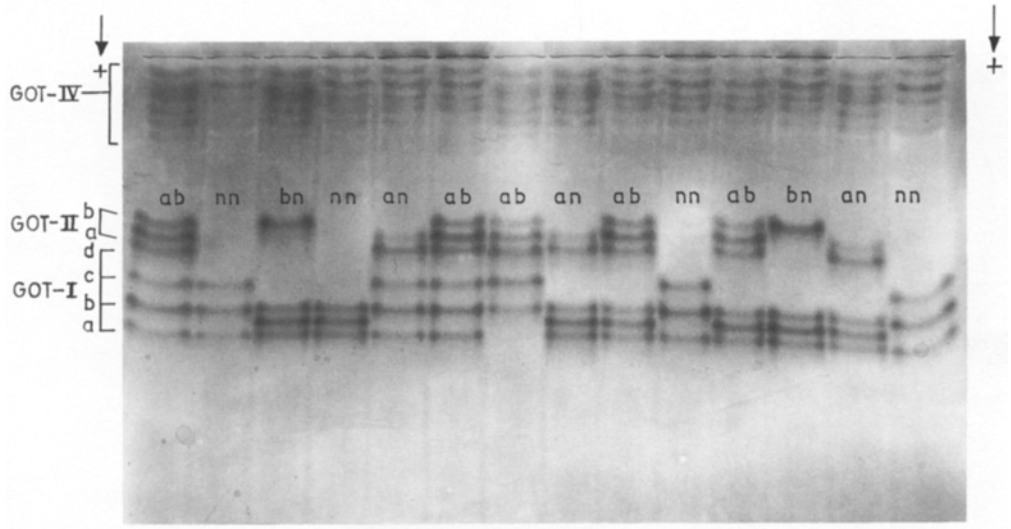

Fig. 4. Glutamate oxaloacetate transaminase zymograms of progeny F48: "Jonathan" (*GOT-2 an*) × "Idared" (*GOT-2 bn*) where homozygous *nn* seedlings did not show any activity in GOT-II zone

Table 2. Segregation for *GOT-4*

Family	Parental phenotypes	Progeny phenotypes			Expected ratio	χ^2	*P*
F106 (T30-9[a] × T31-12[a])	ab × ab	8aa	26ab	8bb	1:2:1	2.38	0.30
F37 ("Granny Smith" × "Kent")	ab × aa	21aa	24ab		1:1	0.20	0.65
F46 ("Discovery" × "Red Jade")	ab × aa	10aa	6ab		1:1	1.00	0.32
F70 (A172-2[b] × A814-137[a])	ab × aa	17aa	11ab		1:1	1.28	0.26
F633 ("S. Seedless" × "G. Carpenter")	ab × aa	30aa	24ab		1:1	0.67	0.42
F36 ("N. Spy" × "Winter Majetin")	ab × ab	31 (bb + 2ab)		13aa	3:1	0.48	0.49
F49 ("Winter Majetin" × N. Spy")	ab × ab	20 (bb + 2ab)		13aa	3:1	3.15	0.06
F93 ("Jonathan" × A849-7[d])	ab × ab	25 (bb + 2ab)		4aa	3:1	1.94	0.16
F107 ("Fiesta" selfed)	ab × ab	35 (bb + 5ab)		10aa	3:1	0.19	0.65

[a] "Cox" × "Idared"
[b] "James Grieve" × "OR33T90"
[c] "Cox" × A467-74
[d] "Edward VII" × A423-2

was accompanied by the *b* allele. The most obvious difference between *bb* and *bn* genotypes was in the intensity of the b band. When the *b* allele was present with the *n* allele the corresponding b band was weaker than when it was in the homozygous state. Tris-HCI pH 8.7, incorporated into the running gel, improved clarity in this zone sufficiently to distinguish between the two genotypes. The hypothetical position of the main null band was deduced from the position of the hybrid bands, which were assumed to be midway between their components. Further evidence for a null allele at this locus occurred in the Jonathan (*an*) × Idared (*bn*) progeny where the expected *nn* seedlings (no activity in the GOT-II zone) were identified (Fig. 4).

Table 2 shows the segregation of the GOT-IV enzymic bands in nine progenies. These results are in agreement with the hypothesis that these bands are coded by a single gene (*GOT-4*) with two alleles (*a* and *b*) which determine the phenotypes A and B respectively (Fig. 2). The heterozygote *ab* determines the C phenotype. The presence of an intermediate intensively staining band indicates that the GOT-IV enzyme is dimeric. When genotypes *ab* and *bb* were not distinguishable the pooled data were used to calculate the chi-square value.

Analysis of linkage

Linkage between the GOT loci *GOT-2* and *GOT-4* and 20 other loci was examined; these included loci for isoen-

Table 3. Estimates of linkage between GOT and LAP loci

Family	Proposed parental genotypes*	Progeny phenotypes**	No. of sdls.	χ^2	(df)	*P*	r	(SE)
	GOT-2/LAP-2							
F5 & F6	*bn*/*ab* × bb/aa	22 bb/aa 7 bb/ab 5 bn/aa 9 bn/ab	53	15.91	(1)	< 0.01	0.22	(0.057)
F11 & F21	*bn*/*ab* × bb/aa	7 bb/aa 3 bb/ab 2 bn/aa 13 bn/ab	25	8.53	(1)	< 0.01	0.20	(0.080)
F30	*bn*/*ab* × bb/aa	6 bb/aa 0 bb/ab 1 bn/aa 6 bn/ab	13	9.55	(1)	< 0.01	0.08	(0.074)
F34	*bn*/*ab* × bb/aa	14 bb/aa 1 bb/ab 2 bn/aa 14 bn/ab	31	20.26	(1)	< 0.01	0.10	(0.053)
F93	*an*/*ab* × bb/aa	14 ab/aa 1 ab/ab 1 bn/aa 14 bn/ab	30	22.53	(1)	< 0.01	0.07	(0.046)
F101	*an*/*ab* × *ab*/*an*	6 aa/(aa + an) 0 aa/ab 1 aa/bn 8 ab/(aa + an) 1 ab/ab 0 ab/bn 0 an/(aa + an) 1 an/ab 0 an/bn 0 bn/(aa + an) 1 bn/ab 3 bn/bn	21	19.47	(6)	< 0.01	0.18	(0.072)
F135	*bn*/*ab* × bb/an	34 bb/(aa + an) 7 bb/ab 5 bb/bn 5 bn/(aa + an) 20 bn/ab 13 bn/bn	71	30.90	(2)	< 0.01	0.20	(0.044)
			Pooled estimate				0.13	(0.024)
	GOT-4/LAP-1							
F36 & F49	ab/bb × *ab*/*bc*	3 aa/bb 31 (ab + bb)/bb 23 aa/bc 19 (ab + bb)/bc	76	17.62	(1)	< 0.01	0.12	(0.058)
F46	*ab*/*bc* × aa/bb	1 aa/bb 6 ab/bb 7 aa/bc 0 ab/bc	14	10.50	(1)	< 0.01	0.07	(0.069)
F93	ab/bb × *ab*/*bc*	0 aa/bb 19 (ab + bb)/bb 4 aa/bc 6 (ab + bb)/bc	29	8.82	(1)	< 0.01	0.12	(0.094)
			Pooled estimate				0.10	(0.040)

* Within each parental genotype the first two alleles are those of the first locus; alleles written in *italics* are assumed to be in coupling phase

** Genotypes within brackets are not distinguishable

zymes and loci for agronomic characters. Close linkage (Table 3) was found only between *GOT-2* and *LAP-2* ($r = 0.13 \pm 0.023$) and *GOT-4* and *LAP-1* ($r = 0.10 \pm 0.040$) loci coding for leucine aminopeptidase (Manganaris, in preparation).

Tests for linkage between *GOT-1* and *GOT-2* were made on eight progenies. None were significant at the 5% level. Seven progenies were examined for linkage between *GOT-1* and *GOT-4* and one for linkage between *GOT-2* and *GOT-4*. None were significant. In addition linkage tests between all combinations of the three GOT loci and the other three isoenzymic loci, *IDH-1*, *LAP-1*, and *LAP-2* showed only the three linkage groups and suggests that they are located on three different chromosomes.

Discussion

The identification of two further GOT loci incorporating five alleles is a significant step towards delineating the genetic basis of isoenzymic variation in apple.

An active band was not identified in the position designated b by Weeden and Lamb (1985). Bands observed in that position were apparently "hybrid" bands involving a null allele. The nomenclature of the bands was changed accordingly; the b bands in this work correspond to the c band described by Weeden and Lamb (1985). Thus new genotypes should be ascribed to Golden Delicious (*bn*), Jonathan (*an*) and Idared (*bn*). A null allele for *GOT-2* (*Aat-p*) was postulated by Weeden and Lamb (1987), their results were deduced from a single progeny derived from Rome Beauty (*an*) where the genotypes *aa* and *an* were not distinguished. Genotypes were not ascribed to any other cultivars in that work.

The three clearly identified GOT loci, *GOT-1*, *GOT-2* and *GOT-4* provide a sound basis for linkage studies, their independent segregation suggesting that they are carried on distinct chromosomes. *GOT-1* is linked to *IDH-1* and an *S* locus (Manganaris and Alston 1987) and two further linkage groups have now been established involving GOT and LAP loci. It is possible to begin the construction of linkage maps for apple chromosomes. Only one other well defined group (*ACP-1–ENP-1–l*) has been determined in this crop (Manganaris and Alston 1988) although other isoenzymic linkage groups have been proposed (Weeden and Lamb 1987).

Three segregating loci with a combined total of 11 alleles including two semi-null alleles have now been identified which establishes variation in GOT isoenzymes as an important source of molecular markers in apple.

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