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Inheritance and linkage relationships of glutamate oxaloacetate transaminase isoenzymes in apple

1. The gene *GOT-1*, a marker for the *S* incompatibility locus *

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Summary. Four zones of enzymatic activity for glutamate oxaloacetate transaminase (GOT) were found in apple tissue. A dimeric gene, *GOT-1*, determining the fastest migrating zone, was identified. Six alleles were found, including a near null allelle which produced detectable heterodimeric bands but not homodimeric bands. A marked deficit or absence of certain genotypes in all backcrosses and in some crosses between unrelated varieties was attributed to the close linkage ($r = 0.02 \pm 0.005$) of *GOT-1* with the incompatibility *S* locus. *GOT-1* was also closely linked with the isocitrate dehydrogenase locus *IDH-1* (0.03 ± 0.01). Proposed incompatibility genotypes for four cultivars, and the linked *GOT-1* alleles are 'Cox': S_1b/S_2d, 'Idared': S_3a/S_4c, 'Fiesta': S_3a/S_2d and 'Kent': S_3a/S_1b.

Key words: *Malus pumila* Mill – Glutamate oxaloacetate transaminase – Incompatibility – Genes *GOT-1* – *IDH-1* – *S* – Linkage

Introduction

Most apple varieties are self-incompatible (Brown 1975); this phenomenon is determined by a multiallelic *S* gene (Kobel 1954) having a gametophytic effect (Frankel and Galun 1977). However, most workers have been unable to demonstrate a clearcut distinction between compatibility and incompatibility in apple, since environmental and physiological factors have variable effects on pollen tube growth (Modlibowska 1945; Williams and Maier 1977; Petropoulou 1985). It is therefore difficult to identify the *S* locus using such conventional methods as assessing apple fruit set after controlled pollination and measuring pollen tube growth in styles (Spiegel-Roy and Alston 1982).

A large number of genes coding for isoenzymes of different electrophoretic mobility have been identified in apple (Chevreau 1985; Weeden and Lamb 1985). The delineation of these genes is rarely affected by environmental and physiological factors and since their alleles are mostly codominant it is possible to distinguish all genotypes (Tanksley 1983). However, null alleles may confuse genetic interpretation since they fail to produce electrophoretically detectable homo- or heterodimers (Goodman et al. 1981). Where close linkage occurs between genes coding for isoenzymes and genes determining economic characters, they can be used readily to identify desirable genotypes. Linkages found in other crops between isoenzyme and incompatibility loci include peroxidase isoenzymes in *Secale cereale* (Wricke and Wehling 1985) and *Nicotiana alata* (Labroche et al. 1983) and phosphoglucose isomerase in *Lolium perenne* (Cornish et al. 1980).

Glutamate oxaloacetate transaminase (GOT, EC2.6.1.1.) or aspartate aminotransferase (AAT) is known to be polymorphic in several genera and isoenzymic genes have been identified (Gottlieb 1982). Polymorphism of this enzyme was shown to occur in apple by Weeden and Lamb (1985). On the basis of band phenotypes in 54 apple cultivars and results of progeny tests (unpublished), these authors described two gene loci, *AAT-1* and *AAT-2*, with four (a, b, c, d) and three (a, b, c) alleles respectively.

Further data on the genetic basis of polymorphism for this enzyme in apple and linkage of one of the gene loci involved with the *S* incompatibility locus and a locus for isocitrate dehydrogenase, *IDH-1* with two (*a*, *b*) alleles (Chevreau 1985), are reported in this paper.

Materials and methods

Plant material

Progenies studied included some raised from crosses made in the field and under glass at the Brogdale Experimental

* The results reported in this paper are part of a PhD Thesis by the first author

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Horticulture Station, Faversham, in pollination studies with new varieties; others were raised at East Malling specifically for this work. Supplementary material came from the breeding programme at East Malling. Crosses made were between siblings, between unrelated varieties, between varieties with one parent in common, and back-crosses. F_2 seedlings from the partly self-fertile cultivars 'Fiesta' ('Cox'×'Idared') and 'Early Victoria', and partly self-fertile clones of 'Cox' (Campbell and Lacey 1982) were also examined.

Sample preparation

Cotyledons and actively growing leaves were used. Tissue (200 mg) was crushed into a fine powder in a mortar using liquid nitrogen to facilitate maceration and to keep the temperature low. Immediately, 1.5 ml of extraction solution (0.05 M Na-phosphate buffer pH 7.1, 0.2 M sucrose, 10% w/v insoluble PVP, 0.14 M 2-mercaptoethanol) was mixed with this powder and left in a refrigerator to thaw. Homogenization was completed after thawing and the resulting extract was poured into plastic tubes and centrifuged at 35,000 g (30 min, 4 °C). The supernatant was transferred to plastic vials and either used immediately or kept in a freezer at −20 °C. A small amount of bromophenol blue was added as a tracking dye. Ice was used to keep the temperature low during preparation.

Electrophoretic procedure

Samples were run in polyacrylamide slab gels (18×14× 1.5 cm) prepared according to Davis (1964), however, a sample gel was not used. Tris-glycine pH 8.8 (0.19 M), incorporated into the "running" gel instead of tris-HCl, increased the activity of the bands. Samples were run at constant voltage (100 V) until they passed into the "stacking" gel (≃30 min) and then at 150 V until they entered the "running" gel (≃30 min more) before the voltage was increased to 350 V for 4 h. The temperature of the electrode buffer was kept at 4 °C with the use of a cooler. At the end of electrophoresis, gels were stained for GOT in an incubator at 30 °C (Siciliano and Shaw 1976). Stained gels were washed and fixed in 7% acetic acid.

Starch-gel electrophoresis with L-histidine/citric acid buffer pH 6.5 incorporated in the gel (Cardy et al. 1980) was used for the separation of IDH.

Squash preparations

Ten flowers at the balloon stage were collected from each cross, de-petalled, emasculated, put in damp "Oasis" (floral products, Smithers Oasis, France F-67000), pollinated and stored immediately at a constant temperature of 20 °C for 4 days. Fresh pollen was used, the viability being tested before use with fluorescein diacetate (Heslop-Harrison and Heslop-Harrison 1970). At sampling, all tissues were removed from healthy flowers except for styles and ovules, which were immersed in 5% sodium sulphite and autoclaved immediately for 25 min (Currier 1957). As much as possible of the surrounding ovary tissue was removed carefully and the styles were spread on a slide, flooded with 0.1% aniline blue stain and squashed under a coverslip. Preparations were examined microscopically at x60–x100. The pollen tubes which reached the base of the style and the ovule for each flower were counted. Compatibility was assessed on the basis of the number of pollen tubes reaching the ovule.

Field assessment of compatibility was made on 50 to 100 flowers/cross. One or two flowers in each cluster were chosen at the balloon stage and the rest were removed. The flowers were de-petalled, emasculated, pollinated and immediately covered with paraffin wax paper bags, which were removed after two weeks. Fruit set was recorded 50 days after full bloom.

Statistical analysis

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

Results

Description of electrophoretic patterns

The electrophoretic separation of GOT revealed four zones of enzymatic activity, designated GOT-I, GOT-II, GOT-III and GOT-IV in sequential order from the anode (Fig. 1). Clear resolution was obtained in the GOT-I zone (Fig. 2), where at least one band and a maximum of three bands were usually observed. A single band was observed in any one of five positions, or three bands involving any two of these positions with the addition of an intermediate 'hybrid' band. In certain cultivars and their progenies a faint band was observed cathodally to one of the principal bands (Fig. 3). In addition to the four bands, a, b, c, d designated by Weeden and Lamb (1985) a fifth band, e was located close to the a band (Fig. 4).

Genetic control of GOT-I electrophoretic variants

In twelve progenies (Table 1), 1 : 1 : 1 : 1 ratios of GOT-I phenotypes could be explained on the basis of segregation for a single multi-allelic gene, each band being

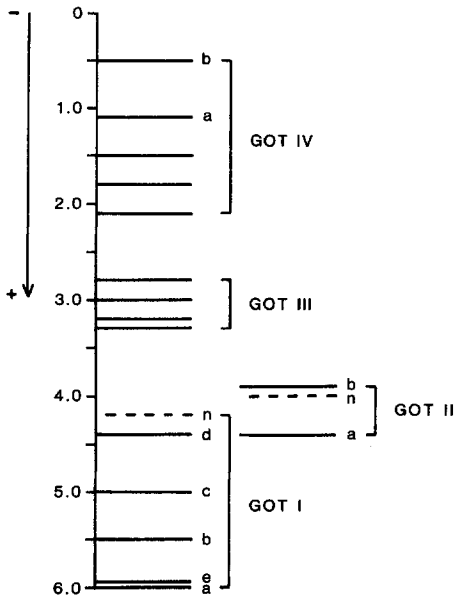

Fig. 1. Schematic representation showing the migration distance in cm of GOT electrophoretic bands. Bands with intermediate mobilities present in heterozygotes are not shown

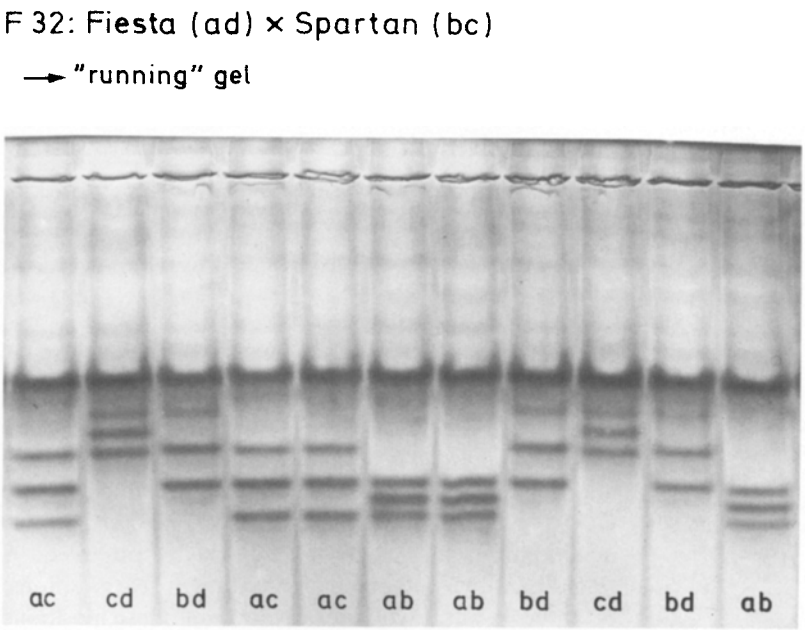

Fig. 2. Glutamate oxaloacetate transaminase zymograms of progeny from F32 'Fiesta' (*ad*)×'Spartan' (*bc*) showing all expected phenotypes. Anode is towards the bottom of the figure

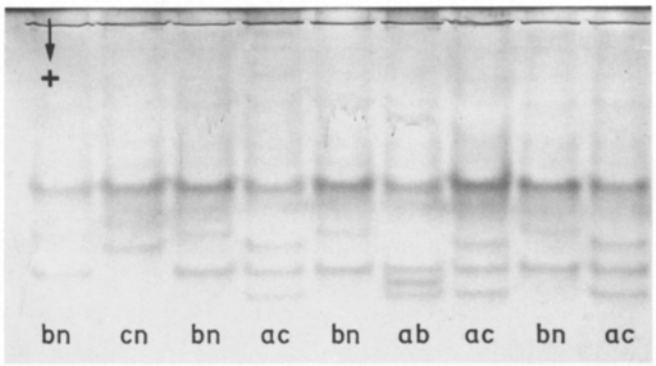

Fig. 3. Glutamate oxaloacetate transaminase zymograms of progeny from F36 'Northern Spy' (*an*)×'Winter Majetin' (*bc*). A faint heterodimeric band appears above the b and c bands when the n allele is present. Anode is towards the bottom of the figure

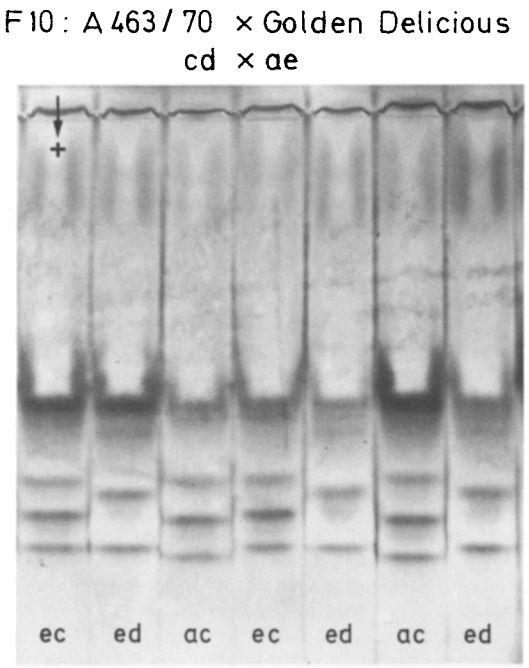

Fig. 4. Glutamate oxaloacetate transaminase zymograms of progeny from F10 A463/70 (*cd*)×'Golden Delicious' (*ae*), showing segregation of the a and e bands. Anode is towards the bottom of the figure

controlled by a different allele. This gene is now designated *GOT-1* with five alleles *a, b, c, d* and *e* corresponding to the a, b, c, d and e zymogram bands, and an *n* allele responsible for the faint bands. Segregation of faint bands in the progenies examined and their positions in the gel agree with the hypothesis that a near-null allele is present, which does not produce homodimeric bands but produces faint intermediate heterodimeric bands. As a heterodimeric band may be expected to migrate into a position midway between its two parental bands, the hypothetical position of the *n* allele can be deduced (Fig. 1).

In 22 progenies two of the four phenotypes expected on the basis of single gene segregations were in a marked deficit or missing (Table 2). The most likely explanation is close linkage between *GOT-1* and the incompatibility locus, with both parents having one *S* allele in common. Pollen bearing this *S* allele would be ineffective and linked *GOT-1* alleles would be in deficit in seedling progenies. This theory would account for

Table 1. Segregation for *GOT-1* (fully-compatible and selfed crosses)

Family		Parental phenotypes	Progeny phenotypes				Expected ratio	χ^2	df	P
F9	('Golden Delicious' × A463-70[a])	ae × cd	3 ce	6 ac	3 ad	2 de	1:1:1:1	2.57	3	0.46
F10	(A463-70[a] × 'Golden Delicious')	cd × ae	6 ce	5 ac	1 ad	4 de	1:1:1:1	3.50	3	0.32
F34	('Idared' × 'Spartan')	ac × bc	12 ab	9 ac	12 bc	13 cc	1:1:1:1	0.78	3	0.85
F30	('Idared' × 'Golden Hornet')	ac × bd	4 ab	4 ad	0 bc	5 cd	1:1:1:1	4.54	3	0.21
F32	('Fiesta' × 'Spartan')	ad × bc	3 ab	5 ac	3 bd	2 cd	1:1:1:1	1.46	3	0.69
F36	('N. Spy' × 'Winter Majetin')	an × bc	14 ab	13 ac	12 bn	7 cn	1:1:1:1	2.52	3	0.47
F49	('Winter Majetin' × 'N. Spy')	bc × an	8 ab	15 ac	13 bn	11 cn	1:1:1:1	2.28	3	0.52
F70	(A172-2[b] × A814-137[c])	cd × ad	4 ac	10 ad	8 cd	6 dd	1:1:1:1	2.86	3	0.41
F71	(A140-7[d] × A172-2[b])	bd × cd	7 bc	2 bd	7 cd	2 dd	1:1:1:1	5.55	3	0.13
F72	(604[e] × A853-1[f])	an × bc	6 ab	2 ac	5 bn	4 cn	1:1:1:1	2.06	3	0.56
F93	('Jonathan' × A849-7[g])	bc × ad	9 ab	7 ac	6 bd	11 cd	1:1:1:1	1.79	3	0.62
F102	('N. Spy' × 'Worcester')	an × en	2 ae	4 an	3 en	4 nn	1:1:1:1	0.85	3	0.84
F41	('Cox' × 'Baskatong')	bd × bd	21 bb	28 bd	12 dd		1:2:1	3.06	2	0.22
F25	('Gloster' × 'Golden Hornet')	cc × bd	14 bc	13 cd			1:1	0.04	1	0.85
F46	('Discovery' × 'Red Jade')	nn × bd	12 bn	14 dn			1:1	0.15	1	0.69
F69	(3759[h] × 'Baskatong')	bb × bd	9 bb	10 bd			1:1	0.05	1	0.82
F106	(T30-9[i] × T31-12[i])	cd × cd	6 cc	18 cd	17 dd		1:2:1	6.51	2	0.04
F107	('Fiesta'[i] selfed)	ad × ad	5 aa	30 ad	10 dd		1:2:1	6.11	2	0.05
F47	('Early Victoria' selfed)	bd × bd	19 bb	24 bd	0 dd		1:2:1	17.37	2	< 0.01
F63	('Cox' self-fertile clones)	bd × bd	0 bb	51 bd	1 dd		1:2:1	48.12	2	< 0.01

[a] 'Granny Smith' × 'Bountiful' ('Cox' × ?)
[b] 'James Grieve' × OR33T90
[c] 'Cox' × A467/74
[d] *'M. zumi'* o.p. × 'Edward VII'
[e] 'Court Pendu Plat' selfed (?)
[f] 'Edward VII' × A363/30
[g] 'Edward VII' × A423/2
[h] *'M. hupehensis robusta'* o.p.
[i] 'Cox' × 'Idared'

Table 2. Segregation for *GOT-1* (semi-compatible crosses)

Family		Proposed *S* and *GOT-1* genotypes	Progeny phenotypes				Expected ratio	χ^2 (a)	P
F1	(A463-70[b] × 'Cox')	$S_?c/S_2d \times S_1b/S_2d$	16 bc	12 bd	0 cd	0 dd	1:1:1:1	0.57	0.45
F3	('Cox' × A463-70[b])	$S_1b/S_2d \times S_?c/S_2d$	12 bc	9 cd	1 bd	0 dd	1:1:1:1	0.42	0.51
F2	(A463-70[b] × 'Granny Smith')	$S_?c/S_2d \times S_3a/S_?c$	12 ac	15 ad	0 cc	0 cd	1:1:1:1	0.33	0.56
F5	('Idared' × T31-12[c])	$S_3a/S_4c \times S_4c/S_2d$	14 cd	13 ad	1 ac	0 cc	1:1:1:1	0.04	0.85
F6	(T31-12[c] × 'Idared')	$S_4c/S_2d \times S_3a/S_4c$	14 ac	13 ad	1 cd	0 cc	1:1:1:1	0.04	0.85
F11	('Idared' × 'Fiesta'[c])	$S_3a/S_4c \times S_3a/S_2d$	9 ad	19 cd	0 aa	0 ac	1:1:1:1	3.57	0.06
F21	('Fiesta' × 'Idared')	$S_3a/S_2d \times S_3a/S_4c$	10 ac	8 cd	0 aa	0 ad	1:1:1:1	0.22	0.64
F12	(T30-24[c] × 'Cox')	$S_3a/S_1b \times S_1b/S_2d$	8 ad	7 bd	0 ab	0 bb	1:1:1:1	0.07	0.80
F17	('Cox' × T30-24[c])	$S_1b/S_2d \times S_3a/S_1b$	10 ab	5 ad	1 bb	0 bd	1:1:1:1	1.67	0.20
F13	T32-2[c] × 'Cox')	$S_3a/S_2d \times S_1b/S_2d$	5 ab	8 bd	0 ad	0 dd	1:1:1:1	0.69	0.40
F14	('Cox' × T32-2[c])	$S_1b/S_2d \times S_3a/S_2d$	7 ab	6 ad	0 bd	0 dd	1:1:1:1	0.08	0.78
F18	('Cox' × 'Fiesta'[c])	$S_1b/S_2d \times S_3a/S_2d$	15 ab	5 ad	1 dd	0 bd	1:1:1:1	5.00	0.03
F33	('Gala' × 'Elstar')	$S_?e/S_2d \times S_?a/S_2d$	13 ad	10 ae	1 ed	0 dd	1:1:1:1	0.39	0.53
F37	('Granny Smith' × 'Kent')	$S_3a/S_?c \times S_3a/S_1b$	19 ab	24 bc	2 ac	0 aa	1:1:1:1	0.58	0.44
F23	('V. Bella' × 'Katja')	$S_?a/S_?c \times S_?d/S_?n$	10 ad	6 cd	0 an	0 cn	1:1:1:1	1.00	0.32
F48	('Jonathan' × 'Idared')	$S_?b/S_4c \times S_3a/S_4c$	24 ab	19 ac	3 cc	0 bc	1:1:1:1	0.58	0.44
F27	('Delprim' × 'Katja')	$S_?a/S_?n \times S_?d/S_?n$	11 ad	14 dn	0 an	0 nn	1:1:1:1	0.36	0.55
F29	('Katja' × 'Delprim')	$S_?d/S_?n \times S_?a/S_?n$	11 ad	8 an	0 dn	0 nn	1:1:1:1	0.47	0.49
F115	('Kent' × 'Fiesta')	$S_3a/S_1b \times S_3a/S_2d$	12 ad	14 bd	0 aa	0 ab	1:1:1:1	0.15	0.69
F135	('Idared' × A679-12[d])	$S_3a/S_4c \times S_3a/S_?e$	42 ae	43 ce	0 aa	0 ac	1:1:1:1	0.01	0.91
F633	('Sp. Seedless' × 'G. Car.')	$S_?b/S_?c \times S_?a/S_?n$	37 ab	45 ac	0 bn	0 cn	1:1:1:1	0.78	0.38
F132	('Katja' × 'W. Angel')	$S_?d/S_?n \times S_?c/S_?d$	18 dd	21 dn	1 cd	0 cn	1:1:1:1	0.23	0.63

[a] Chi-square test for the two most numerous genotypes (1 df)
[b] 'Granny Smith' × 'Bountiful' ('Cox' × ?)
[c] 'Cox' × 'Idared'
[d] 'Worcester' × A363/38

Table 3. Estimates of linkage between *GOT-1* and *IDH-1*

Family	Proposed parental *GOT-1*/*IDH-1* genotypes (a)	Type of cross	Progeny phenotypes						No. of sdls.	χ^2	df	*P*	r	SE
Fully-compatible crosses:														
F30	ac/bb × *bd*/*ab*	3	4 ab/ab 0 cd/ab	0 ab/bb 5 cd/bb	0 ad/ab	4 ad/bb	0 bc/ab	0 bc/bb	13	13.00	3	< 0.01	0.00	(0.000)
F32	ad/bb × *bc*/*ab*	3	3 ab/ab 0 cd/ab	0 ab/bb 2 cd/bb	0 ac/ab	5 ac/bb	3 bd/ab	0 bd/bb	13	13.00	3	< 0.01	0.00	(0.000)
F34	ac/bb × *ab*/*ab*	3	6 aa/ab 0 bc/ab	0 aa/bb 4 bc/bb	0 ab/ab	5 ab/bb	4 ac/ab	0 ac/bb	19	19.00	3	< 0.01	0.00	(0.000)
F36	an/bb × *bc*/*ab*	3	13 ab/ab 0 cn/ab	1 ab/bb 7 cn/bb	0 ac/ab	13 ac/bb	12 bn/ab	0 bn/bb	46	42.25	3	< 0.01	0.02	(0.022)
F49	*bc*/*ab* × an/bb	3	8 ab/ab 0 cn/ab	0 ab/bb 11 cn/bb	1 ac/ab	14 ac/bb	12 bn/ab	1 bn/bb	48	39.48	3	< 0.01	0.04	(0.029)
F93	*bc*/*ab* × ad/bb	3	9 ab/ab 0 cd/ab	0 ab/bb 11 cd/bb	1 ac/ab	5 ac/bb	6 bd/ab	0 bd/bb	32	28.67	3	< 0.01	0.03	(0.031)
Semi-compatible crosses:														
F12	*ab*/*ab* × bd/ab	2	0 ad/aa 0 ab/aa	0 ad/ab 0 ab/ab	2 ad/bb 0 ab/bb	0 bd/aa 0 bb/aa	5 bd/ab 0 bb/ab	1 bd/bb 0 bb/bb	8	4.44	1	0.03	0.13	(0.117)
F17	*bd*/*ab* × ab/ab	2	0 ab/aa 1 bb/aa	4 ab/ab 0 bb/ab	0 ab/bb 0 bb/bb	0 ad/aa 0 bd/aa	0 ad/ab 0 bd/ab	3 ad/bb 0 bd/bb	8	16.10	4	< 0.01	0.00	(0.000)
F48	*bc*/*ab* × ac/bb	1	9 ab/ab 0 cc/ab	1 ab/bb 2 cc/bb	0 ac/ab	8 ac/bb	0 bc/ab	0 bc/bb	20	16.36	2	< 0.01	0.06	(0.054)
F132	dn/bb × *cd*/*ab*	3	0 cd/ab 0 cn/bb	17 dd/ab 0 dn/bb	0 cn/ab	21 dn/ab	1 cd/bb	1 dd/bb	40	20.12	2	< 0.01	0.03	(0.025)
									Pooled estimate				0.03	(0.012)

(a) Alleles bold type are assumed to be in coupling phase

Table 4. Pollen tube growth in detached flowers and fruit set of test crosses

Cross[a]	*GOT-1* genotypes	No. of flowers examined	Pollen tubes per flower stylar base	Pollen tubes per ovule	Fruit set %	
1) T31-12 × T30-9	cd × cd	4	1.50	0.50	9.8	
2) T30-9 × T31-12	cd × cd	7	5.28	1.10	22.0	
3) T30-43 × 'Fiesta'	ad × ad	8	0	0	–	
4) 'Fiesta' × T30-43	ad × ad	8	0.87	0.12	33.9	A
5) T32-2 × 'Fiesta'	ad × ad	9	2.77	0	0	
6) 'Fiesta' × T32-2	ad × ad	8	5.12	0.25	30.6	
7) T30-43 × T32-2	ad × ad	7	0	0	0	
8) T32-2 × T30-43	ad × ad	8	0.12	0	0	
9) 'Cox' × T30-43	bd × ad	6	7.00	1.50	–	B
10) 'Cox' × T32-2	bd × ad	6	7.16	4.66	64.0	
11) 'Cox' × 'Idared'	bd × ac	6	5.83	2.80	69.2	C
12) 'Spartan' × 'Idared'	bc × ac	7	4.71	3.40	86.0	

[a] T30-9, T30-43, T31-12, T32-2 and Fiesta = Cox × Idared

the contrasting phenotypic arrays in the reciprocal progenies in Table 2 (F1/F3, F5/F6, F11/F21, F12/F17, F13/F14, F27/F29). The overall proportion of crossover genotypes in Table 2 was 12 out of 665 plants.

A homogeneity test ($X^2 = 19.6$, 21 d.f.) showed that these progenies could be pooled to calculate the recombination fraction ($r = 0.02 \pm 0.005$).

Seedlings from some of the progenies in Tables 1 and 2 were subsequently examined for *IDH-1* genotypes. Some seedlings were omitted from the second test because suitable plant material was no longer available. Joint segregation data for *GOT-1* and *IDH-1* are shown in Table 3. Data from 10 progenies showed that *GOT-1* and *IDH-1* were closely linked ($r = 0.03 \pm 0.012$). Aberrant segregation for *IDH-1* in the three semi-compatible progenies F12, F17 and F132 could be attributed to linkage with the *S* locus ($r = 0.06 \pm 0.035$). From these results it can be deduced that the gene order is:

$$Sx \leftarrow 0.02 \rightarrow GOT\text{-}1 \leftarrow 0.03 \rightarrow IDH\text{-}1$$
$$\leftarrow \qquad 0.06 \qquad \rightarrow$$

Further evidence in support of this gene order is found in F132 (*IDH-1*: bb × ab, Table 3) thirty-eight seedlings had genotype *ab* and only two had genotype *bb*, one of them (*GOT-1* : *cd*) must have resulted from crossing over between *GOT-1* and the *S* locus while the other (*GOT-1* : *dd*) must have resulted from crossing over between *GOT-1* and *IDH-1*.

Seedlings from self-pollination of the self-fertile clones of 'Cox' (*GOT-1* : *bd*) included fifty-one with *bd* genotype, one with *dd* genotype and none with *bb* genotype (Table 1) suggesting that either the pollen tubes which reached the ovule selectively fertilised eggs with different *S* alleles or indicating preferential survival of heterozygotes. The examination of the same seedlings for other enzyme systems confirmed the selfed pollinated origin and excluded the occurrence of apomixis as a cause. Significant deficiencies ($P < 0.10$) in the expected ratios for *GOT-1* were found in the two other selfed families (F47, F107, Table 1). In 'Early Victoria' (*GOT-1* : *bd*) only pollen carrying the *b* allele appears to be functional giving nearly equal numbers of *bd* and *bb* genotypes. Heterozygous genotypes predominated in the selfed progeny of 'Fiesta' (*GOT-1* : *ad*).

Pollen tube growth and fruit set tests

Pollen tube growth and fruit set tests included three types of cross (Table 4); between selections and varieties with (A) two *GOT-1* alleles in common (four pairs of reciprocal crosses) (B) one *GOT-1* allele in common (semicompatible crosses) and (C) with, in one case, two different *GOT-1* alleles and in the other, one *GOT-1* allele in common, both fully compatible combinations.

Crosses (C) between unrelated parents (11, 12) and backcrosses (B) (9. 10) gave similar results on the basis of mean numbers of pollen tubes reaching the ovule and percentage fruit set. In the sibling crosses (A) both parents had the same GOT-1 alleles and in most cases only a small number of pollen tubes reached the base of the style. However, in two crosses (2 and 6) the number of pollen tubes at the stylar base was comparable to those recorded in group B and C. Similarly, the number of pollen tubes reaching the ovule in group A was very low, with only one cross (2) showing comparable results to crosses in groups B and C. Results for fruit set which are incomplete showed, overall, a much lower set in group A than that observed in groups B and C.

Discussion

Polymorphism for GOT (also designated AAT, Aspartate aminotransferase) in apple has been described previously (Chyi and Weeden 1984; Weeden and Lamb 1985) but detailed genetical accounts have not been presented. This earlier work located the positions of all the bands identified in the present work except the e band and the hybrid bands resulting from interactions with null alleles.

Heterozygotes of *GOT-1* produced phenotypes with both parental bands and a third intermediate band with more intense staining which supports the previous finding that the enzyme has a dimeric subunit structure in apple (Weeden and Lamb 1985). GOT has also been found to be dimeric in other crops including maize (Scandalios et al. 1975), *Stephanomeria exigua* (Gottlieb 1973), *Camellia japonica* (Wendel and Parks 1982) and tomatoes (Rick and Fobes 1975).

Close linkage between *GOT-1* and the incompatibility *S* locus is proved by the differing arrays of *GOT-1* phenotypes in reciprocal progenies (Table 2). This close linkage ($r = 0.02 \pm 0.005$) enables *GOT-1* to be used as a molecular marker for genotyping apple according to *S* alleles.

From the data in Table 2, the following cultivar genotypes are proposed: 'Cox' S_1b/S_2d, 'Idared' S_3a/S_4c, 'Fiesta' S_3a/S_2d and 'Kent' S_3a/S_1b.

In the backcross F48 ('Jonathan' $bc \times$ 'Idared' S_3a/S_4c) the *cc* and *bc* genotypes were in deficit suggesting that 'Jonathan' carries the S_4 allele but not S_3.

Similarly, 'Granny Smith' (*ac*) was shown to carry S_3 linked with *c* (from F2 and F37) but not S_1 (from F37), 'Elstar' (*ad*) and 'Gala' (*ed*) carry the S_2 linked with *d* (from F33). From Table 1, it can be assumed that 'Golden Delicious' (*ae*) does not carry S_2 and 'Spartan' (*bc*) does not carry S_2, S_3, or S_4.

In pollination tests, the incompatibility reactions were not as clearly defined as in cherries (Crane and Brown 1938). In back-crosses where the parents were expected to have one *S* allele in common, the number of pollen tubes counted and fruit set were similar to those in crosses between unrelated parents, as could be expected if half the pollen was compatible and pollination conditions were good. Thus the two types of cross could not be distinguished this way.

Crosses between siblings with the same *GOT-1* genotype generally showed low fruit set (Table 4). However, there were reciprocal differences. Three crosses (5, 7, 8) appeared fully cross-incompatible while four (1, 2, 4, 6) gave fruit set of 10–34%. Although lower than in unrelated varieties (69–86%) such variability in fruit set suggests that the effect of *S* alleles on apple pollen growth can be modified by environmental and additional genetical and physiological factors. Petropoulou (1985) attributed the high level of fertility in 'Fiesta', both crossed and selfed, to high stylar receptivity and to the ability of the pollen to grow at low temperatures (10 °C). 'Fiesta' appears self-fertile, setting very well on selfing (50%). In this case the significant deviation ($P < 0.10$) in the expected segregation of the two *GOT-1* alleles (Table 1) was largely due to a high proportion of heterozygous genotypes. Early 'Victoria' selfed gave an approximately 1 : 1 ratio of *bb* : *bd* which with a complete absence of *dd* phenotypes suggests that this cultivar may carry an allele for self-fertility (S_f) linked to *GOT-1 b*.

The 'Cox' self-fertile clones gave most striking results, homozygotes for *GOT-1* being almost completely absent. Apomixis can be ruled out because of the six enzymes examined in the Cox selfed progeny, all segregated in the expected ratios except the *S*-linked *GOT-1* and *IDH-1*. A second stage may be suggested for the incompatibility reaction which occurs in the ovule permitting fertilisation only between gametes with different *GOT-1* alleles.

Few cases of cross-incompatibility have been reported in apples (Knight 1963). However, significant differences in the fruit set between crosses have been observed in the breeding programme at East Malling. In most cases these differences could be explained by reference to *GOT-1* genotypes. In field pollination tests (Spiegel-Roy and Alston 1982) some cultivars with common parents do not interpollinate well. Under low temperature conditions poor pollen growth will have a significant effect on fruit set, especially if only 50% of pollen grains are compatible. Such effects are subject to modification through inherent variations in the ability of pollen to grow at low temperatures. Schmadlak (1965) tried to establish indices of affinity between varieties by comparing the growth of pollen tubes and the relative distribution of pollen tube tips in the style. The subdivision of the style into segments was helpful in assessing incompatibility to a certain extent. However, this procedure is laborious and could be inconclusive since there is large variability from style to style. Kobel (1954) classified pollen tubes as compatible, incompatible and semi-compatible; in the latter case about half of the pollen tubes reached the base of the style. Based on these observations, he identified eleven *S* alleles. Such a clear distinction in pollen tube growth was not observed in this work. Since *GOT-1* analyses provide a more definite means of distinguishing *S* alleles in apple than pollination tests, we have used them to establish the *S* genotypes of apple cultivars. So far, as no progeny tests or *GOT-1* analyses have been made on the varieties studied by Kobel (1954), we are not able to relate the *S* alleles in this work to the series he proposed.

Of nineteen crosses between supposedly unrelated cultivars, five (F37, F23, F135, F633, F132, Table 2) showed a semi-compatible segregation for *GOT-1* suggesting that these crosses were between parents with an *S* allele in common. It seems that the number of *S* alleles amongst cultivated apple varieties, may be rather limited suggesting that cultivated apples are derived from a fairly narrow genetic base.

In conclusion, the present analysis has established two marker genes *GOT-1* and *IDH-1* for the *S* in-

compatibility locus. The high polymorphism observed in *GOT-1* in addition to its close linkage with the *S* locus provides a sound base for genotyping apple varieties for the *S* incompatibility locus.

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