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The use of the isoenzymic marker gene *Got-1* in the recognition of incompatibility *S* alleles in apple

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Abstract The *S* incompatibility system of apple was confirmed through the application of the gene *Got-1* for glutamate oxaloacetate transaminase as a marker for the *S* locus. The 11 *S* alleles proposed by Kobel et al. (1939) were confirmed through anomalous segregations for *Got-1* observed in 14 semi-compatible crosses and regular segregations observed in 2 fully compatible crosses. The *S* allele genotypes of 'Idared' (S_3 S_7), 'Cox' (S_5 S_9) and 'Fiesta' (S_3 S_5) were determined and found to fall within the original series. By associating parental incompatibility genotypes with the segregation of *Got-1* alleles, we were able to deduce the coupling of *S* and *Got-1* alleles in 9 varieties.

Key words Apple · Incompatibility alleles
Molecular markers · Genes *Got-1* · *S* · Linkage

Introduction

While apple varieties are largely self-incompatible (Brown 1975), several cases of cross-incompatibility have been reported, often but not always between varieties known to be related (Kobel et al. 1939; Modlibowska 1945; Crane and Lawrence 1952; Spiegel-Roy and Alston 1982).

Kobel et al. (1939) studied the pollen tube growth of ten apple varieties in various cross combinations and proposed a multiallelic *S*-gene system of gametophytic effect for apple. Knight et al. (1962) suggested that an incompatibility locus is closely linked to the *Er* locus, which codes for resistance to woolly aphid (*Erisoma lanigerum*) in the variety 'Northern Spy'. Spiegel-Roy and Alston (1982) produced results supporting a gametophytic basis for incompatibility in apple. Manganaris and Alston (1982) found clear evidence for an incompatibility locus in apple as a result of genetic studies of glutamate oxaloacetate transaminase (GOT). A marked deficit of some GOT-1 genotypes, noted in all backcrosses, could be attributed to a close linkage (r=0.02± 0.005) between *Got-1* and the *S* locus. Six *Got-1* alleles, *a*, *b*, *c*, *d*, *e* and *n*, were found. Isoenzymic analysis of leaf tissues is a convenient method of clarifying *S* alleles in apple and can be used to complement pollination tests and pollen tube growth studies.

In the investigation reported in this paper, the *S* alleles of apple are clarified by relating the series proposed by Kobel et al. (1939) to the *Got-1* markers identified by Manganaris and Alston (1987), and used by them to determine the incompatibility genotypes of 'Cox's Orange Pippin', 'Fiesta', 'Idared' and 'Kent', varieties not previously studied in this way.

Materials and methods

A series of testcrosses were made involving 10 of the varieties used by Kobel et al. (1939): 'Adam's Pearmain' (AP), 'Berner Rosenapfel' (BR), 'Champagner Reinette' (CR), 'Danziger Kantapfel' (DK), 'Oberrieder Glanzreinette' (OG), 'Ontario' (O), 'Roter Sauergrauech' (RS), 'Transparent von Croncels' (TC), 'Wellington' (W) and 'Weisser Klarapfel' (WK), and 2 varieties 'Cox's Orange Pippin' (C) and 'Idared' (I), for which Manganaris and Alston (1987) proposed distinct incompatibility genotypes. Three types of crosses were made: between varieties without an *S* allele in common, fully compatible; between varieties with only one *S* allele in common, semi-compatible; and crosses of unknown constitution, to test for homology between the *S* alleles of 'Cox' and 'Idared' and the series identified by Kobel et al. (1939).

Most crosses were made in the orchards of the National Fruit Collection, Brogdale, Faversham, Kent, under protected conditions. Balloon stage flowers were selected and pollinated and then enclosed in polyester bags until all flowering in the orchard was completed. Crosses involving 'Oberrieder Glanzreinette' were made at the Swiss Federal Research Station, Wädenswil.

Seedlings were analysed for GOT activity using tissue extracts from cotyledons and young leaves. The parent varieties were assessed using extracts from actively growing leaves collected from mature trees at the start of the growing season. Polyacrylamide gel

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electrophoresis (PAGE) was used for this analysis using a procedure similar to that of Manganaris and Alston (1987), except that dithiothreitol replaced mercaptoethanol in the extraction buffer. Gels were stained for GOT according to Manganaris and Alston (1987).

Results

GOT analysis

Clear resolution was obtained in both the GOT-I and GOT-II zones, the latter being important in the interpretation of the slowest GOT-I band positions although in cases where the GOT-II zone was not clear the presence of the GOT-I d band could be deduced on the basis of parental genotypes and the position of the hybrid band (Fig. 1). In the 34 progenies analysed, the arrangement of the bands was consistent with that described by Manganaris and Alston (1987); five alleles, *a, b, c, d* and *n,* of *Got-1* and three of *Got-2*, *a, b* and *n*, were recognised. The *Got-1* allele *e* was not present in the 12 cultivars studied. The *Got-1* genotype of 'Weisser Klarapfel' (Syn. 'White Transparent') was found to be *cn* and not *cc* as assigned by Manganaris and Alston (1989). However, the *Got-1* genotypes of 'Cox' (*bd*) and 'Idared' (*ac*) were confirmed. The *Got-1* genotypes were recorded for the other 9 varieties that had not previously been assayed, 'Adam's Pearmain' (*an*), 'Berner Rosenapfel' (*an*), 'Champagner Reinette' (*bn*), Danziger Kantapfel' (*bc*), Oberrieder Glanzreinette' (*ad*), 'Ontario' (*dn*), 'Roter Sauergrauech' (*an*) 'Transparent von Croncels' (*an*) and 'Wellington' (*bd*).

Crosses between varieties used by Kobel et al. (1939)

The segregations for *Got-1* alleles in 2 fully compatible and 14 semi-compatible crosses are shown in Table 1, together with the parental incompatibility genotypes. The segregation of *Got-1* alleles in the fully compatible crosses was close to the expected 1:1:1:1 ratio. All the semi-compatible crosses showed distorted segregations for *Got-1* alleles, resulting in the almost complete absence, or absence of 1 or 2 expected genotypes, from each progeny, a consequence of the linkage of this gene to the *S*-incompatibility locus (Manganaris and Alston 1987). By associating the parental incompatibility genotypes with the segregation of *Got-1* alleles in these crosses, it was possible to deduce the coupling of *S* and *Got-1* alleles in 6 varieties:

'Weisser Klarapfel'	S_1–*n* S_5–*c*
'Transparent von Croncels'	S_2–*n* S_3–*a*
'Berner Rosenapfel'	S_1–*a* S_2–*n*
'Danziger Kantapfel'	S_2–*c* S_7–*b*
'Wellington'	S_8–*d* S_9–*b*
'Roter Sauergrauech'	S_1–*n* S_3–*a*

Of the 14 semi-compatible crosses 10 showed agreement with the 1:1 ratio expected with respect to the 2 most numerous genotypes in the presence of linkage. The 4 remaining crosses had χ^2 values that were significant at the 5% level. Only 2 recombinants were found amongst the 376 plants, these arose in 'Ontario' × 'Berner Rosenapfel' and 'Roter Sauergrauech' × 'Berner Rosenapfel'. The recombination co-efficient for the combined progenies in this group was 0.006±0.0042.

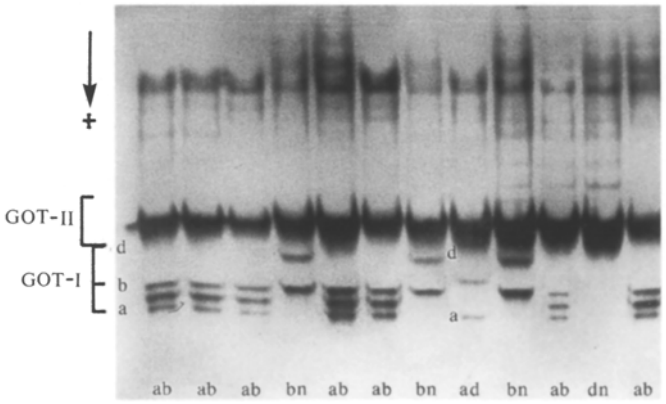

Fig. 1 Glutamate oxaloacetate transaminase zymograms from leaf extracts of 'Roter Sauergrauech' (*Got-1an*)×'Cox' (*Got-1bd*) showing all four phenotypes, *ab*, *ad*, *bn* and *dn*

Crosses involving 'Cox' and 'Idared'

In 3 of the 7 progenies involving 'Idared' (Table 2), 'Transparent von Croncels' × 'Idared', 'Roter Sauergrauech' × 'Idared' and 'Idared' × 'Danziger Kantapfel', the distribution of *Got-1* genotypes differed from the 1:1:1:1 expected in the absence of linkage. They were therefore derived from semi-compatible crosses. From the 4 progenies showing a 1:1:1:1 segregation for *Got-1*, 'Champagner Reinette' × 'Idared', 'Ontario' × 'Idared', 'Wellington' × 'Idared' and 'Idared' × 'Berner Rosenapfel', it may be concluded that none of the following alleles, S_1, S_2, S_4, S_8 or S_9, are present in 'Idared'. It appears that 'Idared' has an *S* allele in common with each of the following cultivars 'Transparent von Croncels' (S_2 S_3), 'Roter Sauergrauech' (S_1 S_3) and 'Danziger Kantapfel' (S_2 S_7). Thus, the incompatibility genotype for 'Idared' appears to be S_3 S_7.

In only 2 of the 11 progenies with 'Cox' as a parent did the segregation for *Got-1* alleles differ from 1:1:1:1. These were 'Wellington' (S_8 S_9) × 'Cox' and 'Cox' × 'Weisser Klarapfel' (S_1 S_5) which were thus 'semi-compatible' crosses. None of the other 9 progenies involving 'Cox' showed a *Got-1* segregation different from 1:1:1:1, as would be expected if the parents had no *S* allele in common. It therefore appears that none of the *S* alleles in these parents, S_1, S_2, S_3, S_4, S_6, S_7, S_8, S_{10} and S_{11}, occur in 'Cox' and that therefore the incompatibility genotype of 'Cox' is S_5 S_9.

Discussion

The linkage (Manganaris and Alston 1987) between *Got-1* and the incompatibility locus *S* described by Kobel et al.

Table 1 Segregations for *S* incompatibility alleles and *Got-1* alleles in crosses between varieties used by Kobel et al. (1939). See Materials and methods for variety abbreviations

Cross	*S* incompatibility genotypes	*Got-1* genotypes	Total	Observed genotypes	Expected ratio[a]	χ^{2} [b]	χ^{2} [c]
Fully compatible crosses							
BR × OG	$S_1S_2 \times S_3S_6$	*an* × *ad*	28	6*aa*:7*ad*:11*an*:4*dn*	1:1:1:1	3.71	–
WK × OG	$S_1S_5 \times S_3S_6$	*cn* × *ad*	14	7*ac*:3*an*:1*cd*:3*dn*	1:1:1:1	5.43	–
Semi-compatible crosses							
BR × TC	$S_1S_2 \times S_2S_3$	*an* × *an*	17	13*aa*:4*an*:0*nn*	1:2:1	24.64***	4.76*
RS × TC	$S_1S_3 \times S_2S_3$	*an* × *an*	36	0*aa*:14*an*:22*nn*	1:2:1	28.65***	1.78
RS × BR	$S_1S_3 \times S_1S_2$	*an* × *an*	39	1*aa*:20*an*:18*nn*	1:2:1	8.53*	0.03
DK × TC	$S_2S_7 \times S_2S_3$	*bc* × *an*	19	13*ab*:6*ac*:0*bn*:0*cn*	1:1:1:1	24.16***	2.58
BR × WK	$S_1S_2 \times S_1S_5$	*an* × *cn*	31	15*ac*:0*an*:16*cn*:0*nn*	1:1:1:1	31.06***	0.03
RS × WK	$S_1S_3 \times S_1S_5$	*an* × *cn*	46	26*ac*:0*an*:20*cn*:0*nn*	1:1:1:1	47.57***	0.78
CR × BR	$S_2S_4 \times S_1S_2$	*bn* × *an*	14	11*ab*:3*an*:0*bn*:0*nn*	1:1:1:1	23.14***	4.57*
CR × TC	$S_2S_4 \times S_2S_3$	*bn* × *an*	27	12*ab*:15*an*:0*bn*:0*nn*	1:1:1:1	27.67***	0.33
CR × DK	$S_2S_4 \times S_2S_7$	*bn* × *bc*	28	13*bb*:0*bc*:15*bn*:0*cn*	1:1:1:1	28.29***	0.14
O × W	$S_1S_8 \times S_8S_9$	*dn* × *bd*	24	6*bd*:18*bn*:0*dd*:0*dn*	1:1:1:1	36.00***	6.00*
O × BR	$S_1S_8 \times S_1S_2$	*dn* × *an*	36	0*ad*:1*an*:10*dn*:25*nn*	1:1:1:1	44.67***	6.43*
O × RS	$S_1S_8 \times S_1S_3$	*dn* × *an*	23	14*ad*:9*an*:0*dn*:0*nn*	1:1:1:1	25.17***	1.09
O × WK	$S_1S_8 \times S_1S_5$	*dn* × *cn*	14	10*cd*:4*cn*:0*dn*:0*nn*	1:1:1:1	19.14***	2.57
OG × RS	$S_3S_6 \times S_1S_3$	*ad* × *an*	22	0*aa*:0*ad*:11*an*:11*dn*	1:1:1:1	22.00***	0.00

*, *** Indicates significant departure from expected at the 5% and 0.1% level, respectively
[a] Expected ratio in absence of linkage between *Got-1* and the incompatibility *S* locus
[b] Chi-square test for 1:2:1 or 1:1:1:1 segregation assuming no linkages *S-Got-1* (2 or 3 *df*)
[c] Chi-square test for 1:1 expected segregation for the two most numerous genotypes assuming linkage *S-Got-1* (1 *df*)

Table 2 Segregations for *S* incompatibility alleles and *Got-1* alleles in crosses involving 'Cox' and 'Idared'. See Materials and methods for variety abbreviations

Cross	*S* incompatibility genotypes	*Got-1* genotypes	Total	Observed genotypes	Expected ratio[a]	χ^{2} [b]	χ^{2} [c]
Crosses involving 'Idared' Fully compatible							
CR × I	$S_2S_4 \times S_3S_7$	*bn* × *ac*	24	5*ab*:9*an*:1*bc*:9*cn*	1:1:1:1	7.33	–
O × I	$S_1S_8 \times S_3S_7$	*dn* × *ac*	48	17*ad*:12*an*:13*cd*:6*cn*	1:1:1:1	5.17	–
W × I	$S_8S_9 \times S_3S_7$	*bd* × *ac*	13	7*ab*:1*ad*:3*bc*:2*cd*	1:1:1:1	6.38	–
I × BR	$S_3S_7 \times S_1S_2$	*ac* × *an*	26	6*aa*:6*ac*:5*an*:9*cn*	1:1:1:1	1.38	–
Semi-compatible							
TC × I	$S_2S_3 \times S_3S_7$	*an* × *ac*	16	0*aa*:9*ac*:0*an*:7*cn*	1:1:1:1	16.50***	0.25
RS × I	$S_1S_3 \times S_3S_7$	*an* × *ac*	51	1*aa*:27*ac*:1*an*:22*cn*	1:1:1:1	44.29***	0.51
I × DK	$S_3S_7 \times S_2S_7$	*ac* × *bc*	19	1*ab*:11*ac*:0*bc*:7*cc*	1:1:1:1	17.00***	0.89
Crosses involving 'Cox' Fully compatible							
BR × C	$S_1S_2 \times S_5S_9$	*an* × *bd*	34	12*ab*:10*ad*:3*bn*:9*dn*	1:1:1:1	5.29	–
DK × C	$S_2S_7 \times S_5S_9$	*bc* × *bd*	14	1*bb*:6*bc*:5*bd*:2*cd*	1:1:1:1	4.86	–
TC × C	$S_2S_3 \times S_5S_9$	*an* × *bd*	8	0*ab*:1*ad*:2*bn*:5*dn*	1:1:1:1	7.00	–
RS × C	$S_1S_3 \times S_5S_9$	*an* × *bd*	64	18*ab*:18*ad*:11*bn*:17*dn*	1:1:1:1	2.13	–
CR × C	$S_2S_4 \times S_5S_9$	*bn* × *bd*	56	10*bb*:14*bd*:12*bn*:20*dn*	1:1:1:1	4.00	–
O × C	$S_1S_8 \times S_5S_9$	*dn* × *bd*	33	8*bd*:9*bn*:9*dd*:7*dn*	1:1:1:1	0.33	–
C × BR	$S_5S_9 \times S_1S_2$	*bd* × *an*	14	4*ab*:2*ad*:5*bn*:3*dn*	1:1:1:1	1.43	–
C × RS	$S_5S_9 \times S_1S_3$	*bd* × *an*	14	3*ab*:2*ad*:3*bn*:6*dn*	1:1:1:1	2.57	–
C × AP	$S_5S_9 \times S_{10}S_{11}$	*bd* × *an*	30	12*ab*:8*ad*:2*bn*:8*dn*	1:1:1:1	6.80	–
Semi-compatible							
W × C	$S_8S_9 \times S_5S_9$	*bd* × *bd*	53	2*bb*:27*bd*:24*dd*	1:2:1	18.28***	0.18
C × WK	$S_5S_9 \times S_1S_5$	*bd* × *cn*	59	0*bc*:28*bn*:0*cd*:31*dn*	1:1:1:1	59.31***	0.15

*** Indicates significant departure from expected at the 5% level
[a] Expected ratio in absence of linkage between *Got-1* and the incompatibility *S* locus
[b] Chi-square test for 1:2:1 or 1:1:1:1 segregation assuming no linkages *S-Got-1* (2 or 3 *df*)
[c] Chi-square test for 1:1 expected segregation for the two most numerous genotypes assuming linkage *S-Got-1* (1 *df*)

(1939) was confirmed through anomalous segregations for *Got-1* observed in 14 semi-compatible crosses and by normal ratios, 1:1:1:1, in 2 fully compatible crosses. In all, 19 semi-compatible crosses were studied. The frequency of crossingover (r=0.014 ± 0.0052) was not significantly different from that reported by Manganaris and Alston (1987). Pooling the two sets of data gave an estimated recombination fraction was 0.016 ± 0.0037.

Eleven *S* alleles have been identified in apple, but the *Got-1* marker gene has only 6 alleles, moreover the effects of the limited crossingover between the two loci will have accumulated during the process of domestication and development of new varieties. It is thus not possible to relate individual *Got-1* alleles to specific *S* alleles or groups of *S* alleles. However, *Got-1* is a very effective marker for the *S* locus, and its alleles can be used to identify fully compatible siblings and derivatives.

The 3 semi-compatible progenies from 'Idared' (Table 2) provide evidence of the coupling of *S* alleles and *Got-1* alleles in 'Idared'. In 2 of these, TC × I and RS × I, the deficiency of *Got-1aa* and *Got-1an* seedlings suggests that the *Got-1a* allele from 'Idared' is coupled with the S_3 allele in that variety and that therefore S_1 and *Got-1c* are also coupled in 'Idared'. A similar analysis of the 2 semi-compatible crosses from 'Cox', W × C and C × WK, reveals a low level of transmission of *Got-1b* from 'Cox'. This suggests that in 'Cox' *Got-1b* is coupled with S_9 and that *Got-1d* is coupled with S_5. It is therefore almost certain that the *S* genotype of 'Fiesta' ('Cox' × 'Idared') *Got-1ad*, is S_3 S_5.

Kobel et al. (1939) observed that 17 of 34 crosses between varieties of distinct origin were semi-compatible, and Manganaris and Alston (1987) found that, of 19 progenies from unrelated cultivars, 5 had a semi-compatible type segregation for *Got-1*. In the experiment involving 'Cox' and 'Idared' (Table 2), 5 of 18 crosses between these varieties and others of distinct origin were semi-compatible. Hence, it appears that the number of *S* alleles in the cultivated apple is limited. This is supported by the view of Manganaris and Alston (1987) after observing that the cross between the commercial variety 'Katy' and the ornamental crab apple 'White Angel' was semi-incompatible.

The number of *S* alleles so far identified in apple is larger than in sweet cherry, where only 6 incompatibility alleles have been identified and where 10 incompatibility groups, out of a possible 15, have been recognised from amongst 150 varieties (Matthews and Dow 1969). The 11 *S* alleles identified by Kobel et al. (1939) suggest 55 possible incompatibility groups in apple, of which 10 were identified. The present work confirms 3 more in compatibility groups, S_5 S_9 ('Cox'), S_3 S_7 ('Idared') and S_3 S_5 ('Fiesta').

Although a high level of isoenzymic polymorphism exists within *Malus*, it is usually limited in commercial varieties to one or two alleles at each locus (Manganaris 1989). *Got-1* is an exception, as all 6 alleles are well distributed amongst commercial varieties (Manganaris and Alston 1989); this is possibly associated with its close linkage to the *S* locus, which has the highest level of polymorphism recorded in *Malus* (Kobel et al. 1939). Both loci might lie in hypervariable chromosomal regions. Parallel situations occur in *Nicotiana* and *Secale* where highly polymorphic peroxidase systems are linked to incompatibility loci (Labroche et al. 1983; Wricke and Wehling 1985).

The effects of the incompatibility mechanism in apple are not as sharply expressed as those in cherry, and have therefore not been studied so thoroughly. This work has clarified the genetics of the gametophytic control of incompatibility in apple through the effective use of *Got-1*, a gene with alleles that show clear electrophoretic reactions, as a marker for the *S* locus and individual *S* alleles. It confirms the suggestion (Kobel et al. 1939) of a series of *S* alleles in apple.

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