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Isozyme segregation in five apple progenies and potential use for map construction

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Abstract In the framework of the European Apple Genome Mapping Project, five apple progenies were scored for 53 isozyme markers and two phenotypic monogenic characters, R_t (anthocyanin production in all tissues) and C_o (columnar habit). The collaboration between two teams (in France and Greece) enabled a clarification of the synonymy between existing isozyme loci designations and the description of 12 new loci. The two phenotypic loci and the majority of the isozyme loci segregated in accordance with the expected monogenic ratios. Twenty percent of the scored segregations involved markers segregating for both parents, but only 9% involved more than two alleles. Thus, only a few isozyme markers (*Aat-1*, *Fdh-1*, *Pgd-1*, *Pgm-1*, *Prx-3*, *Prx-C1* and *Sod-4*) were particularly informative for mapping purposes. Joint segregation analysis located 36 isozyme markers together with the two phenotypic loci, R_t and C_o, in 11 linkage groups, most of which could be related to already published maps. Thus, despite their limited number, polymorphic apple isozyme markers are valuable as anchor points to establish a reliable correspondence between genetic maps built from separate progenies.

Key words Apple · Isozymes · Compact habit · Linkage · Genome mapping

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Introduction

Apple (*Malus* × *domestica* Borkh.) is a major temperate fruit crop which belongs to the *Maloideae* sub-family of the *Rosaceae*. It is considered as a disomic polyploid (2n = 34), probably of allopolyploid origin. The prolonged juvenile period of apple seedlings (4–5 years), combined with a strong self-incompatibility and a high level of heterozygosity, makes genetic studies of apple long and difficult (Brown 1992). In this context, genetic markers can be of great value for apple breeders. A high rate of polymorphism has already been reported for various enzymes in apple, and has been used for the identification of scion varieties (Weeden and Lamb 1985; Menendez et al. 1986 a; Korban and Bournival 1987), rootstocks (Menendez et al. 1986 b; Vinterhalter and James 1986; Samimy and Cummins 1992), or ornamental *Malus* selections (Marquard and Chan 1995). Isozymes also proved useful to characterize the genetic variability of some wild *Malus* species, and to devise germplasm collection strategies (Dickson et al. 1991; Lamboy et al. 1996). A few close linkages have been established between isozymes and single genes coding for agronomical traits, such as the self-incompatibility gene *S* (Manganaris and Alston 1987; Battle et al. 1995), the scab resistance gene V_f (Manganaris et al. 1994) and the mildew resistance gene Pl_w (Battle and Alston 1996). A further step towards marker-assisted selection is the establishment of a genetic map for apple, saturated with a range of reliable markers and locating single genes as well as quantitative loci of agronomical importance. Such a map was published for the first time on apple with 409 segregating markers including mostly random amplified polymorphic DNAs (RAPDs) (Hemmat et al. 1994). More recently, the same team published genetic linkage maps of three apple cultivars consisting mostly of RAPD markers (Conner et al. 1997). In order to develop a consensus map of the apple genome, a collaborative european project was initiated in 1993 and resulted in an integrated map with 290

markers more than a half of which were co-dominant (Maliepaard et al. 1998). In this respect, two teams (France and Greece) were in charge of isozyme analysis. This provided a unique opportunity to clarify the gel interpretation of 19 enzyme systems and of designation problems on more than 50 isozyme loci. In total, 544 individuals were analysed and more than 11 000 isozyme genotypes were generated. The present paper synthesizes the results obtained on apple isozymes during this project and emphasizes their interest in the context of genetic map establishment.

Materials and methods

Five families, progenies from controlled crosses, were studied (Table 1). They were chosen as reference populations for the development of a consensus apple-genome map, in the framework of the European Apple Genome Mapping Project (EAGMAP). The parents included apple varieties as well as hybrids under selection. Prima is a scab-resistant variety of complex parentage. Fiesta is a recent variety derived from the cross Cox's Orange Pippin × Idared. Double Red Northern Spy and Crimson Spy are two sports from the variety Northern Spy. 3762 is a hybrid obtained from the open pollination of *Malus robusta*. It carries a dominant gene for anthocyanin production in all plant tissues, labelled R_t (Brown 1992). Finally, SA 572/2 is a scabresistant hybrid of complex origin, involving several interspecific crosses. It carries the R_t gene and also presents a compact columnar habit determined by a single dominant gene C_o (Brown 1992). Segregation for these two simple phenotypic traits was easily recorded in four families.

Isozyme electrophoresis was conducted mostly on fresh leaf extracts. However, a few isozymes were scored from cotyledon extracts (*Acp-1*) and from bark extracts (*Me*, *Adh-2*). In the french laboratory, extraction and gel preparation was according to Chevreau and Laurens 1987. In the case of the greek team, the protocols were as published in the Manganaris and Alston papers (1987, 1988 a, b, 1992 a, b, c, 1997). Acrylamide gels were used to separate aspartate amino-transferase (AAT), acid phosphatase (ACP), alcohol dehydrogenase (ADH), catechol oxidase (CO), endopeptidase (ENP), esterase (EST), formate dehydrogenase (FDH), leucine aminopeptidase (LAP), peroxidase (PRX), shikimate dehydrogenase (SKD) and superoxide dismutase (SOD). Starch gels were used to separate aconitase (ACO), isocitrate dehydrogenase (IDH), malic enzyme (ME), 6-phosphogluconate dehydrogenase (PGD), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM) and triose phosphate isomerase (TPI). Diaphorase (DIA) was resolved in both types of gels. Staining protocols were according to Wendel and Weeden (1989). Only catechol oxidases were stained according to Schwennesen et al. (1982).

Single-factor segregation at each locus and joint segregation of pairs of loci for possible linkage were examined using the LINKEM 1.0 software (Vowden and Ridout 1994). Single-factor segregation was tested by the chi-square statistic. Joint segregation of pairs of loci was tested by the likelihood ratio test for linkage.

Results and discussion

Description of the isozymes studied and locus interpretation

Most of the 53 isozyme loci studied in this work have already been described in previous papers of the two teams involved (Chevreau et al. 1985; Chevreau and Laurens 1987; Manganaris and Alston 1987, 1988 a, b, 1992 a, b, c, 1997), or else in papers relating to the work of a third team (Weeden and Lamb 1987; Hemmat et al. 1994). A clarification of the synonymy between locus designation by the three groups has been attempted (Table 2). Twelve additional loci were described for the first time during the present study. Among them, catechol oxidase (CO) was previously described only once in apple, but without genetic interpretation (Barnes 1993). CO is a monomeric enzyme which can be detected simultaneously with peroxidase (PRX) loci when the staining solution is based on HankerYates reagent which contains catechol. In this study, CO was stained specifically with catechol and without H_2O_2. Two loci were described and the presence of null alleles was postulated (Fig. 1A). Formate dehydrogenase (FDH) is a dimeric enzyme which has not been completely described in apple, although one *Fdh* locus was mentioned by Hemmat et al. (1994). In the present study, two *Fdh* loci were characterized, *Fdh-1* corresponding to the previous *Fdh* locus. Intergenic bands were formed between subunits encoded by the two loci, thus suggesting a duplicated pair of genes (Fig. 1B). Only one enzyme system, aconitase (ACO), still presents some interpretation problems. ACO is a monomeric enzyme which was not completely described in apple, although three loci were mentioned in the first apple-genome map (Hemmat et al. 1994). Four loci were characterized during the present study, two of them segregating for the presence or absence of a null allele.

Segregation of phenotypic traits and isozyme loci

Anthocyanin production in all tissues was scored without ambiguity in the four families carrying this

Table 1 Plant material – description of the families analysed

Code	Female parent	Male parent	Size of the family	No. segregating izozyme loci	Phenotypic traits segregating
J	Prima	Fiesta	157	25	–
R	Fiesta	3762	147	31	Red tissue
Y	Fiesta	SA 572/2	124	29	Red tissue, columnar
P	Double Red Northern Spy	SA 572/2	58	33	Red tissue, columnar
G	Crimson Spy	3762	58	13	Red tissue

Table 2 Definition of isozyme loci with reference to previous publications and new loci described in the present study

Isozyme name and EC code		Loci already described and synonymous	References	New loci in this study
AAT	EC 2.6.1.1	*Aat-1* (= *Got-1* = *Aat-c*), *Aat-2* (= *Got-2* = *Aat-p*), *Aat-3*, *Aat-4*	(4) (5) (12)	*Aat-5*
ACO	EC 4.2.1.3	*Aco-1*, *Aco-2*, *Aco-3*	(3)	*Aco-4*
ACP	EC 3.1.3.2	*Acp-1*, *Acp-2*, *Acp-3*	(2) (3) (6)	*Acp-4*, *Acp-5*
ADH	EC 1.1.1.1	*Adh-1*, *Adh-2*	(1) (7)	–
CO	EC 1.10.3.-	–	–	*Co-1*, *Co-2*
DIA	EC 1.6.99.-	*Dia-1*, *Dia-2*, *Dia-3*, *Dia-4*, *Dia-5*	(12)	*Dia-6*
ENP	EC 3.4.9.9	*Enp-1*	(2) (6)	–
EST	EC 3.1.1.-	*Est-1*, *Est-2*, *Est-3*, *Est-4*, *Est-C* (cathodic)	(2) (3) (9)	*Est-5*
FDH	EC 1.2.1.2	*Fdh-1* (= *Fdh*)	(3)	*Fdh-2*
IDH	EC 1.1.1.42	*Idh-1*, *Idh-2*, *Idh-3*	(1) (7) (12)	–
LAP	EC 3.4.11.1	*Lap-1*, *Lap-2*, *Lap-3*, *Lap-4*	(2) (8)	–
ME	EC 1.1.1.40	*Me*	(12)	–
PGD	EC 1.1.1.44	*Pgd-1* (= *Pgd-p*) , *Pgd-2* (= *Pgd-c1*), *Pgd-3* (= *Pgd-c2*)	(12)	–
PGI	EC 5.3.1.9	*Pgi-1* (= *Gpi-p*), *Pgi-2* (= *Gpi-c1*)	(1) (12)	–
PGM	EC 5.4.2.2	*Pgm-1* (= *Pgm-p1*), *Pgm-2*, *Pgm-3* (= *Pgm-c1* = *Pgm-r*), *Pgm-4* (= *Pgm-c2*)	(12)	–
PRX	EC 1.11.1.7	*Prx-1*, *Prx-2* (= *Prx-A*), *Prx-3*, *Prx-4*, *Prx-5*, *Prx-6*, *Prx-7*, *Prx-C1* (cathodic) (= *Prx-C*)	(3) (10)	*Prx-C2*
SKD	EC 1.1.1.25	*Skdh*	(3)	–
SOD	EC 1.15.1.1	*Sod-1*, *Sod-3*, *Sod-4*	(1) (11)	*Sod-2*, *Sod-5*
TPI	EC 5.3.1.1	*Tpi-1* (= *Tpi-p1*), *Tpi-2*, *Tpi-3*, *Tpi-4*, *Tpi-5* (= *Tpi-c2*)	(12)	–

(1) Chevreau et al. 1985
(2) Chevreau and Laurens 1987
(3) Hemmat et al. 1994
(4) Manganaris and Alston 1987
(5) Manganaris and Alston 1988 a
(6) Manganaris and Alston 1988 b
(7) Manganaris 1989
(8) Manganaris and Alston 1992 a
(9) Manganaris and Alston 1992 b
(10) Manganaris and Alston 1992 c
(11) Manganaris and Alston 1997
(12) Weeden and Lamb 1987

character. The R_t gene segregated 1 : 1, as expected. Columnar habit was scored clearly on most plants of the Y and P progeny, and the C_o gene segregated in the expected 1 : 1 ratio (Table 3).

In total, 131 segregations (locus/family) were recorded for isozyme loci. A summary of these results is given in Table 4. The segregations presented here were obtained on family samples from 20 to 156 plants, with a mean sample size of 83.8. In most cases, a χ^2 test indicated a good agreement between observed and expected ratios. Less than 11% of the observed segregations diverged significantly ($P < 0.05$) from the expected ratios. These cases occurred in all the families, and were not linked with a particular isozyme locus. About 25% of the isozyme loci segregated only in one family, thus providing limited genetic information. Thirteen loci segregated in four or five families and can thus be considered as very informative genetic markers. Two important parameters of the quality of information from a given marker are its degree of polymorphism (number of alleles detected) and the type of genetic segregation which can be scored. In this study, 127 segregating alleles were observed : 108 were codominant (active alleles) and 19 were recessive (null alleles). The number of segregating alleles detected per locus varied from 2 to 5. As apple is an outcrossing species with a high degree of heterozygosity, 2–4 alleles may be involved in any segregation within a full-sib family. The 131 segregations observed can be classified into seven types (Table 4). Types (1) and (2) are the less-informative segregations, because the marker segregates only for one parent. Unfortunately, the vast majority of the segregations scored in this study belong to these two groups (Fig. 2). All the other types of segregations involve markers segregating for both parents. Among them, segregation types (4), (5) and (7) are ideal because 3–4 alleles are involved, which permits one to attribute the correct parental gamete to all genotypes present in the family. In this respect, the more informative isozyme markers in this study were *Aat-1*, *Fdh-1*, *Pgd-1*, *Pgm-1*, *Prx-3*, *Prx-C1* and *Sod-4*.

Linkage relationships and comparison with previous maps

Joint segregation analysis was performed on all informative pairs of loci. Thirty joint segregations with

LOD score values higher than 3.0 were obtained (Table 5). The majority of them (16) mapped to the parent SA 572/2, eight were on Fiesta, five on 3762, two on Northern Spy and only one on Prima. In total, 36 isozyme markers could be located on 11 linkage groups, together with the two phenotypic loci C_o and R_t (Table 6). Taking into account the synonymy between isozyme loci established in Table 2 and the overall results of the European mapping project (Maliepaard et al. 1998), a correspondence with the linkage groups previously published by Hemmat et al. (1994) and Conner et al. (1997) was attempted. The linkage group A in our study (L1 in the EAGMAP map) clearly corresponds to RB-8. Two additional isozyme markers have been located on this group. The linkage group E in our study (L8 in the EAGMAP map) shares similarities with RB/WA-7. A similar linkage between *Aat-2*, *Lap-2* and *Pgi-2* has also been reported in pear, a closely related species (Chevreau et al. 1997). However, this group could also be related to WA-4 because of the presence of *Pgm-3* and *Acp-1*. The relation between these two segments of group E should be further investigated. Linkage group F (L10 in the EAGMAP map) shares similarities with WA/RB-6 because of the presence of the *Est-1* and *Tpi-1* loci. However, the columnar C_o gene also maps in this group in our study, whereas in the recent study of Conner et al. (1997) it belongs to 10-Wi. However, as no common isozyme markers were used in both studies of this team, homology between linkage groups cannot be ascertained. Interestingly, another branching-habit gene (T_b) responsible for terminal bearing in Rome Beauty has been located on WA-6 (Lawson et al. 1995). The linkage groups G and H of the present study belong to the same L17 group on the EAGMAP map, which shares homologies with WA-1. This could indicate that the small linkage group WA-17 containing the *Fdh* locus is part of the WA-1 group. Linkage group I is probably related to WA-3, because of the presence of *Aco-3* and *Idh-2*. The phenotypic marker gene R_t was also clearly located for the first time on this linkage group. Interestingly, another morphological trait involving fruit skin color (R_f) also maps to the end of 3-WM/NY-58 in the american study. The presence of *Prx-C1* on this group in our study does not correspond with its position on WA-5. There might be a difference between the *Prx-C* locus scored by Hemmat et al. (1994) and the *Prx-C1* locus scored by us. Linkage group J in our study shares an isozyme marker with WA-3 (*Est-C*) and another one with 4-WM/NY-67 (*Dia-2*). In this case again, a different interpretation of the diaphorase and/or esterase zymograms by the two teams might be the simplest explanation.

It is interesting to note that there are similarities between markers on linkage groups H and I. Group I

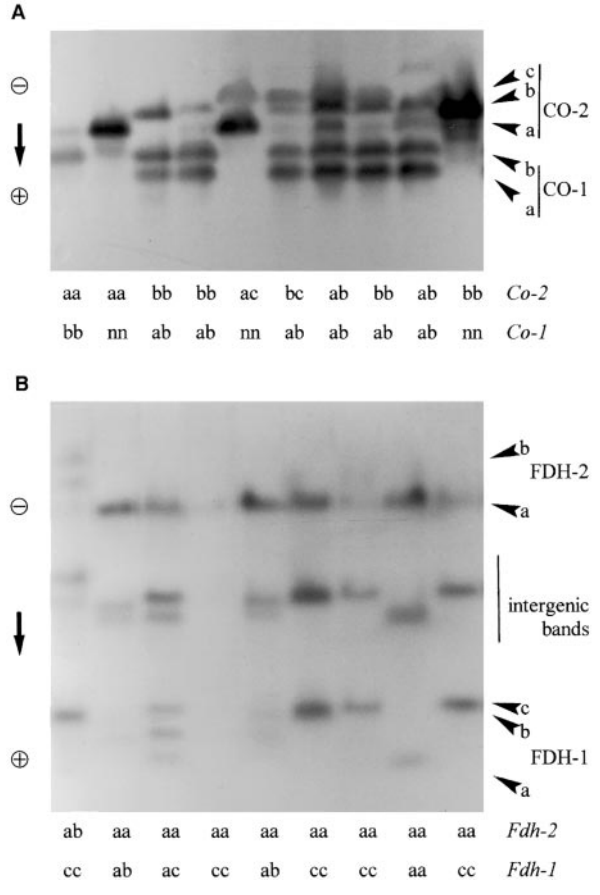

Fig. 1 Isozyme polymorphism of catechol oxidase and formate dehydrogenase in apple. **A** Catechol oxidase from leaf extracts of several apple genotypes, from left to right : M26, MM106, Evereste, M9 open-pollinated, Elstar, Golden Delicious, Granny Smith, Jonagold, Gala, Novole. **B** Formate dehydrogenase from leaf extracts of several apple genotypes, from left to right : P15 hybrid, M26, MM106, Evereste, M9 open-pollinated, Elstar, Golden Delicious, Granny Smith, Jonagold

Table 3 Segregation of phenotypic traits

Locus	Family	Genotypes ♀×♂	Observed segregation	Expected ratio	χ^2	P
C_o	Y	$c_oc_o \times C_oc_o$	49 C_oc_o : 55 c_oc_o	1 : 1	0.35	0.56
	P	$c_oc_o \times C_oc_o$	20 C_oc_o : 23 c_oc_o	1 : 1	0.21	0.65
R_t	R	$r_t\ r_t \times R_t\ r_t$	66 $R_t\ r_t$: 56 $r_t\ r_t$	1 : 1	0.82	0.37
	Y	$r_t\ r_t \times R_t\ r_t$	54 $R_t\ r_t$: 38 $r_t\ r_t$	1 : 1	2.78	0.10
	P	$r_t\ r_t \times R_t\ r_t$	25 $R_t\ r_t$: 21 $r_t\ r_t$	1 : 1	0.35	0.56
	G	$r_t\ r_t \times R_t\ r_t$	35 $R_t\ r_t$: 23 $r_t\ r_t$	1 : 1	2.48	0.12

Table 4 Summary of segregation of isozyme loci

Isozyme locus	Alleles detected	Segregation type[a] and P value of the χ^2 test in family				
		J	R	Y	P	G
Aat-1	a, c, d, e, n	(5) 0.26	(4) 0.28	(2) 0.77	(2) 0.33	(4) 0.11
Aat-2	a, b	(1) 0.52	n.s.	n.s.	n.s.	n.s.
Aat-3	a, b	n.s.	(1) 0.84	n.s.	n.s.	n.s.
Aat-4	a, b	(1) 0.87	(1) 0.72	(1) 0.53	(3) 0.74	n.s.
Aat-5	a, b	n.s.	n.s.	n.s.	n.s.	(1) 0.60
Aco-1	a, b	n.s.	n.s.	n.s.	(1) 0.37	(1) 0.69
Aco-3	a, n	(1) 0.19	(1) 0.14	(1) 0.32	(3) 0.35	n.s.
Aco-4	a, n	n.s.	n.s.	(1) 0.21	(1) 0.21	n.s.
Acp-1	a, n	n.s.	n.s.	(1) 0.64	(1) 0.42	n.s.
Acp-2	a, b, n	n.s.	(2) 0.15	(2) 0.71	(2) < 0.001	n.s.
Acp-4	a, b	n.s.	(3) 0.49	(4) 0.10	n.s.	n.s.
Acp-5	a, b	n.s.	(1) 0.66	n.s.	n.s.	n.s.
Adh-2	a, b	n.s.	(1) 0.76	n.s.	n.s.	n.s.
Co-1	b, n	n.s.	n.s.	(1) 0.03	(1) 0.08	n.s.
Co-2	a, b, n	(1) 0.33	n.s.	(2) 0.39	(2) 0.38	n.s.
Dia-1	a, b	(1) 0.77	n.s.	n.s.	(1) 0.19	n.s.
Dia-2	a, b	(1) 0.15	n.s.	n.s.	(1) 0.71	n.s.
Dia-5	a, b	(1) 0.50	n.s.	n.s.	n.s.	n.s.
Dia-6	a, b	(1) 0.15	n.s.	n.s.	n.s.	n.s.
Enp-1	a, b	n.s.	n.s.	n.s.	(1) 0.40	(1) 0.60
Est-1	a, b	(1) 0.10	(3) 0.88	(3) 0.79	(1) 0.58	(1) 0.43
Est-4	a, b, n	n.s.	(2) < 0.001	(1) 0.27	(1) 0.67	n.s.
Est-5	a, b	n.s.	(1) 0.15	n.s.	n.s.	n.s.
Est-C	a, n	(1) 0.67	n.s.	n.s.	n.s.	n.s.
Fdh-1	a, b, c, d	(1) 0.29	(4) 0.89	(4) 0.02	n.s.	(4) 0.06
Fdh-2	a, b	n.s.	(1) 0.81	(1) 0.18	(1) 0.69	n.s.
Idh-2	a, b	n.s.	n.s.	(1) 0.26	n.s.	n.s.
Idh-3	a, b	n.s.	n.s.	(1) 1.00	(1) 0.21	n.s.
Lap-1	b, c, d, n	n.s.	(2) 0.45	(1) 0.11	(1) 0.72	(2) 0.04
Lap-2	a, c, n	(1) 0.63	n.s.	n.s.	(6) 0.19	n.s.
Me	a, b	(1) 0.05	(1) 0.68	n.s.	(1) 0.32	n.s.
Pgd-1	a, b, d, f	(2) 0.009	(4) 0.17	(3) 0.74	(1) 0.002	(1) 1.00
Pgd-2	a, c	n.s.	n.s.	(1) 0.51	(1) 0.09	n.s.
Pgi-2	b, c	n.s.	n.s.	(1) 0.85	(1) 1.00	n.s.
Pgm-1	a, c, d	(1) 0.63	(1) 0.25	(1) 0.23	(3) 0.41	(4) 0.007
Pgm-2	a, n	(1) 0.45	(6) 1.00	(6) 0.96	(6) 0.87	n.s.
Pgm-3	e, n	n.s.	n.s.	(1) 0.05	(1) 0.77	n.s.
Pgm-4	a, b, c, n	n.s.	n.s.	(2) 0.26	(2) < 0.001	n.s.
Prx-1	a, b	n.s.	n.s.	n.s.	(1) 0.53	n.s.
Prx-2	b, c, e	(2) 0.30	(2) 0.46	(2) 0.24	(1) 1.00	(1) 0.19
Prx-3	a, b, n	(2) 0.30	(7) 0.29	(2) 0.32	(2) 0.51	(7) 0.02
Prx-4	a, b, c	n.s.	(1) 0.47	n.s.	(1) 1.00	n.s.
Prx-C1	a, b, n	(1) 0.32	(7) 0.03	n.s.	n.s.	n.s.
Prx-C2	a, b	(1) 0.02	(1) 0.40	n.s.	n.s.	n.s.
Skdh	a, b	n.s.	(1) 1.00	n.s.	n.s.	n.s.
Sod-1	a, b	n.s.	(1) 1.00	n.s.	n.s.	(1) 1.00
Sod-2	a, b	n.s.	(1) 0.13	n.s.	n.s.	n.s.
Sod-3	a, n	(1) 0.26	n.s.	(1) 0.05	(6) 0.30	(1) 0.60
Sod-4	a, b, n	(7) < 0.001	(1) 0.23	(1) 0.38	n.s.	n.s.
Sod-5	a, b	n.s.	(1) 0.86	n.s.	n.s.	n.s.
Tpi-1	a, b	n.s.	(1) 0.32	(1) 0.86	(1) 0.59	n.s.
Tpi-3	a, n	(1) 0.87	(1) 0.02	n.s.	n.s.	n.s.
Tpi-5	a, b	(1) 0.87	(3) 0.36	(1) 0.14	(1) 0.12	n.s.

[a] Type of segregation:
(1): aa × ab, segregating 1 : 1, with 2 alleles
(2): aa × bc, segregating 1 : 1, with 3 alleles
(3): ab × ab, segregating 1 : 2 : 1, with 2 alleles
(4): ab × ac, segregating 1 : 1 : 1 : 1, with 3 alleles
(5): ab × cd, segregating 1 : 1 : 1 : 1, with 4 alleles
(6): an × an, segregating 3 : 1, with 1 allele + 1 null allele
(7): an × ab, segregating 2 : 1 : 1, with 2 alleles + 1 null allele
n.s.: non segregating, both parents homozygous

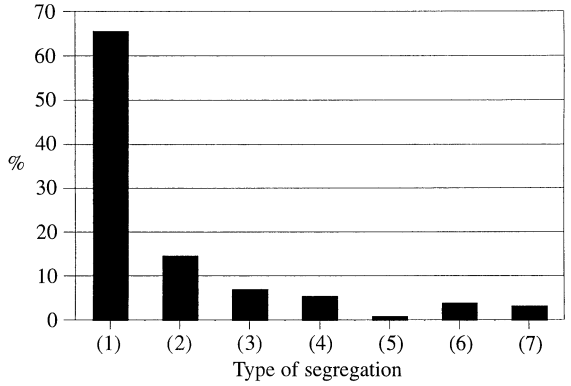

Fig. 2 Distribution of the 131 observed segregations in seven genetic types, as defined in Table 4. (*1*): aa × ab, segregating 1 : 1, with 2 alleles. (*2*): aa × bc, segregating 1 : 1, with 3 alleles. (*3*): ab × ab, segregating 1 : 2 : 1, with 2 alleles. (*4*): ab × ac, segregating 1 : 1 : 1 : 1, with 3 alleles. (*5*): ab × cd, segregating 1 : 1 : 1 : 1, with 4 alleles. (*6*): an × an, segregating 3 : 1, with 1 allele + 1 null allele. (*7*): an × ab, segregating 2 : 1 : 1, with 2 alleles + 1 null allele

comprises *Aco-3*, *Pgd-2* and *Fdh-2*, wheras group H comprises *Fdh-1* and *Aco-4*. In addition, linkage between *Fdh-1* and *Pgd-3* was reported in group WA-17 (Hemmat et al. 1994), which corresponds to group H. Furthermore, it has been suggested on the basis of the existence of intergenic hybrid bands, that *Pgd-2* and *Pgd-3* are duplicated loci (Weeden 1986), as are *Fdh-1* and *Fdh-2* (this study). This evidence of homoeology between linkage groups is in good agreement with the allopolyploid origin of the *Maloideae* genome.

Conclusion

The results presented in this study illustrate an extensive analysis of the isozyme-marker segregations conducted by the two teams in collaboration. It enables us

Table 5 Summary of linkage analysis

Pair of loci	Mapped parent	Family	R value	LOD score
R_t : Est-5	3762	R	0.06	22.1
R_t : Idh-2	SA572	Y	0.16	7.0
R_t : Prx-C1	3762	R	0.15	9.2
C_o : Est-1	SA572	Y	0.16	5.3
Aat-1 : Dia-6	Fiesta	J	0.22	6.8
Aat-2 : Lap-2	Prima	J	0.09	25.2
Acp-1 : Pgm-3	SA572	Y	0.08	20.4
		P	0.11	6.8
Acp-2 : Pgm-4	SA572	Y	0.07	20.4
		P	0.06	10.1
Aco-3 : Idh-3	SA572	Y	0.22	4.5
Aco-3 : Pgd-2	SA572	Y	0.17	6.5
		P	0.00	–
Aco-4 : Fdh-1	SA572	Y	0.11	4.3
Co-1 : Co-2	SA572	Y	0.12	9.3
		P	0.06	6.4
Dia-2 : Est-C1	Fiesta	J	0.16	9.1
Dia-2 : Prx-C2	Fiesta	J	0.30	4.1
Est-1 : Tpi-1	3762	R	0.14	5.6
Est-1 : Tpi-3	Fiesta	J	0.03	8.0
Est-4 : Sod-3	SA572	Y	0.21	7.6
Est-5 : Prx-C1	3762	R	0.20	5.9
Est-C1 : Prx-C2	Fiesta	J	0.19	6.3
Fdh-2 : Idh-3	SA572	Y	0.08	10.6
		P	0.00	–
Fdh-2 : Pgd-2	SA572	Y	0.09	15.9
		P	0.06	3.8
Idh-2 : Pgd-2	SA572	Y	0.27	3.0
Idh-3 : Pgd-2	SA572	Y	0.06	13.3
		P	0.17	3.2
Lap-1 : Lap-2	SA572	P	0.00	4.1
Lap-1 : Pgi-2	SA572	Y	0.17	6.5
		P	0.10	4.5
Pgi-2 : Pgm-3	SA572	P	0.21	3.4
Pgm-1 : Tpi-5	3762	R	0.18	4.4
Prx-2 : Prx-3	Fiesta	J	0.00	46.6
		R	0.00	44.2
		Y	0.00	36.7
	DNRS	P	0.02	14.1
		G	0.05	12.3
Skd-1 : Sod-5	Fiesta	R	0.13	3.9
Sod-4 : Tpi-5	Fiesta	R	0.19	3.8

Table 6 Linkage groups and correspondence with previously published maps

Family	Linkage groups detected in the present study [a]	Homology with published apple maps		
		Maliepaard et al. (1998)[b]	Hemmat et al. (1994)	Conner et al. (1997)
A	*Sod-5* + (*Skdh----Tpi-5----Pgm-1*) + *Sod-4*	L1-Pr/Fi	RB-8	–
B	*Prx-2----Prx-3*	L3-Fi	RB-10 (p.c.[c])	–
C	*Sod-3----Est-4*	L4-Pr	WA-5	–
D	*Co-1----Co-2*	L5-Pr	–	–
E	*Lap-1* + (*Aat-2----Lap-2----Pgi-2----Pgm-3----Acp-1*)	L8-Pr	RB/WA-7	7-NY-67
F	*Tpi-3* + (*Est-1----Tpi-1*) + *C_o*	L10-Fi	RB/WA-6	10-WM
G	*Aat-1----Dia-6*	L17-Pr/Fi	WA-1	1-WM/NY-58
H	*Fdh-1----Aco-4*	L17-Fi	WA-17	–
I	(*Aco-3----Pgd-2----Idh-3----Fdh-2----Idh-2*) + (*Prx-C1----R_t----Est-5*)	–	WA-3	–
J	*Dia-2----Est-C----Prx-C2*	–	WA-3	4-WM/NY-67
K	*Acp-2----Pgm-4*	–	–	–

[a] Inside linkage groups, markers linked in known order are joined with (----), markers with undetermined position are joined with (+)
[b] The EAGMAP map published by Maliepaard et al. (1998) includes isozyme data from the present study for the family Prima × Fiesta
[c] p.c.: personal communication from Dr. N.F. Weeden, cited in Maliepaard et al. (1998)

to draw conclusions about the interest and limits of this type of marker for mapping the apple genome. Among the potential advantages of isozymes is their known reliability.

The experience gained during this study led us to conclude that if some easy to score loci are in fact reliable, mis-interpretation of gels can still occur for enzymes with particularly complex patterns. The tentative establishment of synonymy between the isozyme locus designations employed by different teams working on apple has still to improve. One of the main advantages of this type of marker is its level of polymorphism in an outcrossing species such as apple. In the present study, up to five alleles per locus were observed. The monogenic segregation ratios were also of good quality. The proportion of distorted segregations (11%) was lower than that reported for apple RAPD markers by Conner et al. (1997): 17–24%. The availability of co-dominant markers heterozygous in both parents of a family, and furthermore carrying specific parental alleles, makes isozymes very important anchor points to integrate separate maps generated on different parents. Our study demonstrated the efficiency of isozymes to determine homologous linkage groups from unrelated maps, but it also revealed that in apple only a minority of isozyme markers have the appropriate qualities. Finally, the main drawback of isozyme markers lies in their limited number. In the present study, the number of scorable apple isozyme loci has been extended to 53, and this is probably close to the maximum number of loci that can be reliably detected. Restriction fragment length polymorphism (RFLP) and microsatellites offer other opportunities to extend the number of co-dominant polymorphic markers to complete the consensus apple genome map, but their development still requires much effort.

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