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ORIGINAL PAPER

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Inheritance of the *Md-ACS1* gene and its relationship to fruit softening in apple (*Malus* × *domestica* Borkh.)

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Abstract The 1-aminocyclopropane-1-carboxylic acid synthase (ACS) gene is a member of the ACS gene family that is involved in apple (*Malus* × *domestica* Borkh.) fruit ripening. Presence of an allele (*Md-ACS1-2*) of this gene is associated with low internal ethylene concentration in some apple cultivars. In this study, inheritance of *Md-ACS1* was determined for 50 apple cultivars/advanced selections and 101 F_1 seedlings from five populations. Following this, the softening pattern of apples stored at 20°C for up to 40 days was examined using 35 fruiting cultivars/selections of defined *Md-ACS1* status. *Md-ACS1* is inherited in a Mendelian fashion and was found to be linked to fruit softening. Maturity season of genotypes also significantly affected fruit softening. Late-season genotypes in the *Md-ACS1-2/2* class had the slowest rate of softening, while early-season *Md-ACS1-1/1* genotypes had the most rapid softening rate. The implications of these results are discussed in relation to parental selection and breeding for storage ability in apple.

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Introduction

Softening is a critical determinant of the potential of a fruit cultivar to last through prolonged storage and in the consumer's home. Excessive softening is considered undesirable in apple and can lead to lower sensory values for firmness, juiciness, crispness and crunchiness, as well as increased mealiness (Abbott et al. 1984; Harker et al. 1997, 2002; Jaeger et al. 1998) and reduced consumer acceptability (Liu and King 1978). Fruit softening is often assessed using a puncture test in apple, also known as a flesh firmness, fruit firmness or fruit pressure test (Johnston et al. 2002a). Many markets are increasingly using fruit firmness as a guide to ensure that apples delivered to customers have the required textural characteristics year-round (Johnston et al. 2002a). Failure of growers to meet these standards can result in shipment rejections, a damaged reputation as a supplier of quality apples and reduced financial returns.

The onset of ethylene production during fruit ripening is widely believed to be the key driver of cellular structural changes that lead to softening (Abeles and Biles 1991). The role of ethylene in apple ripening has been studied using several approaches, but the most conclusive evidence that ethylene promotes apple fruit softening has resulted from experiments using inhibitors of ethylene action. Apples treated with 1-methylcyclopropene softened slower and had reduced internal ethylene concentration (IEC) relative to untreated fruit in storage (Fan et al. 1999; Rupasinghe et al. 2000; Watkins et al. 2000). Suppression of the biosynthesis of this gaseous hormone is one of the mechanisms by which controlled atmospheres extend the storage life of apples (Saftner et al. 2002). The hormone is synthesized in plants from S-adenosyl-L-methionine (SAM) via a short pathway catalysed by two enzymes: 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO). The conversion of SAM to ACC by ACC synthase is the first step in ethylene biosynthesis and is generally considered to be the rate-limiting step (Lau et al. 1986). Several ACC

synthase genes have been reported in apple, but *Md-ACS1* has been found to be predominantly expressed in the ripening fruit (Rosenfield et al. 1996; Harada et al. 1997, 2000). Homozygosity of an allele (*Md-ACS1-2*) of this gene isolated from apple (Sunako et al. 1999) resulted in low levels of ethylene production in some apple cultivars (Harada et al. 2000). For example, 'Fuji', an *Md-ACS1-2* homozygous cultivar that is a low ethylene producer could be stored up to 8 months at 0–4°C (Fan et al. 1997).

Many studies have been carried out to-date to determine the biological causes of fruit softening (Johnston et al. 2002a). Both genetic and environmental factors are important. Genetic factors will influence the inherent firmness of the fruit, as well as the rate of softening during storage, but many environmental factors such as storage temperature will modify the inherent behaviour of fruit (Saftner 1999; Saftner et al. 2002). In terms of genetic factors, the role of *Md-ACS1* in fruit softening may be critical. The aim of the present study was to determine the mode of inheritance of *Md-ACS1* and to examine its relationship to storage ability in apple. We believe this information will assist apple breeders to make an informed decision on the choice of parents for use in developing varieties with long-term storage potential.

Materials and methods

Determination of *Md-ACS1* genotype and its inheritance in F_1 progenies

Plant material

This study was based on 50 apple cultivars and advanced selections (Table 1) located in the orchards of NIFTS, Morioka, Japan. In addition, segregation of *Md-ACS1* was studied in five F_1 populations with a total of 101 glasshouse-raised seedlings in second and third leaf stages. The populations were carefully chosen to represent a range of crosses with parents homozygous and heterozygous for *Md-ACS1* (Table 2).

DNA isolation, amplification and analysis

Genomic DNA was isolated from young immature leaves and quantitated using the method described by Gardiner et al. (1996). The following oligo-nucleotide primers numbered according to GenBank accession number U89156 (Sunako et al. 1999) were used for PCR amplification: ACS1-5′F 5′AGAGAGATGCCATTTTT-GTTCGTAC3′ 861-887; ACS-5′R 5′CCT ACAAACTTGCGTGG-GGATTATAAGTGT3′ 1379-1350. The methods of Sunako et al. (1999) and Harada et al. (2000) were used for PCR amplification and identification of *Md-ACS1* alleles present in cultivars/selections and F_1 progenies.

The reaction was carried out in a 50-µl volume with 100 ng of genomic DNA, 0.2 µM of each primer, 200 µM of each dNTP, 1×PCR reaction buffer and 2.5 U of Ampli*Taq* DNA polymerase. The PCR amplification was carried out in a GeneAMP thermocycler (PCR System 9700) using the following amplification conditions: initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 58°C for 1 min and 72°C for 2.5 min, with a final 10 min extension at 72°C.

The PCR amplified products were resolved on a 1.5% agarose gel using *Md-ACS1-1* and *Md-ACS1-2* markers as size standards. *Md-ACS1-1* is 489 bp in length, while *Md-ACS1-2* is 655 bp (Sunako et al. 1999). Any individual that exhibited only one PCR fragment size was classified as homozygous: *Md-ACS1-2/2* if the fragment was 655 bp and *Md-ACS1-1/1* if the fragment was 489 bp. The banding profile from heterozygous individuals exhibited both fragments (Fig. 1).

Storage trial

Thirty-five out of the 50 cultivars/selections described in the previous section produced fruit in 2001, and these were used for the storage study (see Table 1).

Fruit were harvested at commercial maturity from August to November 2001. About 30–40 fruit per tree/cultivar or selection were evaluated. Five fruit were randomly chosen immediately after harvest, weighed and assessed for firmness, brix, starch pattern index and acidity. A small skin area (up to 15 mm in diameter) was removed from two opposite sides of each fruit around the equator corresponding to the blushed and shaded portions, respectively, and firmness (the maximum force after the penetration of a probe into the flesh) was measured using an Effegi penetrometer (model FT327, McCormick Fruit Tech, Washington) fitted with a 11.2-mm-diameter probe. Juice from the tip of the penetrometer probe from each fruit was transferred onto a digital refractometer (model PR-100, Atago, Tokyo) for measurement of per cent soluble solids. About 10 g of cortical tissue from the opposite sides of each fruit were peeled and cored, and the crude juice was extracted with a juicer (model MJ-C68, National Osaka). Following filtration, about 2-ml aliquot of the juice was titrated (with an auto-titrator model AUT-301, TOA Electronics, Tokyo) to an endpoint of pH 9.5 using 0.1 N NaOH. Titration results were calculated as malic acid (g) per 100 ml of sample juice. Starch content was assessed using standard methods (e.g. Lau 1985) that had been slightly modified. About 5-mm-thick slices from each fruit cut through the equator were dipped for about 20 min in starch-iodine solution, and the resulting starch pattern was scored on a 0–5 scale, where 0 (no blue colour) indicates no starch, and 5 (sliced section completely blue) indicating extreme starch.

The remaining fruit were transferred to an incubator (model 540-DL-2WS, Hashimoto, Tokyo) and stored at 20±2°C and 80±5%RH. Temperature and humidity were controlled by an air-conditioner (model MSZ-AXV22H, Mitsubishi Electrical, Tokyo) and a humidifier (model PH-5F, PS Manufacturing, Tokyo), respectively. Five fruit samples per cultivar/selection (depending on availability) were removed from the incubator at intervals of 5,

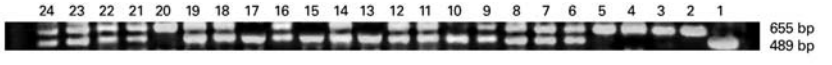

Fig. 1 The *Md-ACS1* PCR banding profile of representative apple cultivars/selections used in the study

Lanes are numbered in ascending order from right to left; 1= *Md-ACS1-1* marker (489 bp), 2 = *Md-ACS1-2* marker (655 bp), 3 = 'Sansa', 4 = 'Gala', 5 = 'Hoozuri', 6 = 'Morioka 61', 7 = 'TSR32T219', 8 = 'Morioka 53', 9 = 'Morioka 55', 10 = 'Morioka 57', 11 = 'Morioka 58', 12 = 'Morioka 60', 13 = 'Morioka 54', 14 = 'Hacnine', 15 = 'Beninomai', 16 = 'Freedom', 17 = 'Rosehask', 18 = 'Kitarou', 19 = 'Kanki', 20 = 'Senshu', 21 = 'Orin', 22 = 'TSR32T130', 23 = 'Koutarou', 24 = 'Smoothee.'

Table 1 Apple cultivars/selections used in the study, *Md-ACS1* class, maturity season, rootstock used and harvest date

Md-ACS1 class	Maturity season[a]	Cultivar/selection	Code no.	Rootstock	Harvest date (2001)
1/1	Early	Beninomai	3	M26	31 August
1/1	Early	Kitakami	20	Maruba	7 September
1/1	Early	McIntosh	23	Maruba	20 September
1/1	Mid	Hatsuaki	11	Maruba	28 September
1/1	Mid	Morioka57	30	M26	5 October
1/1	Mid	Morioka59	32	M9	5 October
1/1	Mid	Santarou	41	M26	28 September
1/1	-	8H226	1	-	-
1/1	-	Granny Smith	9	-	-
1/1	-	Indo	15	-	-
1/1	-	Morioka 51	26	-	-
1/1	-	Morioka 54	28	-	-
1/1	-	Rosehask	39	-	-
1/2	Early	Kanki	18	M26	20 September
1/2	Early	Mikilife	25	M9	7 September
1/2	Early	Silken	45	M9	20 September
1/2	Early	Tsugaru	50	JM7	14 September
1/2	Late	Golden Delicious	8	M9	26 October
1/2	Late	Hacnine	10	Maruba	26 October
1/2	Late	Hokuto	13	JM7	26 October
1/2	Late	Koutarou	22	JM2	26 October
1/2	Late	Morioka58	31	M9	2 November
1/2	Late	Mutsu	35	JM7	26 October
1/2	Late	Orin	36	M9	2 November
1/2	Mid	JonaGold	16	JM7	20 October
1/2	Mid	Jonathan	17	Maruba	11 October
1/2	Mid	Kitarou	21	JM2	11 October
1/2	Mid	Morioka60	33	M9	11 October
1/2	Mid	Morioka61	34	M26	28 September
1/2	Mid	RedGold	38	M9	11 October
1/2	Mid	Sekaichi	42	-	11 October
1/2	Mid	Starking Delicious	47	JM7	20 October
1/2	-	Freedom	5	-	-
1/2	-	Morioka 53	27	-	-
1/2	-	Morioka 55	29	-	-
1/2	-	Smoothee	46	-	-
1/2	-	TSR32T130	48	-	-
1/2	-	TSR32T219	49	-	-
2/2	Early	Akane	2	Maruba	14 September
2/2	Early	Himekami	12	MM106	20 September
2/2	Early	Sansa	40	M9	7 September
2/2	Late	Fuji	6	M9	9 November
2/2	Late	Hoozuri	14	-	9 November
2/2	Late	Kinsei	19	-	9 November
2/2	Late	Megumi	24	-	9 November
2/2	Late	Ralls Janet	37	M9	17 November
2/2	Mid	Senshu	43	JM7	11 October
2/2	-	Discovery	4	-	-
2/2	-	Gala	7	-	-
2/2	-	Shinsekai	44	-	-

[a] There is no information on maturity season, rootstock and harvest date for cultivars not used in the storage trial

Table 2 Segregation of *Md-ACS1* in five F_1 progenies including the *Md-ACS1* genotype of parents and total number of individuals within each family that belonged to a particular *Md-ACS1* genotype class

Family	*Md-ACS1* parental genotypes	Tree no.		
		1/1	1/2	2/2
Golden Delicious × Akane	1/2×2/2	-	15	16
Indo × TSR32T130	1/1×1/2	16	10	-
Indo × Freedom	1/1×1/2	2	9	-
Sansa × Fuji	2/2×2/2	-	-	6
Fuji × 8H226	2/2×1/1	-	30	-

10, 15, 20, 30 and 40 days, and assessed for firmness with the penetrometer.

Analysis of firmness data to examine softening behaviour of cultivars/selections

Cultivars/selections were grouped by maturity season as well as into the three *Md-ACS1* genotype classes. Individual trees (one tree per cultivar or selection) harvested from August to 20 September, after 20 September to 20 October, and after 20 October, were classified as early, mid-, and late season, respectively (Table 1). In all analyses, tree means were used as the unit rather than individual fruit. Firmness measurements from both sides of all fruit from the

same cultivar/selection assessed on the same day were averaged prior to analysis.

Assessment of differences in softening pattern required the fitting of a time-dependent model. A range of curve shapes were fitted to the data, using SAS Proc Mixed (SAS Institute 2000), with cultivar/selection as a random effect. However, it was found that simple curves (e.g. linear and exponential) did not fit the time course of the cultivars/selections well. The more complex models (quadratic, cubic and exponential quadratic), which fitted much better, were unrealistic because for some *Md-ACS1*/maturity season combinations, the firmness was predicted to increase in some time periods. We therefore used a more realistic non-linear model to describe the change in firmness with time. Benge et al. (2000) considered a number of different kiwifruit softening curves, and found that while a two-parameter 'complementary Michaelis-Menton type' curve did not fit well, a modified version with four parameters involving two successive Michaelis-Menton phases provided a good fit with monotonic decreasing curves. We have taken an approach intermediate between these extremes whereby most of the softening process is described by a simple Michaelis-Menton type curve, but a small quadratic section is spliced on at the start so that a delay prior to rapid softening is possible. This gives a three-parameter model,

$$FF = \begin{cases} FF_0(1 - at^2) & t < t_c \\ FF_0\left(1 - at_c^2\right)\left(1 - \frac{(t-t_c)}{(t-t_c)+t_h}\right), & t \geqslant t_c. \end{cases}$$

where to match the slopes of the quadratic and Michaelis-Menton sections at $t=t_c$ we must have

$$a = \frac{1}{t_c(t_c + 2t_h)}.$$

The three parameters are the firmness at harvest (FF_0), the time where the quadratic and Michaelis-Menton sections join (t_c), and the time after t_c when the level drops to half its value at $t=t_c$ (t_h). These three parameters were estimated for each cultivar or selection using least-squares nonlinear regression (SAS Proc NLIN). Appropriate *F*-statistics were used to test whether this three-parameter model provided significant improvement over simple linear regression and whether cultivars/selections differed significantly within each *Md-ACS1*/maturity season group. Fitted harvest firmness (FF_0), and two derived parameters [the change in firmness over the first 20 days after harvest (DAH), and the firmness at 20 DAH] were analysed using SAS Proc GLM. One cultivar for which data were only available for the first 10 days ('Beninomai') was given a weighting of 0.5 in this analysis, and cultivars where data stopped after 15 days were given a weighting of 0.75. For each parameter, a one-sided *t*-test was used to determine whether there was an increasing trend with *Md-ACS1* genotype class (i.e. from *Md-ACS1-1/1* to *Md-ACS1-1/2* and *Md-ACS1-2/2*), but the significance of any trend with maturity season was estimated with a two-sided test.

Data obtained at harvest for fruit weight, soluble solids, acidity and starch pattern index (used as maturity indicators) were also analysed using SAS Proc GLM.

Results

Determination of *Md-ACS1* genotype of cultivars/selections and F_1 seedlings

The gel image of the *Md-ACS1* profile of some of the cultivars/selections is shown in Fig. 1. The specific *Md-ACS1* alleles exhibited by the cultivars/selections were used to group them into three genotype classes. Out of the 50 individuals genotyped, 13 were classified as *Md-ACS1-1* homozygous, 12 as *Md-ACS1-2* homozygous and the rest were heterozygous (i.e. *Md-ACS1-1/2*) (Table 1).

Inheritance studies using five small F_1 populations showed that the gene is inherited in Mendelian fashion. For example, a cross between 'Fuji' (*Md-ACS1-2/2* genotype) and the selection '8H226' (*Md-ACS1-1/1* genotype) produced heterozygous F_1 progeny only. Also, the F_1 progeny from 'Fuji' × 'Sansa' (two *ACS1-2* homozygotes) were all *ACS1-2* homozygous (Table 2).

Softening behaviour of cultivars/selections and the relationship to *Md-ACS1* and maturity season

Michaelis-Menton curves described the time course of individual cultivars/selections well, accounting for 91% of the variation about cultivar/selection means. However, while separate values of FF_0 and t_h were needed for each cultivar/selection to adequately describe the time course ($P<0.001$), use of different values of t_c for each gave no significant improvement over a global value of $t_c=4$ days. A reduced Michaelis-Menton model with a global t_c requires just one more global parameter than separate linear or exponential curves for each cultivar/selection, but performed significantly better ($P<0.001$). A fixed value of $t_c=0$ gave a significantly poorer fit ($P<0.001$), suggesting that on average, there is a short delay after harvest before the onset of rapid softening. For simplicity, the different *Md-ACS1*/maturity season groupings were therefore compared using two fitted parameters only (FF_0 and t_h), with t_c fixed at 4 days.

At harvest, there was a range in average firmness (N) of individual cultivars/selections, from 54.4 N ('Kitakami', early *ACS1-1/1* genotype) to 79.5 N ('Morioka 60', a mid-season *Md-ACS1-1/2* genotype) (Fig. 2). Other cultivars that had a firmness of 79 N were 'Mutsu' (a late-season *Md-ACS1-1/2* genotype) and 'RedGold'(a mid-season *Md-ACS1-1/2* genotype). From the fitted curves, there was a significant trend for later maturing genotypes to have firmer fruit at harvest ($P<0.05$, Table 3), but with some variation in this pattern. The positive trend with *Md-ACS1* genotype was not significant, nor was the interaction between *Md-ACS1* class and maturity season.

During the first 20 DAH, there was a tendency for *Md-ACS1-1/2* and *Md-ACS1-2/2* genotypes and later-maturing genotypes to soften more slowly (Table 3), but neither of these trends was significant, nor was their interaction. The trend with the *Md-ACS1* class is not present in early maturing fruit, and the increase with maturity season is apparent only in *Md-ACS1-2/2* fruit. By 20 DAH, however, the combined effects of differences in harvest firmness and softening rate in the first 20 days were apparent, so the trends for *Md-ACS1-1/2* and *Md-ACS1-2/2* classes, and for later-maturing genotypes, to be firmer were both significant ($P<0.05$, Table 3). The trend with *Md-ACS1* class is least obvious for early-season cultivars, with early-season *Md-ACS1-1/2* fruit being firmer than might be expected, which may also have contributed to the lack of a clear trend with maturity season for *Md-*

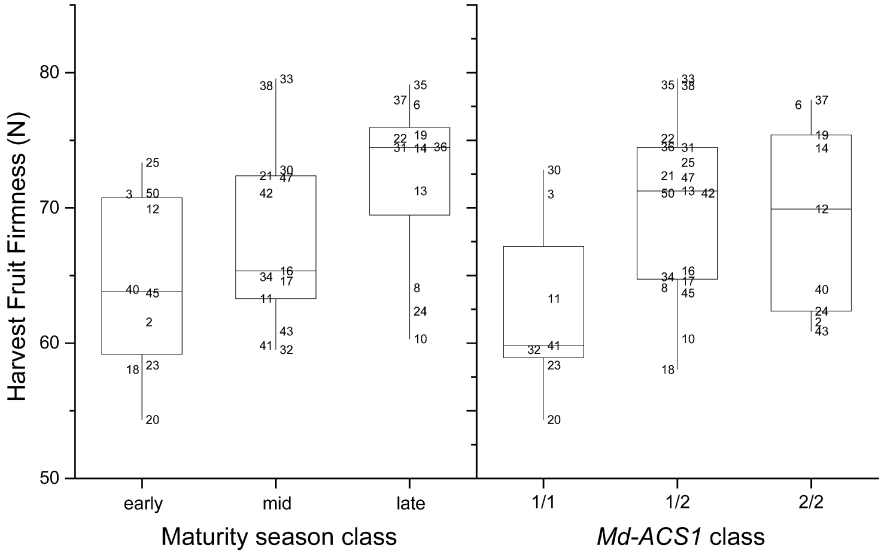

Fig. 2 Distribution of average harvest firmness of apple cultivars/selections within maturity season and *Md-ACS1* classes. Numbers correspond to cultivar/selection names in Table 1. The *bottom*, *inside horizontal* and *top borders* in each box indicate 25% quantile, median value and 75% quantile, respectively

Table 3 Influence of *Md-ACS1* genotype and season of maturity on fruit firmness at harvest, change in fruit firmness in the first 20 days after harvest (DAH), and fruit firmness at 20 DAH. Trends represent the fitted mean change per step in *Md-ACS1* genotype class (1/1, 1/2, 2/2) or maturity season (early, mid, late)

		Season of maturity		
		Early (±SE)	Mid (±SE)	Late (±SE)
Fruit firmness (N) at harvest				
Md-ACS1	1/1	57 (±3.9)	64 (±3.2)	
	1/2	67 (±3.3)	73 (±2.3)	70 (±2.4)
	2/2	64 (±3.6)	61 (±7.1)	73 (±2.7)
Trends: *Md-ACS1* class: $+2.2^{ns}$		Maturity season: +3.4*		
Change in fruit firmness (N) between harvest and 20 DAH				
Md-ACS1	1/1	−28 (±6.9)	−24 (±5.6)	
	1/2	−23 (±5.8)	−26 (±4.0)	−25 (±4.2)
	2/2	−28 (±6.3)	−12 (±12)	−14 (±4.9)
Trends: *Md-ACS1* class: $+3.0^{ns}$		Maturity season: $+2.3^{ns}$		
Fruit firmness (N) 20 DAH				
Md-ACS1	1/1	29 (±6.9)	40 (±5.6)	
	1/2	44 (±5.9)	47 (±4.1)	45 (±4.2)
	2/2	36 (±6.3)	49 (±13)	58 (±4.9)
Trends: *Md-ACS1* class: +5.2*		Maturity season: +5.7*		

Significance trends: * $P<0.05$, *ns* non-significant

ACS1-1/2 fruit. The interaction between *Md-ACS1* class and maturity season was not, however, significant. At 20 DAH, the smallest fitted average firmness (21.2 N) was observed in 'Silken', an early-season *Md-ACS1-1/2* genotype, whereas the highest average firmness (72.1 N) was observed in 'Hoozuri'(a late *Md-ACS1-2/2* genotype), followed by 'Fuji', another late *Md-ACS1-2/2* genotype (Fig. 3). The fitted trends mean that, on average, *Md-ACS1-2/2* genotypes were about 10 N firmer at 20 DAH than *Md-ACS1-1/1* genotypes from the same maturity season, and late-season genotypes were on average 11 N firmer than early-season genotypes from the same *Md-ACS1* class (Table 3). However, there was some variation in these patterns; for example, early-season *Md-ACS1-2/2* fruit appeared to be softer at 20 DAH than their *Md-ACS1-1/2* counterparts, but this difference was not quite significant ($P>0.05$). For all three parameters (i.e. firmness at harvest, change in firmness between harvest and 20 DAH and firmness at 20 DAH), the estimate for the mid-season *Md-ACS1-2/2* group had a much larger standard error than the other groups, because it contains just one cultivar ('Senshu'). Over the whole 40-day storage period, early *Md-ACS1-1/1* genotypes had the most rapid rate of softening, while late *Md-ACS1-2/2* genotypes had the slowest softening rate (Fig. 4).

Furthermore, there were significant differences in softening behaviour among cultivars/selections within the different *Md-ACS1*/maturity season groupings (data not presented). The shape of the softening curves differed significantly within all groups containing more than four cultivars/selections [mid- and late-season *Md-ACS1-1/2* ($P<0.05$), and late-season *Md-ACS1-2/2* ($P<0.01$)]. Variation within groups containing exactly four cultivars/selections (mid-season *Md-ACS1-1/1* and early-season *Md-ACS1-1/2*) was almost significant ($P<0.1$), but for other groupings, the number of cultivars/selections was too small for significant differences to be detected. There was only one tree per cultivar/selection, so it is possible some of this apparent variation between cultivars within a group could be due instead to between-tree variation.

The maturity indicators assessed at harvest, namely fruit weight, soluble solids, acidity and starch pattern index, did not differ significantly among *Md-ACS1* classes, but all, except acidity, differed significantly among maturity season classes ($P<0.05$). These data are not presented, as they provide no additional information on softening behaviour.

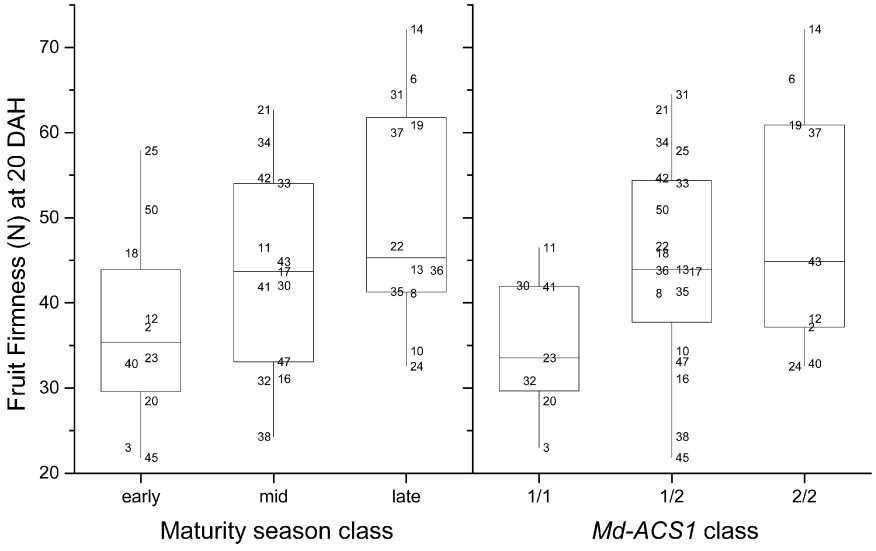

Fig. 3 Firmness of apple cultivars/selections within maturity season and *Md-ACS1* classes at twenty days after harvest (20 DAH) following storage at 20±2°C and 80±5%RH. Numbers correspond to cultivar/selection names in Table 1. The *bottom*, *inside horizontal* and *top borders* in each box indicate 25% quantile, median value and 75% quantile, respectively

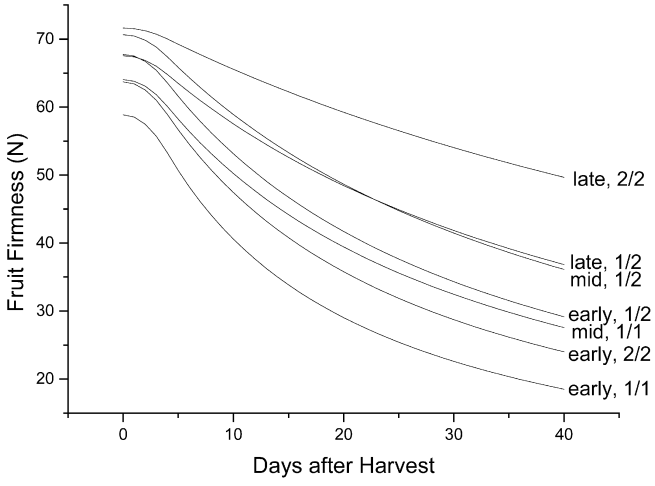

Fig. 4 Fitted fruit softening behaviour of apple cultivars/selections for different *Md-ACS1*/maturity season combinations following storage at 20±2°C and 80±5%RH for 40 days. There was no late *Md-ACS1-1/1* genotype and only one mid-season *Md-ACS1-1/1* genotype, hence no curves for these combinations

Discussion

Maintenance of adequate texture during long storage and/or extended shelf life is a key objective of many apple breeding programmes (DeEll et al. 1999; Laurens and Pitiot 2002; White 2002). In this study, late-season *Md-ACS1-2/2* genotypes (with the exception of 'Megumi') with higher harvest firmness softened slower than their early-season counterparts (Figs. 2, 3). The results are in agreement with the general belief that fruit with higher firmness at harvest are firmer after storage than fruit with lower firmness (Johnston et al. 2002a). However, the predictive accuracy of prestorage firmness measurements can vary substantially between seasons and cultivars (Johnston et al. 2002a). Therefore, a higher harvest firmness may not always lead to longer storage ability as we also observed in this study. For example, early- and late-season *Md-ACS1-1/2* genotypes started with the same average firmness, but more rapid softening of the early-season genotypes led to a difference in firmness after storage (Fig. 4). Similarly, some mid- and late-season *Md-ACS1-1/2* genotypes had a higher average harvest firmness than *Md-ACS1-1/1* and *Md-ACS1-2/2* genotypes of similar ripening classes, but at 20 DAH, the highest firmness was observed in late-season *Md-ACS1-2/2* genotypes, including 'Hoozuri' and 'Fuji' (Fig. 3). Moreso, there was no significant difference in harvest firmness of late-season *Md-ACS1-1/2* and *Md-ACS1-2/2* fruit (Table 3), but their fitted firmness differed significantly by 20DAH. These results suggest that *Md-ACS1* genotype is associated with the variable softening behaviour found in the apple cultivars/selections.

At harvest, firmness increased significantly with maturity season, but there was no significant overall trend with *Md-ACS1* class ($P<0.05$). However, at 20 DAH, both *Md-ACS1* genotype and maturity season significantly influenced firmness, resulting in differences in softening behaviour among genotypes. Late-season genotypes in the *Md-ACS1-2/2* class had the slowest softening rate, while early-season *Md-ACS1-1/1* genotypes had the most rapid softening rate. These results suggest that both *Md-ACS1* genotype and maturity season affect apple fruit softening, and that neither alone can determine softening behaviour. A combination of late maturity and *Md-ACS1-2/2* genotype will more likely soften slower than any other combinations of *Md-ACS1* and maturity season.

We carried out our fruit storage at 20±2°C and 80±5% RH, similar to the conditions under which Harada et al. (2000) carried out their IEC study in apple fruit. We were particularly interested in examining if lower IEC observed in *Md-ACS1-2/2* fruit in that study would correlate with longer storage under similar conditions (Gussman et al. 1993). We are aware that some apple cultivars may require chilling treatment to reach the climacteric stage

(Saftner et al. 2002) and so, the results presented herein cannot be extrapolated to storage at cold temperature. Further studies with a larger sample size may be needed to examine the correlation between storage at ambient temperature and cold storage. However, 'Fuji', a late *Md-ACS1-2/2* genotype, stores well at both ambient temperature and at 0–4°C.

It is generally believed that the onset of ethylene production during fruit ripening controls structural changes in the cell walls of climacteric fruits leading to softening (Abeles and Biles 1991). However, in the case of late *Md-ACS1-2/2* genotypes used in this study [with generally lower fruit IEC compared to *Md-ACS1-1/1* and *Md-ACS1-1/2* genotypes (Harada et al. 2000)], this may not necessarily be true. For example, 'Megumi', a very late *Md-ACS1-2/2* genotype, had about 52% decline in fruit firmness at 20 DAH (Fig. 3), even though it had the lowest IEC (<10 μl/l) in Harada et al.'s (2000) study. In fact, most of the deterioration in firmness in this cultivar occurred at 10 DAH, whereas other late *Md-ACS1-2/2* genotypes retained their firmness up to 40 DAH (data not presented). Similarly, the harvest fruit firmness in this cultivar differed significantly from that of other late *Md-ACS1-2/2* genotypes, but was very close to early *Md-ACS1-2/2* fruit ($P<0.05$), which had a faster rate of softening. Johnston et al. (2001, 2002a, 2002b) noted that onset of rapid softening was consistently associated with IEC exceeding 1.5μl/l in some apple cultivars, whereas in other cultivars, the fruit sensitivity to ethylene appeared to have more of a regulatory role in determining the occurrence of rapid softening than IEC. Lau et al. (1986) and Blankenship and Unrath (1988) observed that apple fruit firmness declined before the IEC increased during on-tree maturation and suggested that ethylene may not be required for on-tree maturation. A low basal rate of ethylene production may have been sufficient to promote on-tree softening in 'Megumi', as has been suggested for the phases of kiwifruit softening when ethylene production is low (Kim et al. 1999). Therefore, it is possible that individual cultivars that fit into the *Md-ACS1-2/2* category may have different sensitivities to ethylene, or there could be other non-ethylene-dependent processes involved in their fruit softening tendencies. Further studies may be necessary to define the relationship of firmness, ethylene, *Md-ACS1*, maturity date and softening in apple fruit, and in particular, among late *Md-ACS1-2/2* genotypes.

Nonetheless, our results have shed light on the apparent relationship between *Md-ACS1*, maturity season and fruit softening. *Md-ACS1* is inherited in Mendelian fashion, and the alleles can be recovered in progenies from two parents homozygous for the marker gene (Table 2). It should therefore be possible to use *Md-ACS1* as a marker for parental selection in conjunction with maturity season to breed for storage ability in apple. The variation in firmness within late *Md-ACS1-2/2* genotypes ($P<0.01$) provides room for improvement of long storage potential via conventional breeding. Likewise, the variation within other *Md-ACS1*/maturity season combinations (in particular, early- and mid-season genotypes) offers the opportunity for prolonging the storage period of progenies from these categories until progeny from late-season *Md-ACS1-2/2* genotypes are ready for harvest.

Conclusion

This study has demonstrated that *Md-ACS1* is inherited in Mendelian fashion and also established a relationship between the gene and fruit softening. Both *Md-ACS1* and maturity season were significant in determining softening behaviour. Late *Md-ACS1-2/2* genotypes are likely to produce low ethylene and will store longer than genotypes in other *Md-ACS1* classes (irrespective of maturity season) when harvest firmness is high. Further studies are needed to compare the softening behaviour of apple genotypes at both ambient and cold temperatures. It should be possible in the interim period, however, to use *Md-ACS1* as a marker to select late *Md-ACS1-2/2* genotypes as parents for developing apples with long-term storage potential.

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