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ORIGINAL PAPER

Md-ACS1 and Md-ACO1 genotyping of apple (*Malus* x *domestica* Borkh.) breeding parents and suitability for marker-assisted selection

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Abstract Fruit ethylene production genotypes for Md-ACS1 and Md-ACO1 were determined for 60 apple cultivars and 35 advanced breeding selections. Two alleles for each gene are commonly found in cultivated apple. Earlier studies showed that genotypes homozygous for the ACS1-2 allele produce less ethylene and have firmer fruit than ACS1-1/2 and ACS1-1/1 genotypes. ACO1 plays a minor role compared to ACS1, with homozygous ACO1-1 having lower ethylene production. In this study, ACS1-2 and ACO1-1 homozygotes had firmer fruit at harvest and after 60 days of 0–1°C cold storage compared to other genotypes. These genotypes, ACS1-2/2 and ACO1-1/1, were observed for the following 8 of 95 cultivars/selections: "Delblush", "Fuji", "Pacific Beauty", "Sabina" and four breeding selections. Cultivars/selections that were homozygous ACS1-2 but not ACO1-1 were: "Ambrosia", "Aurora Golden Gala", "CrimsonCrisp", "Gala", "GoldRush", "Huaguan", "Pacific Rose, "Pacific Queen", "Pinova", "Sansa", "Sonja", "Sundance", "Zestar", and 17 breeding selections. Cultivars with the heterozygous ACS1-1/2 genotype were "Arlet", "Braeburn", "Cameo", "Delicious", "Delorgue", "Empire", "Enterprise", "Ginger Gold", "Golden Delicious", "Granny Smith", "Honeycrisp", "Orin", "Pink Lady", "Silken", "Suncrisp", "Sundowner", "Sunrise" and 11 breeding selections. No cultivars were detected homozygous for both ACS1-1 and ACO1-1, or for both ACS1-2 and ACO1-2. This study is the first large-scale allelic genotyping of both ethylene synthesis genes for a comprehensive set of apple breeding parents used in an ongoing breeding project. The data reported here are important for informative selection of parent combinations and marker-assisted selection of progeny for breeding low ethylene-producing apple cultivars for better storability and improved consumer acceptance.

Keywords Ethylene · Fruit firmness · Marker-assisted selection

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Introduction

Ethylene production rate in apple fruit significantly impacts apple fruit quality, specifically firmness and its retention, during and after storage. Fruit softening is an irreversible process of senescence and excessive softening of apple is undesirable because it results in short shelf life and lower sensory values (Abbott et al. 1984; Harker et al. 1997, Harker et al. 2002; Jaeger et al. 1998). Many environmental factors, horticultural practices, and storage regimes can modify fruit softening behavior; however, a strong genetic basis exists as firmness at harvest and/or after storage varies greatly among cultivars (Saftner et al. 2002; Johnston et al. 2002). The objective of many postharvest practices in the apple industry is to delay the ripening process including firmness loss to achieve a longer marketing period. Retention of desirable firmness after prolonged storage is

one of the key requirements for new cultivars to provide year-round high-quality apples to consumers.

Ethylene regulates several physiological processes related to fruit ripening including changes in skin color, flesh texture, and synthesis of aromatic flavor compounds (Giovannoni 2004). Ethylene biosynthesis during apple fruit ripening is generally regarded as a primary factor leading to softening. Apple is a climacteric fruit, and ripening is characterized by an ethylene burst accompanied by an increase in respiration. The role of ethylene in apple ripening has been studied using ethylene action inhibitors and transgenic approaches. Antisense suppression of the key ethylene biosynthesis genes showed impacts on ripening related processes (Defilippi et al. 2005). Apples treated with the ethylene action inhibitor 1-methylcyclopropene (1-MCP) soften slowly and have reduced internal ethylene concentration relative to untreated fruit (Fan et al. 1999; Watkins et al. 2000; Defilippi et al. 2004). Suppression of the biosynthesis of this gaseous hormone is one of the mechanisms by which controlled atmospheres extend the storage life of apples (Gorney and Kader 1996).

The ethylene biosynthetic pathway was elucidated in the 1980s (Yang and Hoffman 1984). The first step in the ethylene biosynthesis pathway generates 1-aminocyclopropane-1-carboxylic acid (ACC) from *S*-adenosyl-L-methionine (SAM) by ACC synthase (ACS). The second step converts ACC to ethylene by the action of ACC oxidase (ACO). It is generally believed that the first step is the rate-limiting step for ethylene biosynthesis (Lau et al. 1986). Both ACS and ACO enzymes are encoded by multigene families in many plants (Barry et al. 2000; Bleecker and Kende 2000).

In the apple genome, there are at least four members in ACS gene family (Rosenfield et al. 1996; Harada et al. 1997) with Md-ACS1 as the predominant form expressed in ripening fruit tissues (Harada et al. 2000; Wakasa et al. 2006). Two allelic forms of Md-ACS1 are typically observed, i.e., Md-ACS1-1 and Md-ACS1-2 (Sunako et al. 1999). The three allelic combinations, ACS1-1/1, 1-1/2 and 1-2/2, generally confer high, medium, and low ethylene production, respectively (Sunako et al. 1999; Harada et al. 2000; Oraguzie et al. 2004; Costa et al. 2005). Similarly, Md-ACO1 is primarily expressed in fruit tissues among the Md-ACO gene family members (Wakasa et al. 2006). Md-ACO1 has been demonstrated to have a relatively minor, but clear and independent role in ethylene biosynthesis. For example, homozygosity for ACO1-1 further reduces ethylene level within a homozygous ACS1-2 background (Costa et al. 2005). In general, there is a close relationship among ethylene biosynthesis genotype, ethylene production and fruit storability or shelf-life (Harada et al. 2000; Oraguzie et al. 2004; Oraguzie et al. 2007; Costa et al. 2005). Fuji, with this desirable combination of homozygous ACS1-2/2 and homozygous ACO1-1/1, has acceptable firmness through 8 months storage at 0–4°C (Fan et al. 1999).

Deoxyribonucleic acid (DNA)-based molecular markers are currently being used in apple breeding programs, although most are associated with major disease resistance loci (Gardiner et al. 2007; Dirlewanger et al. 2004). Markers used in this study for ACS1 and ACO1 belong to the emerging category of molecular markers termed "functional" or "perfect markers", which are derived from sequence variation of functionally analyzed genes (Andersen and Lübberstedt 2003; Varshney et al. 2005). As their allele effects are known, and they are developed "within or very close to" genes of interest, these markers are ideal for selection of desired genotypes. Integration of marker-assisted selection (MAS) in conventional apple breeding programs should significantly increase breeding efficiency as undesirable genotypes can be eliminated at the very early seedling stage. A means for early selection is particularly advantageous in perennial crops like apple because its fruit quality traits are not expressed during the long juvenile period.

Most fresh and processed apples are stored under various conditions before shipping and processing, to provide a continuous product supply. An apple cultivar with innate low ethylene production in fruit may not only offer better storability, and may also be less dependent on postharvest environments and/or ethylene inhibiting chemical treatments to extend the marketing period. Ethylene biosynthesis genotypes for most of the cultivars and selections used as parents in the Washington State University (WSU) apple breeding program have not been previously reported. Genotyping ethylene biosynthesis potential for elite breeding parents is important for the informed selection of parent combinations to produce cultivars with low ethylene production and better fruit storage quality. In this study, 95 cultivars and selections were genotyped for ethylene biosynthesis potential, using two co-dominant functional markers. The relationships between genotypes and their fruit firmness at harvest and after storage were analyzed. Our results support the practical utilization of these function markers in an apple scion breeding program to efficiently and accurately select ethylene biosynthesis genotypes for low ethylene production, better storability, and shelf life.

Materials and methods

DNA isolation, amplification of ACS1 and ACO1 alleles

Leaves from 95 cultivars and selections collected in the spring of 2006 were frozen in liquid nitrogen and stored at −80°C. Selections from the Washington State University breeding program have "WA" (Washington apple) desig-

nations that are randomly selected codes for actual selections. Genomic DNA was isolated according to Cullings (1992). Polymerase chain reactions (PCRs) were performed in a final mix of 25 µl containing 50 ng of template DNA, 0.25 mM of each deoxyribonucleotide triphosphate (dNTP), 1 mM $MgCl_2$, 0.2 µM of each primer (forward and reverse), 2.5 µl 10 × PCR buffer and 1 U of *Taq* polymerase (New England Biolabs, Ipswich, MA). The thermal cycler (Techne TC-512, GMI, Ramsey, MN) performed the following thermal profile: 94°C for 2 min, 35 cycles of 94°C for 45 s, 58°C for ACS1 primers and 65°C for ACO1 primers for 45 s, 72°C for 2 min, followed by a final extension at 72°C for 7 min. The PCR products were separated on a 2% agarose gel. The sequence of the Md-ACS1 primers followed Harada et al. (2000), and the ACO1 primers by Costa et al. (2005).

The Md-ACS1 marker was developed based on detection of a transposon insertion, possibly the cause of lower gene expression, in the gene's promoter region in the ACS1-2 allele. The size of ACS1-1 is 489 bp and Md-ACS1-2 is 655 bp (Sunako et al. 1999). Cultivars and selections that exhibited only one PCR fragment size were classified as homozygous: ACS1-2/2 if the fragment was 655 bp and Md-ACS1-1/1 if the fragment was 489 bp. Polymorphism of a 62-base pair indel in the third intron of Md-ACO1, possibly the cause of the low of transcription and translation efficiency, was used to generate the ACO marker (Costa et al. 2005). ACO1-1 and ACO1-2 have the fragment size of 525 and 587 bp, respectively (Costa et al. 2005). The examples of banding pattern for both markers are shown in Fig. 1.

Assessment of fruit firmness

Test fruits were harvested in the fall of 2006 near Wenatchee, WA. To standardize fruit maturity, unripe and overripe fruits were excluded based on starch pattern index. Fruits with a uniform external appearance and free of hail damage or other defects were included. Samples of 10 fruits per genotype and/or harvest date were collected. Five fruits were used to estimate firmness at the time of harvest and five fruits were placed in 0–1°C cold storage for 60 days and then evaluated for firmness. Fruit firmness was assessed using a Texture Technologies TA XT2 texture analyzer with an 11-mm diameter Magness Taylor probe. A standard *t* test was carried out using Microsoft Excel for analyzing fruit firmness difference among various ACS1 and ACO1 allelotypes.

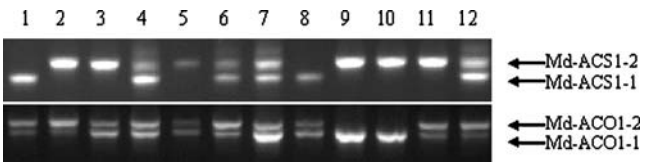

Fig. 1 Banding patterns for Md-ACS1 and Md-ACO1 genotypes for several cultivars. The *top panel* shows Md-ACS1 allelic forms. The *bottom panel* shows Md-ACO1 allelic forms. Cultivars from left are: *1* "Monidal"; *2* "Gala"; *3* "GoldRush"; *4* "Pink Lady"; *5* "Ambrosia"; *6* "Granny Smith"; *7* "Golden Delicious"; *8* "Hatsuaki"; *9* "Fuji:; *10* "Delblush"; *11* "Pinova"; and *12* "Honeycrisp"

Results

Of 60 cultivars/selections evaluated (excluding WA selections), 28 were homozygous ACS1-2/2, 27 were heterozygous ACS1-1/2, and five were homozygous ACS1-1/1 (Table 1). Of the 35 WA selections, 21 were homozygous ACS1-2/2, 11 were heterozygous ACS1-1/2, and three were homozygous ACS1-1/1. The skewed distribution away from ACS1-1/1 genotypes (those with higher ethylene production) reflects the criteria applied by breeders to develop firm, long-storing cultivars.

For ACO1, 13 of 95 cultivars/selections were homozygous ACO1-1/1, 76 were heterozygous ACO 1-1/2, and six were homozygous ACO1-2/2. The preponderance of heterozygotes is common for many apple traits (King et al. 2000). It appears that selection for fruit firmness has had little impact on increasing the frequency of low ethylene producing ACO1-1 homozygotes in apple breeding programs.

For 14 cultivars ("Braeburn", "Delicious", "Fuji", "Gala", "Golden Delicious", "Hatsuaki", "Orin", "Pacific Beauty", "Pacific Rose", "Pacific Queen", "Sansa", "Shensu", "Shinsekai", "Silken") the ACS1 genotypes reported by Harada et al. (2000), Oraguzie et al. (2004, 2007) and Costa et al. (2005) were confirmed. For "Granny Smith" our data (ACS1-1/2) agree with Harada et al. (2000), but not with Oraguzie et al. (2004). For ACO-1 our results confirm the genotypes for "Gala" (ACO1-1/2) and "Fuji" (ACO1-1/1-1) observed by Costa et al. (2005).

The ACS1 allelic genotypes of cultivars and selections were as expected in 47 of 49 cases when the parent genotypes were known (Table 1; Harada et al. 2000; Oraguzie et al. 2004; Oraguzie et al. 2007), e.g, eight cultivars/selections from the cross of Gala and Splendour, both ACS1-2/2, were all ACS1-2/2. Two exceptions were selections WA4 (Gala x Delblush) and WA35 (Fuji x Splendour). These selections were expected to be ACS1-2/2, but were observed to be ACS1-1/2 genotype. Errors in pollen contamination or incorrect parentage may explain the discrepancies. The ACO1 allelic genotypes of cultivars and selections were as expected in 37 cases with no exceptions when their parent genotypes were known (Table 1, Costa et al. 2005).

With few exceptions, ACS1-2/2 genotypes had firmer fruit than ACS1-1/2 genotypes for both freshly harvested

Table 1 Genotypes of Md-ACS1 and Md-ACO1 alleles for 95 apple cultivars and selections

Cultivar/Selection	Parentage	Md-ACS1	Md-ACO1
Fortune (NY429)	Schoharie Spy × Empire	1/1	2/2
Hampshire	Chance seedling	1/1	1/2
Hatsuaki	Jonathan × Golden Delicious	1/1	1/2
Monidal (Delbarestivale)	Red sport of Delcort	1/1	1/2
NY75414-1	Liberty × Macspur	1/1	1/2
WA9	Honeycrisp × Pink Lady	1/1	1/2
WA19	Arlet × Pink Lady	1/1	1/2
WA21	Arlet × Pink Lady	1/1	1/2
Delorgue (Festival)	Delcort × Akane	1/2	2/2
Sunrise	(McIntosh × Golden Delicious) × PCF-3-120	1/2	2/2
WA2	Honeycrisp × Chinook	1/2	2/2
WA5	Enterprise × Honeycrisp	1/2	2/2
WA11	Honeycrisp × Pink Lady	1/2	2/2
Arlet (Swiss Gourmet)	Golden Delicious × Idared	1/2	1/2
Autumn Gold	Chance seedling	1/2	1/2
Braeburn	Chance seedling	1/2	1/2
Cameo (Carousel)	Chance seedling	1/2	1/2
Coop 15	Complex parentage	1/2	1/2
Creston (BC-8M-15-10)	Golden Delicious × NJ381049	1/2	1/2
Elliot	Chance seedling	1/2	1/2
Empire	McIntosh × Delicious	1/2	1/2
Enterprise (Coop 30)	Complex parentage	1/2	1/2
Ginger Gold	Chance seedling	1/2	1/2
Golden Delicious	Chance seedling	1/2	1/2
Granny Smith	Chance seedling	1/2	1/2
Honeycrisp	Unknown	1/2	1/2
NJ 109	Golden Delicious × NJ88	1/2	1/2
NY 79507-72	Empire × Redfree	1/2	1/2
Orin	Golden Delicious × Indo	1/2	1/2
Pink Lady (Cripps Pink)	Golden Delicious × Lady Williams	1/2	1/2
Pristine	Camuzat × PRI 1659-10	1/2	1/2
Shizuka	Golden Delicious × Indo	1/2	1/2
Silken (BC 8S-04-33)	Honeygold × Sunrise	1/2	1/2
Suncrisp (NJ55)	(Cortland × Cox's Orange Pippin) × Golden Delicious	1/2	1/2
Sundowner (Cripp's Red)	Golden Delicious × Lady Williams	1/2	1/2
WA1	Honeycrisp × Chinook	1/2	1/2
WA4	Gala × Delblush	1/2	1/2
WA6	Honeycrisp × Delicious	1/2	1/2
WA8	Pacific Rose × Pink Lady	1/2	1/2
WA23	Gala × Pink Lady	1/2	1/2
WA26	Cameo × BC 8S-27-2	1/2	1/2
Delicious	Chance seedling	1/2	1/1
NJ 90	Complex parentage × Spartan	1/2	1/1
Runkel	Chance seedling	1/2	1/1
WA14	Splendour × Coop 15	1/2	1/1
WA35	Fuji × Splendour	1/2	1/1
Ambrosia	Chance seedling	2/2	1/2
Aurora Golden Gala (BC 8S-69-23)	Gala × Splendour	2/2	1/2
BC 8S-26-50	Gala × Splendour	2/2	1/2
BC 8S-31-56	Gala × Splendour	2/2	1/2
BC SPA493	Gala × Splendour	2/2	1/2
Chinook (BC 8S-27-51)	Splendour × Gala	2/2	1/2
CQR10T17	Complex parentage	2/2	1/2
CQR12T50	Complex parentage	2/2	1/2
CrimsonCrisp (Coop 39)	Complex parentage	2/2	1/2
Gala	Kidd's Orange × Golden Delicious	2/2	1/2

Table 1 (continued)

Cultivar/Selection	Parentage	Md-ACS1	Md-ACO1
Gala Supreme	Chance seedling	2/2	1/2
GoldRush (Coop 38)	Golden Delicious × Coop 17	2/2	1/2
Huaguan (Chinese Champion)	Golden Delicious × Fuji	2/2	1/2
NY 632	NY87 × Splendor	2/2	1/2
Pacific Queen (GS58, Scired)	Gala × Splendour	2/2	1/2
Pacific Rose (GS2085, Sciros)	Gala × Splendour	2/2	1/2
Pinova (Piñata)	(Duchess of Oldenburg × Cox Orange Pippin) × Gold. Del.	2/2	1/2
Sansa	Gala × Akane	2/2	1/2
Senshu	Toko × Fuji	2/2	1/2
Shinsekai	Fuji × Akagi	2/2	1/2
Sonja	Gala × Delicious	2/2	1/2
Splendour	Chance seedling	2/2	1/2
Sundance (Coop 29)	Golden Delicious × 1050 NJ1	2/2	1/2
Zestar (MN 1824)	State Fair × MN 1691	2/2	1/2
WA3	Arlet × NY632	2/2	1/2
WA7	Honeycrisp × Delicious	2/2	1/2
WA10	Fuji × Splendour	2/2	1/2
WA12	Honeycrisp × Enterprise	2/2	1/2
WA13	Coop 25 × Goldrush	2/2	1/2
WA17	Splendour × Open	2/2	1/2
WA18	Gala × Delicious	2/2	1/2
WA20	Arlet × Pink Lady	2/2	1/2
WA22	Arlet × Pink Lady	2/2	1/2
WA24	NJ90 × Goldrush	2/2	1/2
WA27	Cameo × BC 8S-27-2 (Gala × Splendour)	2/2	1/2
WA28	Gala × Pink Lady	2/2	1/2
WA29	Gala × Fuji	2/2	1/2
WA30	Gala × Fuji	2/2	1/2
WA32	Fuji × BC 8S-27-2 (Gala × Splendour)	2/2	1/2
WA33	Fuji × BC 8S-27-2 (Gala × Splendour)	2/2	1/2
WA34	Splendour × Cameo	2/2	1/2
Pacific Beauty (GS494, Sciearly)	Gala × Splendour	2/2	1/1
Delblush (Tentation)	Golden Delicious × Blushing Golden	2/2	1/1
Fuji	Fuji (Ralls Janet × Delicious)	2/2	1/1
Sabina (BC SPA 343)	Sandow × Schoneraus Nordhausen	2/2	1/1
WA15	Splendour × Coop 15	2/2	1/1
WA16	Splendour × Open	2/2	1/1
WA25	Gala × Pink Lady	2/2	1/1
WA31	Gala × Fuji	2/2	1/1

fruit and fruit from 60 days of cold storage (Fig. 2). This observation was consistent over an 8-week range of harvest dates among different cultivars. Mean firmness at harvest and after 60 days of storage were significantly greater for ACS1-2/2 than for ACS1-1/2 (Table 2). Relatively higher firmness of ACS1-2/2 occurred with both ACO1-1/1 and ACO1-1/2. The loss of firmness during storage was greater for ACS1-1/2 compared to ACS1-2/2, but the difference was not always significant. Miller et al. (2004) evaluated fruit firmness at harvest for 18 cultivars genotyped in this study. None of the cultivars with ACS1-1/1 genotype had firm fruit, none of the cultivars with ACS1-2/2 genotype had soft fruit, and most of the ACS1-1/2 allelotypes had intermediate firmness.

The influence of ACO1 genotypes (ACO1-1/1 and ACO1-1/2) on fruit firmness was significant when combined with ACS1-2/2, but not with ACS1-1/2 (Table 2). ACO1-1/1 did not increase firmness when the fruit was already relatively soft due to the ACS1-1/2 allelotype.

Discussion

Genotypes for ethylene biosynthesis potential were determined for 60 cultivar/selections commonly used as parents in breeding programs internationally and 35 advanced selections (WA selections) that are also potential parents from the public breeding program at WSU. ACS1 had a

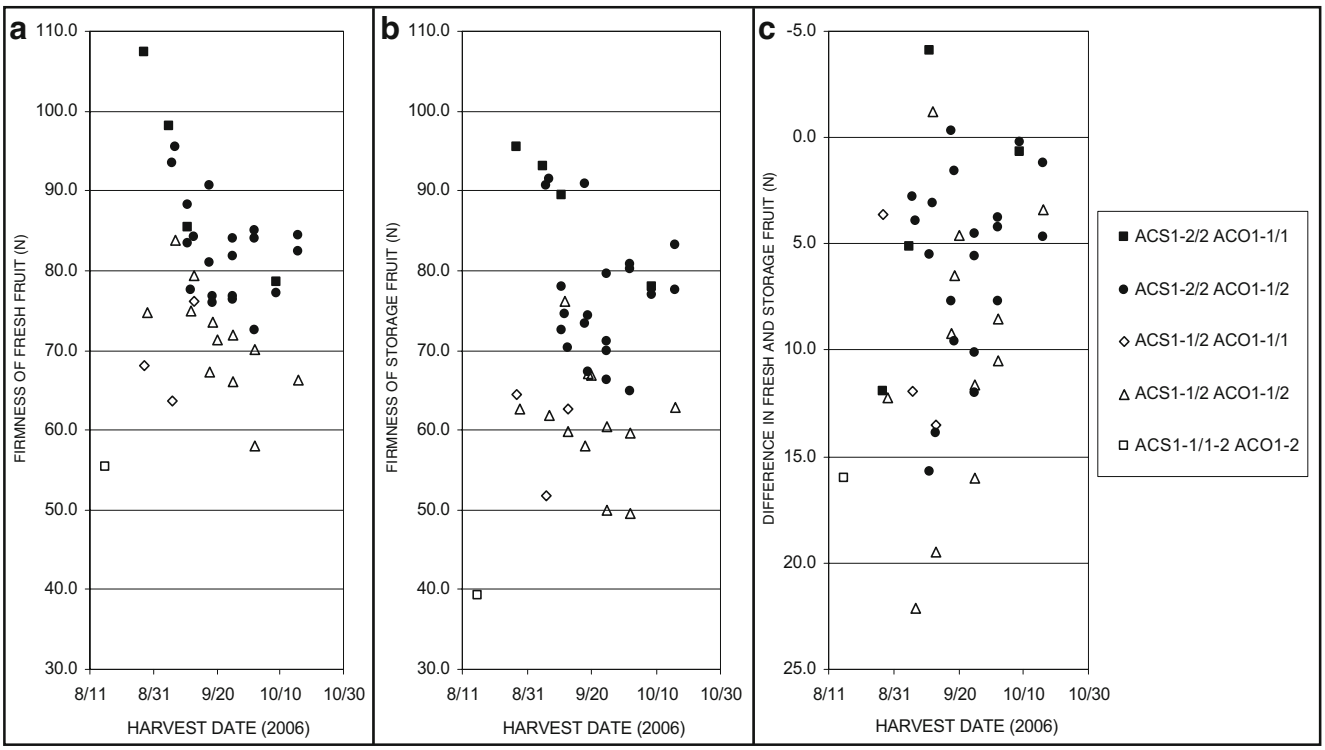

Fig. 2 Fresh fruit firmness (Newtons) at harvest (**a**), firmness after 60 days of cold storage (**b**) and the difference in firmness (fresh minus stored fruit) (**c**) for 40 samples displayed by their ACS1 and ACO1 genotypes across harvest dates

much greater influence on fruit firmness than ACO1. The association between ACS1 and ACO1 allelotypes and observed firmness phenotypes at harvest and after storage supports the practical utilization of both ACS1 and ACO1 functional markers for selecting the progeny at the seedling stage with low ethylene production, firm fruit, and long storage potential.

In conventional apple breeding, the time between the initial cross and new cultivar release is typically 15 to 25 years due to the lengthy juvenile phase and time-consuming phenotypic evaluation. Moreover, due to a high level of heterozygosity, many valuable traits present in one parent are not expressed uniformly in the progeny (Kellerhals et al. 2000; King et al. 2000). Utilization of molecular markers allows a large portion of genotypically undesirable progenies, e.g., those with higher levels of ethylene production, to be eliminated at the early stage of the breeding process. Molecular breeding can also offer the potential of precise design for a cultivar with specific combinations of desirable traits for a target market.

To our knowledge, this is the first report of genotyping two ethylene biosynthesis genes for a comprehensive set of elite apple breeding parents. Traditionally, breeders select the parent combination based on experience with the cultivars and it can be difficult to recover a high proportion of desirable genetic combinations in progeny. The genotype data presented in this paper can be used for informative selection of breeding parent combinations and predict the ratio of desirable low ethylene production genotypes. Subsequently, undesirable genotypes from a segregating population can be eliminated using both markers at a very early stage during the breeding process. Given the fact that fruit firmness and storability are important traits for apple scion breeding, early elimination of unwanted soft-fruited seedlings may allow breeders to concentrate on other fruit quality traits in the remaining smaller population or may allow an increase in the size of the population to be evaluated.

The rate of softening (the difference in firmness during 60 days in storage) was not closely related to ACS1 and ACO1 allelotypes. This suggests that the rate of decrease in firmness is independent of initial firmness (influenced primarily by ACS1) and may be controlled by other genes. The stronger expression of a PG (polygalacturonase) encoding gene was observed to correspond with increased internal ethylene concentration during the early phases of softening for several apple cultivars (Atkinson et al. 1998). Ten apple cultivars with the same Md-ACS1-2/2 genotype showed different patterns of firmness loss, which correlate with Md-PG1 expression levels (Wakasa et al. 2006). This suggests a role of PG in modifying fruit firmness at low ethylene levels. Quantitative trait loci (QTL) analysis indicates that there are several major loci in the apple genome that contribute to observed variation in fruit flesh

Table 2 Relationship between apple fruit firmness (Newtons) at harvest (fresh), after 60 days of cold storage or firmness loss during storage and the allelotypes of ACS1 and ACO1

	ACO1-1		ACO1-1/2
	Fresh Fruit		
ACS1-2	92 (n=4)	*[z]	83 (n=20)
	**		**
ACS1-1/2	69 (n=3)	NS	71 (n=12)
	Stored Fruit		
ACS1-2	89 (n=4)	**	77 (n=20)
	**		**
ACS1-1/2	60 (n=3)	NS	61 (n=12)
	Loss of Firmness		
ACS1-2	3 (n=4)	NS	6 (n=20)
	NS		*
ACS1-1/2	10 (n=3)	NS	10 (n=12)

Values are means of five fruit per cultivars/selections. Values in parentheses are the number of cultivars/selections used in calculating the phenotypic means

[z] Asterisks (*, P=0.05; **, P=0.01) and NS (not significant) refer to the significance of differences between adjacent means using a t test

firmness (King et al. 2000; Seymour et al. 2002; Liebhard et al. 2003). Nevertheless, results from this and previous studies support the conclusion that genotypes of ethylene biosynthesis genes play a primary role in fruit ripening and therefore influence fruit firmness and fruit storability. Our results support the practical use in apple breeding of ACS1 and ACO1 markers in marker-assisted selection for firm apple fruit with improved storability and shelf life.

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