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Ethylene production, shelf-life and evidence of RFLP polymorphisms linked to ethylene genes in melon (*Cucumis melo* L.)

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Abstract Sixty three cultigens from eight market types of the melon (*Cucumis melo* L. subsp. *melo*) groups Cantaloupensis and Inodorus were evaluated for ethylene production rate, shelf-life (postharvest decay), and RFLP polymorphisms. The ethylene production rates of melon fruits at maturity and (after) postharvest decay were measured on individual genotypes. The ethylene production rates of individual genotypes ranged from undetectable to 103 nl/g per h. The mean ethylene production rates of the eight market types, ranked from highest to lowest, were Eastern U.S. type, Charentais, Western U.S. type, Long Shelf-Life cantaloupes (LSL), Galia, Ananas, Honeydew, and Casaba. Ethylene production and postharvest decay rating were positively significantly correlated (r^2=0.87, P=0.05). Orange-fleshed melon fruits produced significantly (P=0.05) more ethylene than did green- or white-fleshed types. Melon fruits with a netted rind had significantly (P=0.05 for orange-flesh fruits and 0.01 for green- or white-flesh fruits) higher ethylene production than did smooth-type fruits. Using probes made from cDNAs encoding ACC oxidase (MEL1) or ACC synthase (MEACS1) genes, RFLPs were detected melon cultigens of the eight marker types showing varying ethylene production rates and different flesh colors. Low ethylene production and green- and white-flesh color were associated (r^2=0.91; P=0.05) with the presence of a putative RFLP-MEL1 allele A_0 (15-kb), whereas high ethylene production and orange-flesh color were associated with allele B_0 (8.5-kb) in the homozygous condition, after probing MEL1 with *Eco*RV-digested genomic DNA. Also, after probing MEACS1 with *Nde*I-digested genomic DNA, RFLP polymorphism revealed five fragments denoted as A, B, C, D and E, with molecular sizes of 5.2-, 4.2-, 3.8-, 3.0- and 1.0-kb, respectively. A two-fragment pattern, AB, and a three-fragment pattern, ACE, the two predominant RFLP patterns, were also associated with low and high ethylene production, respectively. The ACE fragment pattern was also associated with orange-flesh melons. Scoring of both probes allowed for the unique classification of most melon market types consistent with ethylene production and the postharvest decay phenotypes. Therefore, these RFLPs might have utility in marker-assisted selection for the development of melons with enhanced postharvest keeping ability.

Keywords Melon (*Cucumis melo* L.) · Fruit ripening · Ethylene production rate · Postharvest fruit decay · Shelf-life · ACC oxidase · ACC synthase · SSR · RFLP

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Introduction

Gaseous ethylene is an important plant hormone that has a profound effect on many growth and development processes, as well as stress responses, (Yang and Hoffman 1984; Bardy 1987; Abeles et al. 1992; Kende 1993; Ecker 1995) which include shelf-life, shipping potential, storage behavior, and quality of fruits and vegetables (Theologis 1992), and disease regulations (Ecker and Davis 1987; Bent et al. 1992; Lund et al. 1998; Hoffman et al. 1999). This hormone has been extensively studied in many fruit crops because of its important role in fruit ripening (Abeles et al. 1992; Kende 1993; Yang and Hoffman 1984). In melon (*Cucumis melo* L), orange-fleshed varieties (subsp. *melo* Cantaloupensis Group: Western U.S. Shipping type, Charentais) produce a burst of ethylene concurrent with fruit maturity and abscission (Lester and Dunlap 1985; Shellie and Saltveit 1993; Hadfield et al. 1995). In contrast, honeydew and other long shelf-life melon varieties (subsp. *melo* L. Inodorus

Group: greenflesh, Casaba, yellow canary, Piel de Sapo) do not produce a large ethylene burst, and generally do not form an abscission zone at marketable maturity (Pratt et al. 1977, Miccolis and Saltveit 1991, 1995). Honeydew melons have a shelf-life of 3 weeks, and cantaloupes have a shelf-life of 2 weeks (Ginsburg 1965). Many melons from the subsp. *melo* L. Inodorus Group can be stored for several months at ambient temperature (Miccolis and Saltveit 1995).

Biosynthetic ethylene production has been actively studied (Yang and Dong 1993; Zarembinski and Theologis 1994). It involves the conversion of S-adenosylmethionine to 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (Adams and Yang 1979). These last steps of the biosynthetic pathway are controlled by ACC synthase that catalyzes the conversion of S-adenosylmethionine to ACC, and ACC oxidase that catalyzes the conversion of ACC to ethylene (Yang and Hoffman 1984). The onset of ethylene production in ripening climacteric fruits is associated with the concomitant increase in ACC synthase and ACC oxidase activities (Balagué et al. 1993; Kende 1993).

The molecular regulation of melon ripening has been only recently investigated and much attention has been devoted to the isolation of genes involved in the ethylene biosynthetic pathway. The cDNA of the ACC oxidase gene in melon was isolated (Balagué et al. 1993), and the gene family characterized (Lasserre et al. 1996). Also, a cDNA encoding an ACC synthase from melon has been sequenced (Miki et al. 1995; Yamamoto et al. 1995). The expression of ethylene production genes and unknown ripening-related genes during fruit development was documented in subsp. *melo* L. Cantaloupensis Group and Inodorus Group genotypes (Aggelis et al. 1997). However, little is known about the molecular and physiological ripening differences in subsp. *melo* L. Inodorus Group genotypes that result in varying phenotypes. Therefore, a study was designed to: (1) investigate the relationship between ethylene production and postharvest fruit decay in subsp. *melo* L. Cantaloupensis Group and Inodorus Group melon genotypes, and (2) search for potential RFLP polymorphisms associated with ethylene production in these melons.

Materials and methods

Field trials, ethylene production measurements, and postharvest fruit decay ratings

Table 1 lists 63 melon genotypes representing eight market types included in this study. Seeds of 48 melon genotypes were sown in Speedling trays on March 15 and transplanted on April 7, 1994, into a field in a randomized complete block with three replications. Similarly, seeds of 49 melon genotypes, including some repeat genotypes from the 1994 field trail, were sown on March 5 and transplanted on March 27, 1995. Melon plants were grown on raised beds with 0.2-m centers and a width-in-row 30-cm plant spacing. During flowering, plots were monitored every other day, and female flowers were tagged at anthesis. At horticultural maturity (fruit abscission or rind color change), melons were harvested and measured for ethylene production by a procedure modified from Dunlap and Robacker (1993). Three cores, 2.5-cm long and 1.2-cm diameter, of fruit mesocarp tissue from the middle of each fruit at the equatorial plane were removed, weighed, and then placed individually into 15-ml sealed tubes for 20–30 min at 24±1°C. Then a 0.5-ml gas sample from each sealed tube was taken and injected into a gas chromatograph (5890 Series II, Hewlett-Packard, Avondale, Pa.) to measure the ethylene peak area. Ethylene production rate (nl/g per h) was calculated by converting from the peak area based on a standard curve that was generated by injecting 10, 20, 40, 80 and 100 µl of 10-ppm ethylene which equals to 0.1, 0.2, 0.4, 0.8 and 1.0 nl of pure gaseous ethylene, respectively. For each genotype, 3–6 samples were taken from 1 to 2 fruit(s) for ethylene production measurement and the average production rate was calculated. Additional fruits (3–6) were harvested from each genotype, stored at 27–30°C for 7 days, and then rated visually for postharvest decay on a 5-point scale. A rating of 1 equalled no evidence of softening or decay, and ratings of 2, 3 and 4 reflected a decayed surface area of 25%, 50% and 75%, respectively. A rating of 5 indicated fruits that exhibited complete rot and collapse of the fruit.

Greenhouse tests

Seed were sown in 2.5-l pots containing sterile, soil-less media (1 part peatmoss: 1 part perlite: 1 part vermiculite) on October 7, 1994. Thirty two genotypes (Table 1) were arranged in a randomized complete block with three replications. Pots were fertilized with 100 ppm of N from 20:20:20 N:P:K at each irrigation. As vines grew, they were trained on a string and pruned as needed. Hermaphrodite flowers were emasculated prior to opening, and self-pollinated using staminate flowers from the same plant. Fruits were supported in mesh bags during enlargement, and harvested at full maturity (complete abscission, or based on rind color change). The ethylene production rates (nl/g per h) were measured as described above.

Genomic DNA

Young and healthy leaf tissues of greenhouse seedlings were harvested at the 3–5 leaf-stage. Genomic DNA was extracted from either freshly harvested leaves frozen in liquid nitrogen or from freeze-dried leaves (DURA-DRY, FTS Systems, Inc., Stone Ridge, N.Y., USA), following a modified procedure of Baudracco-Arnas (1995). DNA-sample quantity and quality were determined by a UV-VIS scanning spectrophotometer (UV-2101PC, Shimadzu Scientific Instruments, Inc., Md., USA). All DNA samples had an absorbance ratio of A_{260}/A_{280} above 1.8.

DNA-blot analyses

Genomic DNA (10–15 µg) was digested at 37°C for 10 h with each of the following enzymes: *Eco*RI, *Eco*RV, *Hind*III, *Bam*HI, *Nde*I, *Kpn*I, *Pst*I, *Xho*I, *Xba*I, *Sac*II, *Bgl*II, *Avr*II, *Bstx*I, *Pvu*II and *Dra*III. Digested DNA samples were loaded in each lane on a 0.8% agarose gel and electrophoresed at 1.0 V/cm for 12–15 h. After electrophoresis, gels were treated with 10 vol of 0.25 N HCl for 10–15 min and then 0.4 M NaOH for 20 min before blotting onto Hybond-N^+ nylon membranes (Amersham, Life Science, Arlington Heights, Ill. USA) for 2–3 h using a downward capillary, alkaline-blotting technique (Koetsier et al. 1993; Zheng and Wolff 1999).

cDNAs encoding either the ACC oxidase gene (MEL1, Balagué et al. 1993) or the ACC synthase gene (MEACS1, Miki et al. 1995) were used to probe digested genomic DNA. Probe labeling, hybridization, washing, and signal detection were carried-out using a fluorescein labeling and detecting system (Amersham, Life Science, Arlington Heights, Ill. USA) under optimized conditions (Zheng and Wolff 1999). The blots were then exposed on Hyperfilm-MP (1 min to 1 h) and developed.

SSR analyses

Two SSR (simple sequence repeat) markers (Danin-Poleg, personal communication) derived from ACC oxidase clones (MEL1, Balagué et al. 1993; *CM-AC01*, Lasserre et al. 1996) were tested for polymorphisms among eight cultivars ('TAM Yellow Canary', 'TAM Uvalde', 'Delicious 51', 'Greenflesh Honeydew', 'Santa Clause', 'Castella', 'Galia' and 'Viva') representing all melon market types except Ananas (missing data). The *CMAT35* SSR is located at the 3′ non-coding end of the MEL1 cDNA clone, with a core motif $(TA)^3AA(TA)^2C(AT)^7$. The *CMTC51* SSR is located at the first intron of the genomic clone *CMAC01* (derived from the same gene, Lasserre et al. 1996), with a core motif $(CT)^3GA(TC)^9(CT)^5$.

PCR-amplification of microsatellite loci

Reaction mixtures for PCR amplification of microsatellite loci contained 60 ng of plant genomic DNA, 1 mM of Mg^{2+}, 8–10 pmole of 3′ and 5′ primers, 166 µM of dATP, dTTP, dGTP, 2 µM of dCTP, 0.1 µl of 3000 Ci/mmol [α-^{32}P] dCTP, 1× *Taq* Buffer (Advanced Biotechnologies, UK) and 1 unit of Taq DNA polymerase (Advanced Biotechnologies, UK), in a total volume of 15 µl. The amplification program was as follows: 30-s denaturation at 94°C, 30-s annealing at 51°C, and 60-s extension at 72°C for 34 cycles on a thermocycler (PTC-100, MJ Research, Inc.). PCR products (3.0 µl/lane) were separated on a DNA sequencing gel containing 6% polyacrylamide, 8 M urea and 1× TBE, at 60 W constant power for 1.5–2.5 h. After drying, the gels were exposed to a Kodak XAR-5 film (Eastman Kodak, USA).

Statistical analysis

All statistical analyses used in this study were carried out by using the SAS statistical package (SAS Institute 1996, SAS/STAT User's Guide, Version 6.11 for Window, Cary, N.C., USA). Unless otherwise mentioned, all significance tests were based on either the Waller-Duncan test or Duncan's multiple range tests at $P_{0.05}$.

Results

The association between ethylene production and postharvest fruit decay in diverse melon genotypes

The 63 melon genotypes representing eight different melon market types together with their corresponding ethylene production rates (nl/g per h), postharvest decays, flesh colors, rind types, and the associated RFLP polymorphisms of the representative genotypes of each market type, are listed in Table 1. Results showed that individual genotypes had diverse ethylene production rates that ranged from undetectable or trace ethylene production (e.g., from 0 to 3 nl/g per h for most Honeydew and Casaba types), to some degree of ethylene production (from 5 to 15 nl/g per h for most Ananas, Galia, and most LSL types), to a substantially high rate (from 30 to 103 nl/g per h for most Eastern and Western U.S. Shipping and Charentais types). Generally, individual orange-fleshed genotypes (Western U.S. type, Eastern U.S. type, Charentais, and LSL) had several to hundreds-fold higher ethylene production rates than Honeydew and Casaba genotypes. All Eastern U.S. type genotypes had significantly higher rates of ethylene production than Honeydew and Casaba genotypes. Most genotypes from Charentais and some from Western U.S. types ('Cruiser' and 'Topmark') and one from LSL ('NUN0261') also had significantly higher ethylene production rates than Honeydew and Casaba genotypes. Honeydew and Casaba melons, which belong to the subsp. *melo* Inodorus Group, produced little or no detectable ethylene at maturity and did not abscise. In general, the postharvest decay rating of an individual genotype paralleled its ethylene production rate. High ethylene production rates corresponded to high postharvest decay ratings. However, there were fewer significant differences among the entries for postharvest decay ratings than for ethylene production rates (Table 1). When all genotypes were grouped by market types, the relationship between the ethylene production rate and the postharvest decay of the eight different market types showed a significant (P=0.05) and positive linear relationship (r^2= 0.87) (Fig. 1).

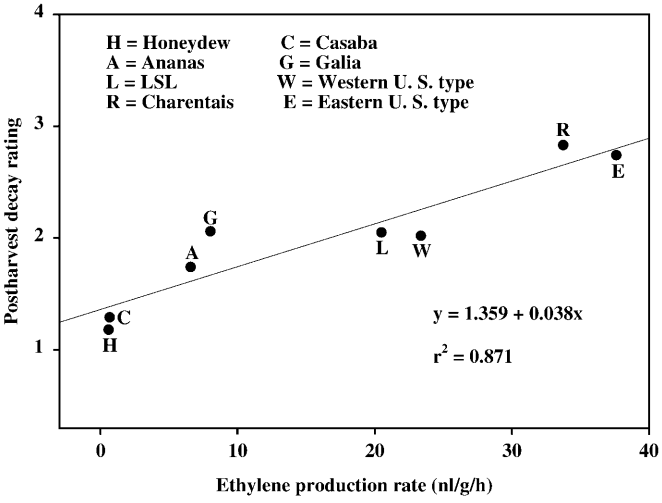

Fig. 1 Linear regression of mean postharvest decay ratings on mean ethylene production rates (1994 and 1995 field experiments) of each market type. Melon fruits were harvested at horticulture maturity and stored at 27–30°C for 7 days before rating visually and mechanically for postharvest decay. A rating of 1 signified no evidence of softening or decay, ratings of 2, 3 and 4 indicated about 25%, 50% and 75% decay of the surface area, and 5 indicated complete rot and collapse of the fruit

Mean ethylene production rates and postharvest decays of the eight market types from two field tests are shown in Table 2. In general, the orange-fleshed, subsp. *melo* Cantaloupensis Group, which consists of Eastern U.S. type, Western U.S. type, Charentais and LSL, had significantly higher ethylene production and postharvest decay ratings than the white-fleshed (Ananas and Casaba) or green-fleshed (Galia and Honeydew) subsp. *melo* Inodorus Group. Ethylene production rates were higher in 1994, which allowed for clearer separation of the different market types than in 1995. Rankings of market types for ethylene production and postharvest decay were similar in the two tests. The only exception was LSL, which had a much higher ethylene production rate in 1995 than 1994 (Table 2). In the 1994 trial, LSL had

Table 1 Melon cultivars representing eight market types and their flesh color, rind type, ethylene production (nl/g per h), postharvest decay rating, and the associated RFLP genotypes[a]

Melon market type	Cultivar/genotype[b]	Flesh color	Rind type	Ethylene production (nl/g/h)[c]	Postharvest decay rating[d]	RFLP polymorphism[e]	
						MEL1	MEACS1
Western U.S.	Cruiser*	Orange	Netted	39.00	1.33	A_0B_0	ACE
	Imperial # 45	Orange	Netted	4.97	2.00	B_0	ACE
	Mission*	Orange	Netted	2.57	1.38	B_0	ACE
	Perlita	Orange	Netted	22.14	2.17	B_0	ACE
	Planter's Jumbo	Orange	Netted	12.78	2.00	B_0	ACE
	Primo *	Orange	Netted	24.08	2.33	B_0	ABCE
	Smith's Perfect	Orange	Netted	10.50	2.00	B_0	AB
	TAM Uvalde*	Orange	Netted	27.42	2.00	B_0	ACE
	Topmark	Orange	Netted	71.22	2.00	B_0	ACE
Eastern U.S.	Athena*	Orange	Netted	31.55	2.00	A_0B_0	AB
	Delicious 51*	Orange	Netted	39.94	3.75	B_0	ACE
	Giant Perfection	Orange	Netted	103.51	4.33	B_0	ACE
	Iroquois*	Orange	Netted	81.07	3.00	A_0B_0	ACE
Charentais	Accent*	Orange	Netted	26.42	4.00	A_0B_0	AB
	Charentais T*	Orange	Smooth	46.97	2.84	B_0	AB
	Doublon*	Orange	Smooth	49.47	2.84	B_0	AB
	NUN1346	Orange	Netted	51.05	4.00	NT	NT
	Ramon	Orange	Netted	40.45	4.00	B_0	ACE
	Viva*	Orange	Netted	18.88	3.21	A_0B_0	ABCE
LSL[f]	Castella*	Orange	Netted	17.82	2.00	B_0	ACE
	Fiola*	Orange	Netted	14.96	2.48	A_0B_0	ACE
	NUN0261	Orange	Netted	49.52	1.63	NT	NT
	NUN0770	Orange	Netted	8.77	1.50	A_0	AB
Galia	Amur	Green	Netted	12.05	1.75	NT	NT
	A-one	Green	Netted	4.42	1.50	NT	NT
	Emeradd Jewel	Green	Netted	0.36	1.17	NT	NT
	Galia*	Green	Netted	8.16	1.34	A_0	ABDE
	Ogen*	Green	Smooth	4.13	2.34	A_0	ADE
	Rocky Ford GF	Green	Netted	6.25	2.25	A_0B_0	ACE
	Rocky Sweet*	Green	Netted	7.34	2.92	A_0	ADE
	Volga	Green	Netted	0.31	1.00	NT	NT
Honeydew	Desio	Orange	Smooth	0.73	1.00	A_0B_0	AB
	HD Green Flesh*	Green	Smooth	0.00	1.00	A_0	ABCE
	HD Orange Flesh*	Orange	Smooth	1.83	2.00	B_0	AB
	Honeybrew*	Green	Smooth	0.00	1.00	A_0B_0	AB
	HD Gold Rind	White	Smooth	0.09	1.42	A_0	AB
	Morning Ice*	Green	Smooth	1.21	1.13	A_0	ABCE
	Rocio*	Green	Smooth	0.25	1.00	A_0	AB
	TAM Dew Improved*	Green	Smooth	0.08	1.00	A_0	AB
	Temptation	Orange	Smooth	0.06	1.17	NT	NT
	UC Honeyloupe	Orange	Smooth	1.73	1.67	A_0	AB
Ananas	Ananas	White	Netted	6.88	2.00	A_0B_0	AB
	Deltex*	White	Netted	2.33	1.33	A_0	AB
	Eden	White	Netted	13.73	1.67	NT	NT
	Israeli	White	Netted	5.65	2.04	A_0	AB
	Spice*	White	Netted	3.05	1.75	A_0	AB

significantly lower ethylene production than the Western U.S. type, Eastern U.S. type or Charentais, and was not significantly different from the Galia, Ananas, Honeydew and Casaba types. In the 1995 trial, however, LSL had the highest ethylene production rate among the eight market types. It was significantly higher than the Western U.S. type, Ananas, Casaba, Galia and Honeydew, but not the Eastern U.S. type and Charentais (Table 2). The ethylene production rates of melons harvested from the greenhouse showed similar results (data not shown).

The associations of flesh-color and rind type with ethylene production and postharvest decay

As shown in Table 1, individual melon genotypes with orange-flesh had higher ethylene production and postharvest decay rates (i.e., more severe decay) than genotypes with green- or white-flesh. When averaged over all genotypes, the ethylene production rates of orange-fleshed genotypes were significantly higher than those of green- or white-fleshed genotypes (Table 3). The relative rank

Table 1 (continued)

Melon market type	Cultivar/genotype[b]	Flesh color	Rind type	Ethylene production (nl/g/h)[c]	Postharvest decay rating[d]	RFLP polymorphism[e]	
						MEL1	MEACS1
Casaba	Amarillo	White	Smooth	0.13	1.00	A_0	AB
	Aril	White	Smooth	0.12	1.00	NT	NT
	Casaba Golden Beauty*	White	Smooth	0.05	1.42	A_0	AB
	Crenshaw*	Orange	Smooth	0.18	2.00	A_0	AB
	Daimiel	White	Smooth	0.01	1.00	NT	NT
	Eagle	White	Smooth	0.29	1.33	NT	NT
	Marygold	White	Smooth	0.03	1.00	A_0	AB
	NUN9829	White	Smooth	2.65	1.67	NT	NT
	Oasis	White	Smooth	0.14	1.25	NT	NT
	Santa Clause*	White	Smooth	0.34	1.00	A_0	AB
	Solo*	White	Smooth	0.08	1.00	A_0	AB
	TAM Mayan Sweet*	Green	Smooth	3.81	1.00	A_0	AB
	TAM Yellow Canary*	White	Smooth	0.02	1.00	A_0	AB
	Toledo*	White	Smooth	0.09	1.00	A_0	AB
	Utopia	White	Smooth	0.02	1.67	NT	NT
	White Crenshaw	White	Smooth	0.04	1.00	A_0	AB
	Zilba	White	Smooth	0.08	1.25	NT	NT
Minimum significant difference[g]		NA	NA	27.53	1.63	NA	NA

[a] A total of 72 melon genotypes were included in this study. Among them nine genotypes were not listed because not enough fruits were produced to generate data. Each the 63 genotypes was tested in at least two of three experiments. Ethylene production and postharvest decay data were collected and analyzed from fruits harvested in 1994 and 1995 field trials

[b] Genotypes marked with an asterisk (*) indicate the 31 out of 32 genotypes tested in greenhouse in 1995, with one genotype ('Persian') not listed in the table

[c] Ethylene production rate was the average of 3 to 6 samples taken from 1 to 2 fruits per genotype

[d] Postharvest decay rating was the average of 3 to 6 fruits per genotype. Melon fruits were harvested at horticulture maturity and stored at 27–30°C for 7 days before rating visually and mechanically for postharvest decay. A rating of 1 signified no evidence of softening or decay, ratings of 2, 3 and 4 indicated about 25%, 50% and 75% decay of the surface area, and 5 indicated complete rot and collapse of the fruit

[e] RFLP-MEL1 polymorphisms resulted from probing pMEL1 cDNA encoding ACC oxidase with genomic DNA digested with *Eco*RV, where fragments A_0 and B_0 were at molecular sizes of 15- and 8.5-kb, respectively. RFLP-MEACS1 polymorphisms resulted from probing pMEACS1 cDNA encoding ACC synthase with genomic DNA digested with *Nde*I, where fragments A, B, C, D and E were at molecular sizes of 5.2-, 4.2-, 3.8-, 3.0- and 1.0-kb, respectively. NT=not tested

[f] LSL=long shelf-life cantaloupe

[g] Minimum significant difference was based on a Waller-Duncan significance test (K=100, P=0.05). NA=not applicable

order of ethylene production rate (nl/g per h) was orange- (34.69, 21.06 and 27.08), green- (6.09, 4.62 and 5.50), and then white-flesh (2.54, 1.17 and 1.96, for the 1994 and 1995 trials and the combined data, respectively). The same relative rank order for postharvest decay ratings of the three flesh colors was found, with orange- (2.38, 2.42 and 2.40), green- (1.59, 1.79 and 1.69), and white-flesh (1.32, 1.57 and 1.43, for the 1994, 1995 trials and the combined data, respectively).

Results also showed that the fruit rind types had significant relationships with ethylene production and postharvest decay rates (Fig. 2). Melons with a netted-rind had significantly (P=0.01) higher ethylene production rates than those with smooth-rind for both green- and white-flesh types. The difference between netted and smooth types was similar in the orange-flesh type, but was not statistically significant. Similarly, for each flesh color, melons with netted-rinds had significantly (P=0.05 for orange-flesh and P=0.01 for green- and white-flesh) greater postharvest decay ratings than those with smooth rinds (Fig. 2). When averaged over all genotypes and flesh colors, melons with netted-rinds had mean ethylene production rates (nl/g per h) of 29.27, 15.55 and 21.83, for the 1994 and 1995 trails and the combined data, respectively, which were significantly higher than those with smooth rinds of 6.51, 9.71 and 7.85, respectively (Table 3). Postharvest decay corresponded closely to ethylene production rate. Netted melons had mean postharvest decay ratings of 2.21, 2.34 and 2.30, for the 1994 and 1995 trails and combined data, respectively, which were significantly higher than those for smooth melons of 1.45, 1.72 and 1.59, respectively (Table 3).

Evidence of RFLP polymorphisms linked to ethylene biosynthetic genes

RFLP polymorphisms were found after probing MEL1 (cDNA of ACC oxidase gene) with genomic DNA digested with *Eco*RV, *Xho*I, *Pst*I, or *Nde*I individually. Among them, polymorphic fragments of 15- and 8.5-kb, designated as A_0 and B_0, respectively (Fig. 3, panel A), resulting from *Eco*RV digestion were the most interesting because they were associated with ethylene production rates, postharvest decays, and flesh color types of diverse melon genotypes (Table 1). Polymorphisms that

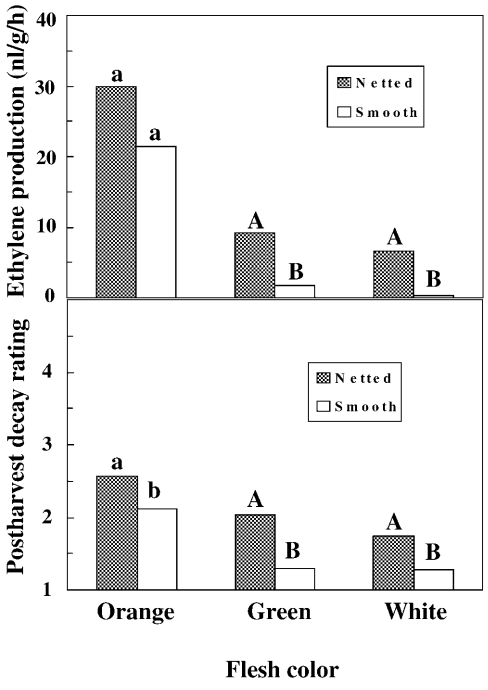

Fig. 2 Ethylene production rates and postharvest decay ratings averaged over all genotypes of netted- or smooth-rind melon fruits in each of the three flesh-colors. The ethylene production rate of individual genotypes was the average of 3–6 samples taken from 1 to 2 fruits per genotype. The postharvest decay rating of individual genotypes was the average of 3–6 fruits per genotype. Melon fruits were harvested at horticulture maturity and stored at 27–30°C for 7 days before rating visually and mechanically for postharvest decay. A rating of 1 signified no evidence of softening or decay, ratings of 2, 3 and 4 indicated about 25%, 50% and 75% decay of the surface area, and 5 indicated complete rot and collapse of the fruit. Ethylene production rates of netted fruits were significantly higher than those of smooth fruits in both green- and white-flesh melons, but not in orange-flesh melons (top panel). Postharvest decay ratings of netted fruits were significantly higher than that of smooth fruits for all three flesh-colors (bottom panel). *Lower and upper case letters* indicate a significant difference between netted and smooth rind within each flesh color at the 0.01 and 0.001 levels, respectively

resulted from probing with MEL1 with genomic DNA digested with *Xho*I, *Pst*I, or *Nde*I were less conclusive (data not shown). DNA-blot analyses also revealed diverse RFLP polymorphisms after probing MEACS1 (cDNA of ACC synthase gene) with genomic DNA digested with *Hind*III, *Nde*I and *Xho*I. The five polymorphic fragments, designated as A, B, C, D and E with molecular sizes of 5.2-, 4.2-, 3.8-, 3.0- and 1.0-kb, respectively, and revealed from *Nde*I blots (Fig. 3, panel B), were the most informative in distinguishing melons from different ethylene production and other ripening phenotypes (Table 1). Polymorphisms that resulted from probing cDNA MEACS1 with genomic DNA digested with either *Hind*III or *Xho*I were less conclusive (data not shown).

The associations between these RFLP polymorphisms and the ethylene production (nl/g per h) and postharvest decay ratings are also shown in Table 4. There were significant interactions between RFLP-MEL1 and RFLP-MEACS1 for both ethylene production and postharvest decay rates. When grouped by RFLP-MEL1 polymorphisms, within the A_0B_0 group, the mean ethylene production rate of melons with ACE fragments was significantly higher than that with AB fragments of RFLP-MEACS1 (Table 4). However, the means of both ethylene production and postharvest decay rates of melons with A_0 or B_0 fragments were not affected by RFLP-MEACS1 polymorphisms. In other words, there were no significant differences among the RFLP-MEACS1 polymorphisms (AB, ABCE, ACE, ADE and ABDE) when melons had the same RFLP-MEL1 polymorphism, with either an A_0 or B_0 fragment. In contrast, when grouped by RFLP-MEACS1 polymorphisms, both the means of ethylene production and the postharvest decay rates of groups with AB or ABCE patterns were significantly different between melons with A_0 and B_0 polymorphisms of RFLP-MEL1 (Table 4). Within either AB or ABCE groups of RFLP-MEACS1, melons with the B_0 fragment

Table 2 Ethylene production rates and postharvest decay ratings grouped as melon market types from fruits harvested in the 1994 and 1995 field trials[a]

Melon market type	1994			1995		
	n[b]	Ethylene production (nl/g per h)	Postharvest decay rating	*n*	Ethylene production (nl/g per h)	Postharvest decay rating
Eastern U.S.	3	82.37 a[c]	3.33 a	6	22.68 a	2.54 a
Charentais	6	43.18 b	3.29 a	8	26.12 a	2.52 a
Western U.S.	7	32.00 b	1.56 c	4	11.57 b	2.54 a
LSL	3	9.15 c	1.25 c	4	29.22 a	2.54 a
Galia	5	11.59 c	2.27 b	7	4.89 b	1.94 ab
Ananas	5	9.16 c	1.67 c	3	3.48 b	1.85 b
Honeydew	6	0.83 c	1.16 c	5	0.25 b	1.21 c
Casaba	12	0.87 c	1.17 c	6	0.28 b	1.45 bc

[a] Ethylene production rate and postharvest decay rating were means of all genotypes in each market type. Melon fruits were harvested at horticulture maturity and stored at 27–30°C for 7 days before rating visually and mechanically for postharvest decay. A rating of 1 signified no evidence of softening or decay, ratings of 2, 3 and 4 indicated about 25%, 50% and 75% decay of the surface area, and 5 indicated complete rot and collapse of the fruit

[b] Number of genotypes of each melon type

[c] Means of each melon market type in each year, where different letters indicate significantly different values based on a Duncan multiple range test (P=0.05)

Table 3 Ethylene production rates and postharvest decay ratings grouped as flesh colors and rind types[a]

Flesh color or rind type		1994		1995		Combined 1994 / 1995	
		Ethylene production (nl/g per h)	Postharvest decay rating	Ethylene production (nl/g per h)	Postharvest decay rating	Ethylene production (nl/g per h)	Postharvest decay rating
Flesh color	Orange	34.69 a[b]	2.38 a	21.06 a	2.42 a	27.08 a	2.40 a
	Green	6.09 b	1.59 b	4.62 b	1.79 b	5.50 b	1.69 b
	White	2.54 b	1.32 b	1.17 b	1.57 b	1.96 b	1.43 b
Rind type	Netted	29.27 a	2.21 a	15.55 a	2.34 a	21.83 a	2.30 a
	Smooth	6.51 b	1.45 b	9.70 b	1.72 b	7.85 b	1.59 b

[a] Ethylene production rate and postharvest decay rating were means of all genotypes with the same flesh color or rind type. Melon fruits were harvested at horticulture maturity and stored at 27–30°C for 7 days before rating visually and mechanically for postharvest decay. A rating of 1 signified no evidence of softening or decay, ratings of 2, 3 and 4 indicated about 25%, 50% and 75% decay of the surface area, and 5 indicated complete rot and collapse of the fruit

[b] Means of each flesh color or rind type in each year with different letters are significantly different based on a Duncan multiple range test (P=0.05)

Table 4 Relationships between the polymorphisms of MEL1- and MEACS1-RFLP and ethylene production rate and postharvest decay[a]

RFLP-MEACS1 fragment	Ethylene production (nl/g per h)			Postharvest decay rating		
	RFLP-MEL1 fragment			RFLP-MEL1 fragment		
	A_0	A_0B_0	B_0	A_0	A_0B_0	B_0
AB	1.48 a[b] A[c]	13.12 a AB	27.19 a B	1.23 a A	2.00 a A	2.42 a A
ABCE	0.61 a A	18.88 ab AB	24.08 a B	1.07 a A	3.21 a B	2.33 a AB
ACE	ND[d]	35.32 b A	34.28 a A	ND	2.27 a A	2.56 a A
ADE	5.74 a	ND	ND	2.63 a	ND	ND
ABDE	8.16 a	ND	ND	1.34 a	ND	ND
Average	2.06 A	22.93 B	31.43 B	1.38 A	2.23 AB	2.51 B
MV[e]		16.75			1.95	

[a] Ethylene production rates and postharvest decay ratings were means over all melon genotypes with the same RFLP fragments for both RFLP-MEL1 and RFLP-MEACS1. RFLP-MEL1 polymorphisms resulted from probing pMEL1 cDNA encoding ACC oxidase with genomic DNA digested with *Eco*RV, where fragments A_0 and B_0 were at molecular sizes of 15- and 8.5-kb, respectively. RFLP-MEACS1 polymorphisms resulted from probing pMEACS1 cDNA encoding ACC synthase with genomic DNA digested with *Nde*I, where fragments A, B, C, D and E were at molecular sizes of 5.2-, 4.2-, 3.8-, 3.0- and 1.0-kb, respectively

[b] Lower- and

[c] upper-case letters indicate significant differences among the columns and rows, respectively, based on a Duncan multiple range test (P=0.05)

[d] ND=not data available

[e] MV=Median value

of RFLP-MEL1 had a significantly higher mean ethylene production rate than those with the A_0 fragment. Although there were fewer significant differences among these RFLP-MEACS1 groups for postharvest decay ratings than for ethylene production rates (Table 4), similar relationships between these RFLP-MEACS1 polymorphisms and postharvest decay ratings also existed. When averaged over all melon genotypes, the A_0 fragment of RFLP-MEL1 was associated with a lower ethylene production rate (2.06 nl/g per h) and a lower postharvest decay rating (1.38), whereas the B_0 fragment was associated with a higher ethylene production rate (31.43 nl/g per h) and a higher postharvest decay rating (2.51). The melon genotypes with both A_0 and B_0 fragments of RFLP-MEL1 showed that both the average ethylene production rate (22.93 nl/g per h) and the postharvest decay rating (2.23) fell between the median values (16.75 nl/g per h and 1.95, respectively) and melons with the B fragment (31.43 nl/g per h and 2.51, respectively).

Polymorphisms for either RFLP-MEL1 or RFLP-MEACS1 were associated with melon market types without regard to flesh colors and rind types (Table 1). All genotypes tested from Western U.S., Eastern U.S. types, Charentais, and most genotypes from the LSL type (except 'NUN0770'), had the B_0 or A_0B_0 fragments of RFLP-MEL1, whereas all genotypes from Casaba, and most genotypes from Galia, Honeydew and Ananas had the A_0 fragment. For RFLP-MEACS1, all genotypes from Ananas, Casaba and Honeydew had AB fragments ('Honeydew Green Flesh' and 'Morning Ice' had additional fragments C and E). All but one genotype each from the Western ('Smith's Perfect') and Eastern U.S. ('Athena') types had ACE fragments. Charentais and LSL types had both AB and ACE fragment patterns. The Galia type had a unique pattern that included a D fragment that was found only in this melon type.

The RFLP polymorphisms were not only related to ethylene production rate, but also to the flesh colors and

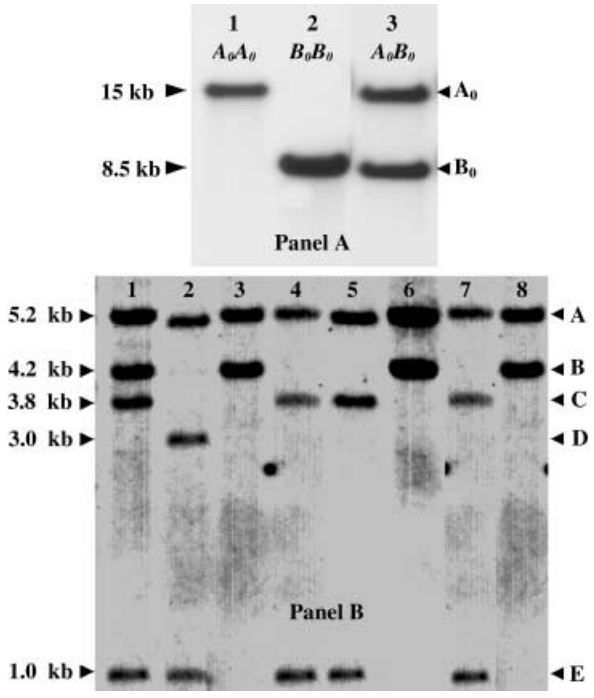

Fig. 3 DNA gel-blotting analyses of RFLP polymorphisms. Panel **A** DNA blots (*Eco*RV digest) probed with MEL1 (cDNA encoding ACC oxidase) showing polymorphisms (A_0=15-kb and B_0=8.5-kb) across melon genotypes. *Lanes 1, 2 and 3* depict 'Honey Dew Green Flesh', 'Doublon' and 'Accent', respectively. A_0A_0, B_0B_0, and A_0B_0 are homozygous and heterozygous genotypes of a putative single locus with two alleles. Panel **B** DNA blots (*Nde*I digest) probed with MEACS1 (cDNA encoding ACC synthase) showing polymorphisms (A=5.2-, B=4.2-, C=3.8-, D=3.0- and E=1.0-kb) across different melon market types. *Lanes 1 through 8* depict 'Honeydew Green Flesh' (Honeydew), 'Rocky sweet' (Galia), 'Doublon' (Charentais), 'Perlita' (Western US Shipping Type), 'Iroquois' (Eastern US Type), 'Ananas' (Ananas), 'Castella' (LSL) and 'TAM Mayan Sweet' (Casaba), respectively

rind types of melon fruits. For RFLP-MEL1, all but one white-flesh and most green-flesh melons had the A_0 fragment, and none of the white- and green-flesh melons possessed only the B_0 fragment, whereas most melons with orange-flesh had the B_0 fragment, either alone or in conjunction with the A_0 fragment (Table 5). Likewise, for the RFLP-MEACS1, all melons with white-flesh, without regard to the rind type, had AB fragments, whereas all ACE fragment profiles were produced by orange-flesh melons, with one exception which was produced by a green-flesh, netted rind genotype ('Rocky Ford Green Flesh') in the Galia type. However, no clear relationship was found between the RFLP-MEACS1 patterns and flesh colors or rind types in the Galia melons.

The RFLP polymorphisms observed for MEL1 and MEACS1, and the associated melon market types and ripening phenotypes, are summarized in Table 6. As mentioned earlier, the A_0 and B_0 fragments of RFLP-MEL1 were associated with white- and orange-flesh, respectively, and melons with the A_0 fragment had a lower ethylene production and less postharvest decay than those with the B_0 or A_0B_0 fragments. For the RFLP-MEASC1, AB and

Table 5 Relationships between the RFLP polymorphisms and melon fruit flesh color type[a]

RFLP polymorphism	Flesh color frequency		
	Orange	Green	White
RFLP-MEL1			
A_0	3	8	12
B_0	15	0	0
A_0B_0	7	2	1
RFLP-MEACS1			
AB	10	4	13
ACE	13	1	0
ABCE	2	2	0
ADE	0	2	0
ABDE	0	1	0

[a]Polymorphisms of RFLP-MEL1 and RFLP-MEACS1 resulted from probing cDNA pMEL1 and pMEACS1 with genomic DNA digested with *Eco*RV and *Nde*I, respectively, using a fluorescein labeling and detection system from Amersham Life Science Inc. RFLP-MEL1 polymorphisms resulted from probing pMEL1 cDNA encoding ACC oxidase with genomic DNA digested with *Eco*RV, where fragments A_0 and B_0 were at molecular sizes of 15- and 8.5-kb, respectively. RFLP-MEACS1 polymorphisms resulted from probing pMEACS1 cDNA encoding ACC synthase with genomic DNA digested with *Nde*I, where fragments A, B, C, D and E were at molecular sizes of 5.2-, 4.2-, 3.8-, 3.0- and 1.0-kb, respectively

ACE fragments were associated with white- and orange-flesh, respectively, and melons with the ADE, or ABDE fragments had a lower ethylene production and less postharvest decay, and non-abscising fruit compared to melons with the C fragment, either ACE or ABCE. Most melons with the ACE fragment were Western U.S. and Eastern U.S. types. Combining polymorphic patterns from both RFLP-MEL1 and RFLP-MEACS1 can be used to differentiate melon market types (Table 6).

SSR analysis

A 110-bp *CMAT35* SSR and a 160-bp *CMTC51* SSR were present in all eight genotypes tested (data not shown). Further genotypes were not tested as no polymorphisms were detected between the genotypes with divergent ethylene production.

Discussion

So far, the most extensively studied model of fruit development and ripening and ethylene biosynthesis is tomato (Theologis 1992; Gray et al. 1994; Wilkinson et al. 1995; Giovannoni et al. 1999). Investigation of the molecular basis of ethylene signal transduction has been focused primarily in the model plant *Arabidopsis* (Ecker 1995). In melon, the molecular basis of ethylene regulation has only recently been investigated and much attention has been devoted to the isolation of genes involved in the ethylene biosynthetic pathway (Balagué et al. 1993; Miki et al.

Table 6 Classification of melon market type based on the most common RFLP and the association with fruit phenotype, ethylene production, and postharvest decay[a]

RFLP polymorphism		Melon market type	Fruit phenotype	Ethylene production (nl/g per h)	Postharvest decay rating
ACC oxidase (MEL1)	ACC synthase (MEACS1)				
A_0	AB	Casaba, Honeydew	White-, or green-fleshed, non-climacteric, no abscission	1.48	1.23
A_0	ABDE or ADE	Galia	Green-fleshed, climacteric, form abscission zone	6.54	2.29
A_0 or A_0B_0	AB or ABCE	Ananas, Charentais, Honeydew	Mixture of high and low ethylene producers, fruit abscise when over-mature	13.12	2.00
B_0 or A_0B_0	ACE	Eastern U.S., Western U.S	Orange-fleshed, climacteric, abscise at mature	30.78	2.41
B_0	AB or ABCE	Charentais	Orange-fleshed, climacteric, abscise when over-mature	32.76	2.56

[1] Ethylene production rates and postharvest decay ratings were means over all melon genotypes with the same RFLP fragments for both RFLP-MEL1 and RFLP-MEACS1. RFLP-MEL1 polymorphisms were resulted from probing pMEL1 cDNA encoding ACC oxidase with genomic DNA digested with *Eco*RV, where fragments A_0 and B_0 had molecular sizes of 15- and 8.5-kb, respectively. RFLP-MEACS1 polymorphisms resulted from probing pMEACS1 cDNA encoding ACC synthase with genomic DNA digested with *Nde*I, where fragments A, B, C, D and E had molecular sizes of 5.2-, 4.2-, 3.8-, 3.0- and 1.0-kb, respectively

1995; Yamamoto et al. 1995; Lesserre et al. 1996; Aggelis et al. 1997). The results from this study were the first to demonstrate the correlation between RFLP polymorphisms and ethylene production in melon. These RFLPs were also associated with flesh color, rind type and postharvest decay characteristics in the melon genotypes examined.

ACC synthase and ACC oxidase are the two enzymes controlling the last two steps of ethylene biosynthesis, respectively. The ACC synthase gene is notoriously polymorphic. In several plants, ACC synthase is encoded by a multigene family (Nakajima et al. 1990; Nakagawa et al. 1991; Rottmann et al. 1991; Liang et al. 1992; Van Der Straeten et al. 1992; Botella et al. 1993; Yamamoto et al. 1995). Our data also seemed to reveal multigene encoding of ACC synthase in melon because within the RFLP-MEACS1 polymorphism group of AB, melons with the high-ethylene-production polymorphism B_0 fragment of RFLP-MEL1 had significantly higher ethylene production rates than those with the low-ethylene-production polymorphism A_0 fragment. These data implied that, other than the ACC synthase gene whose cDNA was used to generate the RFLP-MEACS1 shown in this study, an additional copy or copies of the ACC synthase gene(s) existed and encoded ACC synthase that contributed to the conversion of S-adenosylmethionine to ACC, which was then converted to ethylene by ACC oxidase. The hypothesis of a multigene family encoding ACC synthase in melon was also supported by the single-copy reconstruction assay of the ACC synthase gene. In this assay, *Sty*I and *Xba*I, the two enzymes showing no cutting site along the entire sequence of the cDNA of MEACS1, each resulted in multiple hybridization bands (Zheng and Wolff, unpublished data).

ACC synthase is generally regarded as the rate-limiting step in ethylene biosynthesis (Yang and Hoffman 1984; Zarembinski and Theologis 1994). Our results seemed to suggest that the ACC oxidase gene was the primary determinant, or at least had an equal effect with the specific ACC synthase gene involved in this study, on ethylene production because of several observations. First, the ethylene production rates of melons with polymorphisms of A_0 or B_0 of RFLP-MEL1 were independent of polymorphisms of RFLP-MEACS1 (Table 4). Secondly, within AB or ABCE groups of RFLP-MEACS1, melons with the B_0 fragment of RFLP-MEL1 had significantly higher ethylene production rates than those with the A_0 fragment. Third, both RFLP-MEL1 and RFLP-MEACS1 polymorphisms had a clear relationship with ethylene production rates across the diverse melon genotypes.

The RFLP-MEL1 banding patterns revealed a putative single locus with two alleles. The allele A_0 was associated with low ethylene production, whereas the allele B_0 was associated with high ethylene production. It also seemed to show that the B_0 allele was partially dominant over the A_0 allele because the heterozygous class (A_0B_0) had an ethylene production rate between the median value of the homozygous class values (A_0A_0 and B_0B_0) and the B_0B_0 genotype value. This allelism of the ACC oxidase gene had similar effects on postharvest decay. Although the RFLP-MEACS1 was more complicated than RFLP-MEL1, the ethylene production rate appeared to be controlled by B and C fragments. The fact that melons containing both B and C fragments (i.e., ABCE) of RFLP-MEACS1 had an ethylene production rate close to the median values of melons with AB and ACE fragments suggested that the B and C fragments are two alleles from a single locus which maps to a quantitative trait locus (QTL) of ethylene production in melon. This is not surprising given the fact that 32 independent loci corresponding to genes known, or hypothesized, to influ-

ence ripening and/or ethylene response in tomato were mapped by using RFLP markers (Giovannoni et al. 1999). The ethylene response genes are involved in either ethylene biosynthesis or perception, while additional ripening-related genes include those involved in cell-wall metabolism and pigment biosynthesis (Giovannoni et al. 1999). The allelism of either A_0 and B_0 fragments of RFLP-MEL1 or B and C fragments of RFLP-MEACS1 was further confirmed in segregating populations (F_2, BC_1P_1, and BC_1P_2) derived from a cross between 'TAM Uvalde' (P_1)×'TAM Yellow Canary' (P_2) (Zheng and Wolff, unpublished data). The former is a Western U.S. Shipping genotype with a high ethylene production rate and possessed the B_0 fragment of RFLP-MEL1 and the ACE fragments of RFLP-MEACS1. The latter is a Casaba genotype with a low ethylene production rate and the corresponding A_0 fragment of RFLP-MEL1 and AB fragments of RFLP-MEACS1. A previous report indicated, however, that ethylene production in crosses between cantaloupe and honeydew genotypes was controlled by dominant gene action (Shellie and Wolff 1995). Abscission in a Japanese melon, on the other hand, was controlled by two, incompletely dominant genes (Takada et al. 1975).

The RFLPs were also associated with the flesh colors of melon fruits. An interesting comparison was 'Honeydew Green Flesh' (HDGF) and 'Honeydew Orange Flesh' (HDOF). HDOF was derived from HDGF by simply incorporating an orange flesh into a honeydew background. HDGF had the A_0 fragment of RFLP-MEL1, whereas HDOF had the B_0 fragment. Correspondingly, HDGF had zero (undetectable) ethylene production and no postharvest decay (1.0), whereas HDOF had a 1.83 nl/g per hr ethylene production rate and a postharvest decay rating of 2.0.

Certain cultigens had ethylene production rates and associated RFLP patterns that did not fit the general trend. However, when using both probes, i.e., at least one of the two RFLPs (i.e., RFLP-MEL1 and RFLP-MEACS1) correctly matched the phenotype, all genotypes but three fit into the expected relationship. In other words, a low-ethylene producer did not have high-ethylene production associated fragments of both RFLP-MEL1 (A_0B_0 or B_0) and RFLP-MEASC1 (ACE), but may have had one. On the other hand, melons with both high-ethylene production associated fragment patterns had high-ethylene production rates, except genotypes 'Imperial # 45' and 'Mission'. Another exception was 'NUN0770', which had a higher ethylene production rate (8.77 nl/g per h) than the average (1.48 nl/g per h) of its low-ethylene production associated polymorphisms of combined RFLP-MEL1 (A_0 fragment) and RFLP-MEACS1 (AB fragments). All these three genotypes have been developed for long shelf-life and showed very little postharvest decay. For genotype 'NUN0770', it is possible that reduced postharvest fruit decay or enhanced shelf-life may have been derived from genes not involved in ethylene production. Perhaps either an altered ethylene receptor gene or a specific rot resistance gene was selected. As indicated above, these RFLPs also had a close relationship with flesh colors and rind types. Therefore, it is promising that these RFLPs might be used in marker-assisted selection (MAS) to develop orange flesh, netted melons with reduced postharvest fruit decay severity. Conversion of these RFLPs to PCR-based markers would make MAS in melon more practical.

It should be pointed out that using the cDNA clone (i.e., pMEL1) from the ACC oxidase gene as a probe detected RFLP polymorphisms associated with ethylene production rates while neither SSRs, which were also derived from two non-coding regions of the same gene, detected polymorphism among the same genotypes. Although neither SSR detected any polymorphism among the eight melon genotypes representing the seven market types tested in this study, one (*CMAT35*) of the two SSRs detected polymorphism among several melon genotypes tested elsewhere, while the other SSR (*CMTC51*) detected polymorphism between two cucumber genotypes (Danin-Poleg, personal communication).

Although divergent ethylene production rates in selected melon genotypes have been documented before (Pratt et al. 1977; Lester and Dunlap 1985; Shellie and Saltveit 1993; Hadfield et al. 1995; Miccolis and Saltveit 1995), our data demonstrate a clear and direct correlation between higher ethylene production and more severe postharvest fruit decay in the Cantaloupensis and Inodorus market types of melon cultivars. Ethylene production in melon fruit appears to be the most important factor in determining postharvest decay severity. Flesh color and rind types were associated, or correlated, with ethylene production and postharvest fruit decay (Tables 1 and 3; Fig. 2). Orange flesh was associated with high ethylene production, although there were noted exceptions. For example, orange-fleshed honeydews had much lower ethylene production than other orange-fleshed Western U.S. types and Eastern U.S. types; however, they produced an average of 2× more ethylene than the green-fleshed honeydew (Table 1). Although green- and white-fleshed melons were generally lower ethylene producers than orange-fleshed melons, they can have a climacteric ripening pattern and abscise at maturity (e.g., Ananas, Galia).

Rind type also was correlated with melon ethylene production and postharvest decay rating. The surface meshwork of cracks ('net') found in the melon epidermis consist of an elaborate system of lenticels derived from the subepidermal periderm (Webster and Craig 1976). Our data showed that a netted rind was associated with higher ethylene production and more severe postharvest decay (Table 3, Fig. 2). The increased ethylene production could be due to enhanced gas exchange of the melon mesocarp afforded by the lenticels. Oxygen is required for the ACC oxidase-mediated conversion of ACC to ethylene (Yang and Hoffman 1984). Another direct effect of netting on postharvest fruit decay is the loss of water through the netted rind. Lester and Bruton (1986) showed a 5.7% water loss through the net of the canta-

loupe ‘Magnum 45’ after 20 days of storage, which made the fruit soft and unmarketable. They noted less postharvest fruit decay with the addition of a shrink-wrap polyethylene film over the melons. A netted rind also allows easy entry of fruit rot pathogens through the open cracks in the epidermal tissue, particularly early in development of the net. Gummy stem blight has been observed to infect cantaloupe fruit in the field did not affect honeydew, which has a smooth, intact rind (M.E. Miller, Texas Agricultural Experiment Station, Texas A & M University System, personal communication, 1998).

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References

Abeles FB, Morgan PW, Salveit Jr ME (1992) Ethylene in plant biology. Academic Press, San Diego, California, USA

Adams DO, Yang SF (1979) Ethylene biosynthesis: identification of 1-aminocyclopropanel-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc Natl Acad Sci USA 76:170–174

Aggelis A, John I, Grierson D (1997) Analysis of physiological and molecular changes in melon (*Cucumis melo* L.) varieties with different rates of ripening. J Exp Bot 48:769–778

Balagué C, Watson CF, Turner AJ, Rouge P, Picton S, Pech J, Grierson D (1993) Isolation of a ripening and wound-induced cDNA from *Cucumis melo* L. encoding a protein with homology to the ethylene-forming enzyme. Eur J Biochem 212:27–34

Bardy CJ (1987) Fruit ripening. Annu Rev Plant Physiol 38:155–178

Baudracco-Arnas S (1995) A simple and inexpensive method for DNA extraction from *Cucumis melo* L. Cucurb Genet Coop. Rep 18:50–51

Bent AF, Innes RW, Ecker JR, Staskswicz (1992) Disease development in ethylene-insensitive *Arabidopsis thaliana* infected with virulent and avirulent *Pseudomonas* and *Xanthomonas* pathogens. Mol Plant-Microbe Interact 5:372–378

Botella JR, Schlangnhaufer CD, Arteca JM, Arteca RN, Phillips AT (1993) Identification of two new members of the 1-aminocyclopropane-1-carboxylate synthase multigene family in mung bean. Gene 123:249–253

Dunlap JR, Robacker KM (1993) Suppression of wound-induced ethylene production in muskmelon fruit tissue after vacuum infiltration with aqueous buffer. Plant Growth Reg 13:111–116

Ecker JR (1995) The ethylene signal transduction pathway in plants. Science 268:667–675

Ecker JR, Davis RW (1987) Plant defense genes are regulated by ethylene. Proc Natl Acad Sci USA 84:5202–5206

Ginsburg L (1965) Recommended storage temperatures, percentage relative humidities and storage life for fruit and vegetables. Decid Fruit Grower (S. Africa) 15:80–86

Giovannoni J, Yen H, Shelton B, Miller S, Vrebalov J, Kannan P, Tieman D, Hackett R, Grierson D, Klee H (1999) Genetic mapping of ripening and ethylene-related loci in tomato. Theor Appl Genet 98:1005–1013

Gray J, Picton S, Giovannoni J, Grierson D (1994) The use of transgenic and naturally occurring mutants to understand and manipulate tomato fruit ripening. Plant Cell Environ 17:557–571

Hadfield KA, Rose JKC, Bennett AB (1995) The respiratory climacteric is present in Charentais (*Cucumis melo* cv reticulatus F_1 Alpha) melons ripened on or off the plant. J Exp Bot 46: 1923–1925

Hoffman T, Schmidt JS, Zheng XY, Bent AF (1999) Isolation of ethylene-insensitive soybean mutants that are altered in pathogen susceptibility and gene-for-gene disease resistance. Plant Physiology 119:935–949

Kende H (1993) Ethylene biosynthesis. Annu Rev Plant Physiol Plant Mol Biol 44:283–307

Koestsier PA, Schorr J, Doerfler W (1993) A rapid optimized protocol for downward alkaline Southern blotting of DNA. BioTechniques 15:260–262

Lasserre E, Bouquin T, Hernandez JA, Bull J, Pech JC, Balague C (1996) Structure and expression of three genes encoding ACC oxidase homologs from melon (*Cucumis melo* L.). Mol Gen Genet 251:81–90

Lester GE, Bruton BD (1986) Relationship of netted muskmelon fruit water loss to postharvest storage life. J Amer Soc Hort Sci 111:727–731

Lester GE, Dunlap JR (1985) Physiological changes during development and ripening of ‘Perlita’ muskmelon fruits. Scien Hort 26:323–331

Liang X, Abel S, Keller JA, Shen NF, Theologis A (1992) The 1-aminocyclopropane-1-carboxylate synthase gene family of Arabidopsis thaliana. Proc. Natl Acad Sci USA 89: 11046–11050

Lund ST, Stall RE, Klee HJ (1998) Ethylene regulates the susceptible response to pathogen infection in tomato. Plant Cell 10:371–382

Miccolis V, Saltveit ME Jr (1991) Morphological and physiological changes during fruit growth and maturation of seven melon cultivars. J Amer Soc. Hort Sci 116:1025–1029

Miccolis V, Saltveit ME (1995) Influence of storage period and temperature on the postharvest characteristics of six melon (*Cucumis melo* L., *inodorus* group) cultivars. Postharvest Biol Technol 5:211–219

Miki T, Yamamoto M, Nakagawa H, Ogura N, Mori H, Imaseki H, Sato T (1995) Nucleotide sequence of a cDNA for 1-aminocyclopropane-1-carboxylate synthase from melon fruits. Plant Physiol 107:297–298

Nakagawa N, Mori H, Yamazaki K, Imaseki H (1991) Cloning of a complementary DNA for auxin-induced 1-aminocycopropane-1-carboxylate synthase and differential expression of the gene by auxin and wounding. Plant Cell Physiol 32:1153–1163

Nakajima N, Mori H, Yamazaki K, Imaseki H (1990) Molecular cloning and sequencing of a complementary DNA encoding 1-aminocyclopropane-1-carboxylate synthase induced by tissue wounding. Plant Cell Physiol 31:1021–1029

Pratt HK, Goeschl JD, Martin FW (1977) Fruit growth and development, ripening, and the role of ethylene in the ‘Honey Dew’ muskmelon. J Am Soc Hort Sci 102:203–210

Rottmann WH, Peter GF, Oeller PW, Keller JA, Shen NF, Nagy BP, Taylor LP, Campbell AD, Theologis A (1991) 1-Aminocyclopropane-1-carboxylate synthase in tomato is encoded by a multigene family whose transcription is induced during fruit and floral senescence. J Mol Biol 222:937–961

Shellie KC, Saltveit ME (1993) The lack of a respiratory rise in muskmelon fruit ripening on the plant challenges the definition of climacteric behaviour. J Exp Bot 44:1403–1406

Shellie KC, Wolff DW (1995) Enhancing ripening characteristics of ‘netted’ and ‘honeydew’-type melons. HortScience 30:766 (abstract)

Takada K, Kanazawa K, Takatuka K (1975) Studies on the breeding of melon for resistance to powdery mildew. II. Inherit-

ance of resistance to powdery mildew and correlation of resistance to other characters. Yasai Shikenjo Hokoku 2: 11–31

Theologis A (1992) One rotten apple spoils the whole bushel: the role of ethylene in fruit ripening. Cell 70:181–184

Van Der Straeten D, Rodrigues-Pousada RA, Villarroel R, Hanley S, Goodman HM, Montagu MV (1992) Cloning, genetic mapping, and expression analysis of an *Arabidopsis thaliana* gene that encodes 1-aminocyclopropane-1-carboxylate synthase. Proc Natl Acad Sci USA 89:9969–9973

Webster BD, Craig ME (1976) Net morphogenesis and characteristics of the surface of muskmelon fruit. J Amer Soc Hort Sci 101:412–415

Wilkinson J, Lanahan M, Yen H, Giovannoni J, Klee H (1995) An ethylene-inducible component of signal transduction encoded by *Never-ripe*. Science 270:1807–1809

Yamamoto M, Miki T, Ishiki Y, Fujiami K, Yanagisawa Y, Nakagawa H, Ogura N, Hirabayashi T, Sato T (1995) The synthesis of ethylene in melon fruit during the early stage of ripening. Plant Cell Physiol 36:591–596

Yang SF, Dong JG (1993) Recent progress in research of ethylene biosynthesis. Bot Bull Acad Sinica 34:89–101

Yang SF, Hoffman NE (1984) Ethylene biosynthesis and its regulation in higher plants. Annu Rev Plant Physiol 35:155–189

Zarembinski TI, Theologis A (1994) Ethylene biosynthesis and action: a case of conservation. Plant Mol Biol 26:1579–1597

Zheng XY, Wolff DW (1999) An optimized protocol for rapid and sensitive application of Southern (Northern) hybridization by using a fluorescein labeling and detecting system. Biotechnol Tech 13:431–435