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Genetics of ethylene biosynthesis and restriction fragment length polymorphisms (RFLPs) of ACC oxidase and synthase genes in melon (*Cucumis melo* L.)

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Abstract We investigated the genetics of ethylene biosynthesis and its linkage to the RFLPs of the ACC oxidase and synthase genes in melon (*Cucumis melo* L.). The results suggested that the A_0 and B_0 fragments of RFLP-MEL1 of the ACC oxidase gene were two alleles from a single locus, as were the B and C fragments of RFLP-MEACS1 of the ACC synthase gene. The B_0 allele seemed to be partially dominant over the A_0 allele, whereas B and C alleles appeared to map to quantitative trait loci (QTLs), which most likely contributed to ethylene production. Both RFLPs were linked to ethylene production rates, but they were not linked to each other. The interaction effects of the ACC oxidase and synthase genes on ethylene production were revealed by segregation of RFLP-MEL1 and RFLP-MEACS1. The results of single-copy-reconstruction assays suggested that the ACC oxidase gene is a single copy, whereas the ACC synthase gene is a component of a multigene family in the melon genome. The abscission phenotype appeared to be controlled by an independent locus, with the abscission (full-slip) allele dominant over the non-abscission (not full-slip) allele. These results may facilitate efforts toward mapping the quantitative trait loci (QTLs) of ethylene production. The RFLPs may be used in marker-assisted selection in developing melons with a more-desirable low ethylene production rate for enhancing postharvest storage life.

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Introduction

Ethylene is an endogenous, gaseous regulator with profound effects on many growth and development processes (Yang and Hoffman 1984; Bardy 1987; Abeles et al. 1992; Kende 1993) and stress responses (Ecker and Davis 1987; Bent et al. 1992; Lund et al. 1998). Its physiological effects on fruit ripening, shelf-life, shipping potential, storage practices, and quality of many fruits and vegetables have been well documented (Yang and Hoffman 1984; Abeles et al. 1992; Theologis 1992; Kende 1993). In melon (*Cucumis melo* L.), differences in ethylene production and its associated postharvest characteristics among the different genotypes and/or market types have been reported (Ginsburg 1965; Pratt et al. 1977; Lester and DunLap 1985; Miccolis and Saltveit 1991, 1995; Shellie and Saltveit 1993; Hadfield et al. 1995; Zheng and Wolff 2000).

ACC (1-amino-cyclopropane-1-carboxylic acid) synthase and oxidase are enzymes that control the last two steps of the ethylene biosynthetic pathway, respectively, i.e., S-adenosylmethionine conversion to ACC, and ACC conversion to ethylene (Yang and Hoffman 1984). The biochemical pathway of ethylene production has been well-documented (Adams and Yang 1979; Yang and Dong 1993; Zarembinski and Theologis 1994) and is now largely understood (Theologis 1992; Kende 1993). The onset of ethylene production in ripening climacteric fruits is known to be associated with increased activities of ACC synthase and ACC oxidase (Balagué et al. 1993; Kende 1993). However, the regulation of the ethylene climacteric in fruit development and ripening is far more complicated.

The most extensively studied model for fruit development, ripening, and ethylene biosynthesis is tomato (*Lycopersicum esculentum*) (Theologis 1992; Gray et al.

1994; Wilkinson et al. 1995; Giovannoni et al. 1999), whereas investigation of the molecular basis for ethylene signal transduction has been focused primarily in the model plant *Arabidopsis* (Ecker 1995). In tomato, although ethylene plays a pivotal role in the regulation of climacteric ripening, a large number of loci were found to be involved in the regulation of fruit development and ripening processes. RFLP markers were used to distinguish 32 independent loci, corresponding to genes known, or hypothesized, to influence ripening and/or ethylene responses (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). Investigations into the molecular basis of ethylene regulation in melon have been primarily devoted to the isolation of genes involved in the ethylene biosynthetic pathway. The cDNA encoding an ACC oxidase gene was isolated in melon (Balagué et al. 1993), and the gene family characterized (Lasserre et al. 1996). Also, a cDNA encoding an ACC synthase gene in melon has been sequenced (Miki et al. 1995). The expressions of ethylene production genes and unknown ripening related genes during fruit development of a small sample of *C. melo* L. Cantalupensis Group and Inodorus Group, genotypes were reported (Aggelis et al. 1997).

We recently reported a significant correlation between ethylene production and postharvest fruit decay and the evidence of RFLP linkage to the ethylene production genes among the diverse melon genotypes of Cantalupensis and Inodorus Groups (Zheng and Wolff 2000). The A_0 and B_0 fragments of RFLP-MEL1 were associated with low and high ethylene production, respectively. Although RFLP-MEACS1 revealed a total of five fragments (A, B, C, D and E) in the melon genome, only the B and C fragments seemed to affect ethylene production (Zheng and Wolff 2000). The objective of the present study was to further investigate the genetics of ethylene biosynthesis and its linkage to the RFLPs of ACC oxidase and synthase in melon.

Materials and methods

Germplasm and cross

Based on our earlier results (Zheng and Wolff 2000), 'TAM Uvalde' ('TAMU') and 'TAM Yellow Canary' (TYC') were selected as parental lines, and designated as P1 and P2, respectively. 'TAM Uvalde' is a western shipper type with a high ethylene production rate, and possesses the B_0 fragment of RFLP-MEL1 and the A,C and E fragments of RFLP-MEACS1 (Zheng and Wolff 2000). 'TAM Yellow Canary' is a Casaba type with a low ethylene production rate, and possesses the A_0, and A and B fragments, correspondingly. Reciprocal crosses between the two parental lines were performed to produce the F_1 generation. The F_1 plants were selfed to generate the F_2 population. Backcrosses to each of the parents were also made to produce the backcross populations, BCP_1 and BCP_2. For all crosses, female flowers were emasculated at half to three-quarters pre-anthesis and pollinated with the desired pollen. Forty-four F_2 and each of 20 BCP_1 and BCP_2 individual plants were grown in 1996. Fifty two, 22, and 26 plants of the F_2, BCP_1, and BCP_2 families were grown in 1997. Ten plants each of the parental lines and F_1 generation were also grown at the same time. All plants were grown in 10 L pots containing sterile media (5 parts peat moss: 3 parts perlite: 3 parts vermiculite) in the greenhouse. All plants were fertilized with an equal amount of N (100 ppm) from water-soluble 20:20:20 N:P:K at each irrigation.

Abscising phenotype and ethylene production measurements

Fruits were supported in mesh bags as they enlarged. At horticultural maturity (fruit abscission or rind color change), melons were harvested and measured for ethylene production (Webster and Craig 1976). During the harvesting, abscission phenotypes were recorded as either full slip (FS) or not full slip (NFS). Ethylene production was measured as described by Zheng and Wolff (2000). Three to six samples were taken from one to two fruit(s) for ethylene production measurements and the average production rate was calculated. Significant differences were determined by the Waller-Duncan test.

Genomic DNA

As vines grew, young and healthy leaf tissues and growing tips were harvested and freeze-dried with DURA-DRY (FTS Systems, Inc., Stone Ridge, N.Y., USA) at 4 °C for 24–36 h. The freeze-dried samples were ground in a coffee grinder. Genomic DNA were then extracted from these samples using the procedure of Baudracco-Arnas (1995). DNA sample quantity and quality were determined by an UV-VIS scanning spectrophotometer (UV-2101PC, Shimadzu Scientific Instruments, Inc., Md., USA). All DNA samples had absorbance ratios of $A_{260}{}^{-1}$ A_{280} above 1.8.

DNA gel blotting and RFLP segregation analyses

The cDNAs encoding the ACC oxidase (MEL1, Balagué et al. 1993) or the ACC synthase (MEACS1, Miki et al. 1995) genes were used as probes. For blots to conduct segregation analyses of the RFLP-MEL1 or RFLP-MEACS1 (Zheng and Wolff 2000), the genomic DNAs were digested with either *Eco*RV or *Nde*I (New England BioLabs, Inc. Beverly, Mass., USA), respectively. Chi–square values were calculated using SigmaStat software (Jandel Scientific, San Rafael, Calif.).

For the single-copy reconstruction assays, the cDNA sequences of MEL1 and MEACS1 were first analyzed by GCG package version 8.0 (Genetics Computer Group, Madison, Wis.). Then the restriction endonucleases showing no cutting site(s) in the cDNA sequences were selected. For the single-copy reconstruction assay of the ACC oxidase gene, genomic DNAs of the two parental lines 'TAMU' and 'TYC' were double-digested with *Eco*RI and each of the following nine enzymes: *Eco*RV, *Bam*HI, *Hin*dIII, *Kpn*I, *Pst*I, *Xba*I, *Sac*II, *Bgl*II and *Xho*I. Similarly, for the single-copy reconstruction assay of the ACC synthase gene, genomic DNAs of the parental lines were digested with each of the following five enzymes: *Bgl*I, *Eco*RV, *Kpn*I, *Sty*I and *Xba*I. In all cases, genomic DNAs (10–15 μg) were digested in a 25-μl reaction volume at 37 °C for 10 h. DNA blotting, probe labeling, hybridization, and signal detection were carried-out using a fluorescein labeling and detection system (Amersham, Life Science, Arlington Heights, Ill. USA) under optimized conditions (Koestsier et al. 1993; Zheng and Wolff 1999).

Results

Genetics of the RFLPs in segregating populations

Figure 1 shows the RFLP-MEL1 (Panel A) and the RFLP-MEACS1 (Panel B) in the parental lines ('TAMU' and 'TYC'), F_1, F_2, BCP_1, and BCP_2 indi-

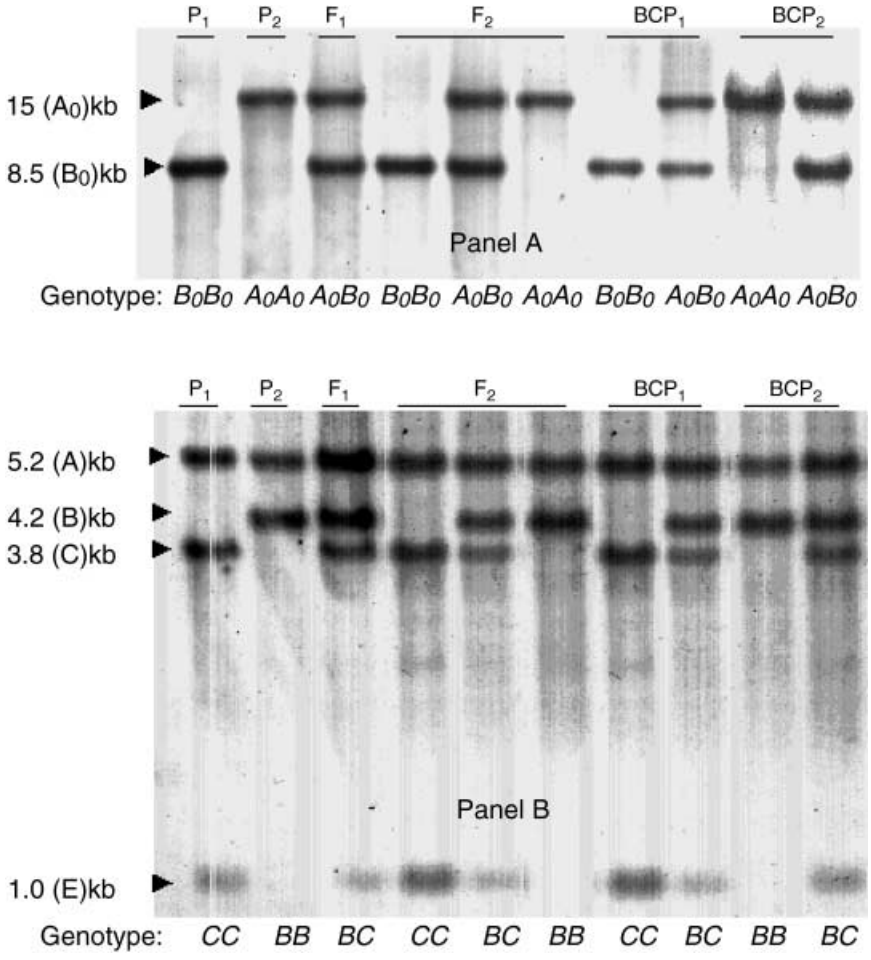

Fig. 1 DNA gel-blotting analyses of the RFLPs in parental lines ('TAMU'–P_1, a Western shipper type with a high ethylene production rate, and 'TYC'–P_2, a Casaba type with a low ethylene production rate, and their genotypes in F_1, F_2, BCP_1, and BCP_2 populations. *Panel A*: DNA blots (*Eco*RV digest) probed with a cDNA of the ACC oxidase gene (RFLP-MEL1) showing the polymorphisms (A_0 = 15 kb and B_0 = 8.5 kb) in the parental lines and F_1 generation, as well as the segregation patterns in F_2, BCP_1, and BCP_2 populations. *Panel B*: DNA blots (*Nde*I digest) probed with a cDNA of the ACC synthase gene (RFLP-MEACS1) showing the polymorphisms (A = 5.2, B = 4.2, C = 3.8 and E = 1.0 kb) in the parental lines and F_1 generation, as well as the segregation patterns in F_2, BCP_1, and BCP_2 populations

Table 1 Chi-square (χ^2) tests of the RFLP genotypes in segregating populations[a]

RFLP[b]	F_2					BCP_1				BCP_2			
	χ^2 (1:2:1), *df*=2					χ^2 (1:2:1), *df*=1				χ^2 (1:2:1), *df*=1			
MEL1	A_0A_0	A_0B_0	B_0B_0	χ^2	*P*	B_0B_0	A_0B_0	χ^2	*P*	A_0A_0	A_0B_0	χ^2	*P*
	9	23	12	0.50	0.78	12	8	0.80	0.37	9	11	0.20	0.66
MEACS1	BB	BC	CC	χ^2	*P*	CC	BC	χ^2	*P*	BB	BC	χ^2	*P*
	10	22	12	0.18	0.91	10	10	0.00	1.00	11	9	0.20	0.66

[a] The χ^2 and *P* values are for χ^2 tests of similarity of the data to noted ratios; accept ratio if $P > 0.05$

[b] 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 B and C were at molecular sizes of 4.2 and 3.8 kb, respectively

viduals. For the RFLP-MEL_1, 'TYC' shows a unique 15-kb fragment (designated as A_0), whereas 'TAMU' had a fragment of 8.5 kb (designated as B_0). Their F_1 progeny had both fragments. The fragments A_0 and B_0 segregated accordingly in F_2, BCP_1, and BCP_2 individuals. Similarly, for the RFLP-MEACS1, each parental line had a unique fragment of 4.2 kb (designated as B) and 3.8 kb (designated as C), respectively, which also segregated accordingly in F_2, BCP_1, and BCP_2 individuals. Since the 5.2-kb fragment (designated as A) was common and the 1.0-kb fragment (designated as E) did not contribute to ethylene production (Zheng and Wolff 2000), only the B and C fragments of RFLP-MEACS1 will be further discussed. Table 1 showed that for either the A_0 and B_0 fragments of RFLP-MEL1, or the B and C fragments of RFLP-MEACS1, segregation patterns fit a Mendelian single-gene model as a 1:2:1 ratio in the F_2 population and as a 1:1 ratio in the backcross populations, indicating that the A_0 and B_0 fragments of RFLP-MEL1, and the *B* and *C* fragments of RFLP-MEACS1, were the two alleles from independent single loci.

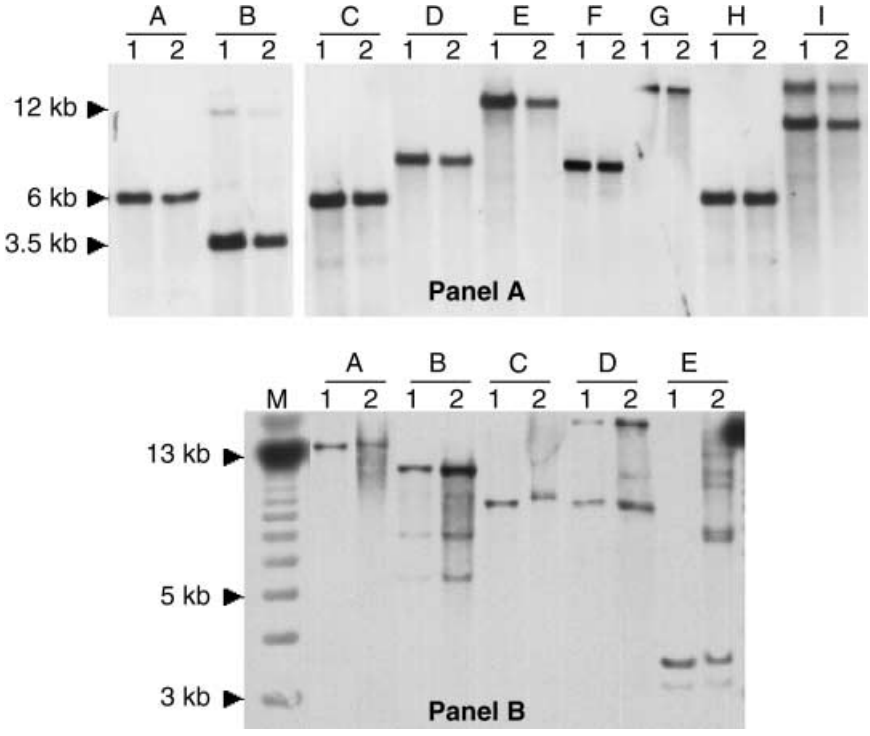

Fig. 2 The single-copy-reconstruction assays of the ACC oxidase gene (*panel A*) and the ACC synthase gene (*panel B*). In order to probe the DNA blot with the cDNA MEL1 for the ACC oxidase gene assay, genomic DNAs of the two parental lines 'TAMU' (*1*) and 'TYC' (*2*) were double-digested with *Eco*RI with each of the following nine enzymes: *Eco*RV (*A*), *Bam*HI (*B*), *Hin*dIII (*C*), *Kpn*I (*D*), *Pst*I (*E*), *Xba*I (*F*), *Sac*II (*G*), *Bgl*II (*H*) and *Xho*I (*I*). Similarly, for the DNA blot to be probed with the cDNA MEACS1 for the ACC synthase gene assay, genomic DNAs of the two parental lines 'TAMU' (*1*) and 'TYC' (*2*) were digested individually with each of the following five enzymes: *Bgl*I (*A*), *Eco*RV (*B*), *Kpn*I (*C*), *Sty*I (*D*) and *Xba*I (*E*)

Single-copy-reconstruction assay

Figure 2 shows a DNA gel-blot analysis of the single-copy-reconstruction assay of the ACC oxidase gene (panel A) or the ACC synthase gene (panel B). For the ACC oxidase gene, except for *Xho*I, double digestion by *Eco*RI and each of the other eight restriction endonucleases (*Eco*RV, *Bam*HI, *Hin*dIII, *Kpn*I, *Pst*I, *Xba*I, *Sac*II and *Bgl*II) resulted in a single predominant hybridization band for both parental lines, indicating that the ACC oxidase gene used in this study is a single-copy gene. In contrast, for the single-copy-reconstruction assay of the ACC synthase gene, digestion with *Bgl*I or *Kpn*I resulted in one predominant hybridization band. However, digestion with *Eco*RV, *Sty*I or *Xba*I resulted in multiple hybridization bands, suggesting that the ACC synthase gene used here is a component of a multigene family.

Table 2 Average ethylene production rates (nl^{-1} g^{-1} h^{-1}) of the parental lines, and the F_1, F_2, BCP_1, and BCP_2 populations[a]

Melon source	Ethylene production (nl^{-1} g^{-1} h^{-1})		
	1996	1997	Combined
'TAMU' (P_1)	51.18	33.51	37.04
'TYC' (P_2)	0.01	0.01	0.01
F_1[b]	18.02 (11.61)	4.46 (4.13)	15.71 (8.65)
F_2	6.59	4.84	6.07
BCP_1	47.99	12.30	30.87
BCP_2	1.27	1.48	1.31

[a] Ethylene production rates are means of values from all individual plants of the parental lines and their F_1, F_2, BCP_1, and BCP_2 families. Individual plant means were based on three readings per fruit
[b] Numbers without the parentheses are averages from F_1 selfed plants, whereas the numbers within the parentheses are averages from all F_1 plants

Evidence of RFLPs mapping to the complex trait of ethylene production

Table 2 lists the average ethylene production rates (nl^{-1} g^{-1} h^{-1}) of the parental lines and the F_1, F_2, BCP_1, and BCP_2 populations of two trials and their combined data. In all cases, ethylene production by 'TAMU' was several thousand-fold higher than that by 'TYC'. Ethylene production rates in the F_1 generation were in-between the two parental lines. However, the ethylene production rates of F_1 individuals seemed to be affected by the pollen sources in the 1996 trial. The F_1 fruits resulting from pollination with pollen from the same plant (i.e., selfing) had an average ethylene production of 18.02 nl^{-1} g^{-1} h^{-1} (Table 2). However, the F_1 fruits, resulting from pollination with pollen from the high ethylene producing parental line 'TAMU' had an average ethylene production rate of 10.10 nl^{-1} g^{-1} h^{-1} as compared to 3.44 nl^{-1} g^{-1} h^{-1} resulting from pollination with pollen from the low ethylene producing parental line 'TYC'. In 1997, the average ethylene production rates of F_1 fruits and F_2 fruits seemed to be lower than the expected theoretical value, based on additive gene action. The ethylene production rates in backcross populations BCP_1 and BCP_2 were 47.99 and 1.27 nl^{-1} g^{-1} h^{-1} respectively in 1996, and 12.30 and 1.48 nl^{-1} g^{-1} h^{-1} respectively in 1997, with a 2 year average of 30.87 and 1.31 nl^{-1} g^{-1} h^{-1}, respectively.

The ethylene production rate of each RFLP genotype in the F_2 population is shown in Fig. 3. For the RFLP-MEL1, melons with the heterozygous genotype A_0B_0 had an ethylene production rate (4.81 nl^{-1} g^{-1} h^{-1}) between the median value (3.44 nl^{-1} g^{-1} h^{-1}) of the homozygous class values (A_0A_0 and B_0B_0) and the high ethylene producing genotype B_0B_0 value (5.11 μl^{-1} g^{-1} h^{-1}) in the F_2 population (Panel A). In the BCP_1 population, the genotypes A_0B_0 and B_0B_0 had ethylene production rates of 8.89 and 13.24 nl^{-1} g^{-1} h^{-1}, respectively. The genotypes A_0B_0 and A_0A_0 had ethylene production rates of 3.71 and 0.18 nl^{-1} g^{-1} h^{-1}, respectively, in the BCP_2 population. For the RFLP-MEACS1, melons with the heterozygous genotype *BC* had an ethylene production rate (4.33 nl^{-1} g^{-1} h^{-1}) that was close to the median value (4.23 nl^{-1} g^{-1} h^{-1}) of the homozygotes of the high ethylene producing genotype *CC* (6.71 nl^{-1} g^{-1} h^{-1}) and the low ethylene producing genotype *BB* (1.75 nl^{-1} g^{-1} h^{-1}) in the F_2 population (Panel B). In the BCP_1, the genotypes *BC* and *CC* had 9.57 and 12.56 nl^{-1} g^{-1} h^{-1} ethylene production rates, respectively. However, in the BCP_2 population, the ethylene production rates of the genotypes *BC* (0.45 nl^{-1} g^{-1} h^{-1}) and *BB* (2.17 nl^{-1} g^{-1} h^{-1}) did not fit the expected values. Figure 3, panel C, also shows the ethylene production rates averaged over both the RFLP-MEL1 and the RFLP-MEACS1 genotypes. Melons with low ethylene producing genotypes of both RFLPs, i.e., the A_0A_0 genotype of RFLP-MEL1 and the BB genotype of RFLP-MEACS1, had the lowest ethylene production rate (0.08 nl^{-1} g^{-1} h^{-1}). In contrast, melons with high ethylene producing genotypes, i.e., the B_0B_0 genotype of RFLP-MEL1 and the *CC* genotype of RFLP-MEACS1, had the highest ethylene production rate (8.48 nl^{-1} g^{-1} h^{-1}). The ethylene production rates of melons with all other RFLP genotypes were between the lowest and the highest rates, and ranged from 1.86 to 6.57 nl^{-1} g^{-1} h^{-1}. The results also revealed interaction effects between RFLP-MEL1 and RFLP-MEACS1 on ethylene production. The ethylene production rates can be sorted initially by the RFLP-MEACS1 genotypes and then by the RFLP-MEL1 genotypes. In other words, regardless of the RFLP-MEL1 genotypes, melons with the homozygous genotypes (*BB*) or (*CC*) of RFLP-MEACS1 had the lowest and highest ethylene production rates, respectively, with the heterozygous genotype (*BC*) being intermediate. Within a given genotype of RFLP-MEACS1, melons with the homozygous genotype (A_0A_0) or (B_0B_0) of RFLP-MEL1 had the lowest and highest ethylene production rates, respectively, with the heterozygous genotype A_0B_0 being intermediate.

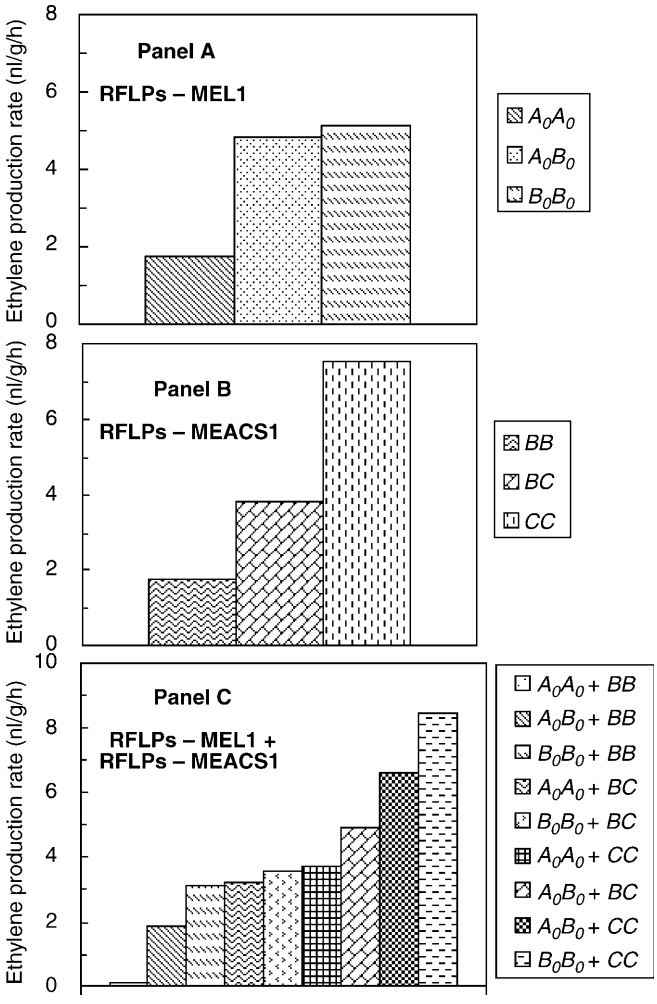

Fig. 3 Ethylene production rates (nl^{-1} g^{-1} h^{-1}) averaged over the RFLP-MEL1 genotypes (A_0A_0, A_0B_0, B_0B_0) (*Panel A*), over the RFLP-MEACS1 genotypes (*BB*, *BC*, *CC*) (*Panel B*) or the combined RFLP genotypes (*Panel C*) in the F_2 population. During plant growth, young leaves and growing tips were harvested for DNA extractions. The genomic DNAs were then used for the RFLP analyses. At horticultural maturity (fruit abscission or rind color change) melons were harvested and measured for ethylene production

Genetics of abscission characteristics

The phenotypes of fruit abscission were recorded during harvest at horticultural maturity for both trials. It was found that, for the F_2 population in the 1996 trial, the number of the NFS and the FS were 10 and 42, respectively, whereas in 1997, 7 out of 22 fruits were not-full-slip (NFS) and the remaining 15 were full-slip (FS). Together, there were 17 fruits with NFS and 57 fruits with FS, which fit closely into a ratio of 1:3, with χ^2 (1:3) = 0.16, *df* = 1, and *P* = 0.69. Although not all melon fruits with low ethylene production rates were NFS, all NFS melon fruits produced low or relatively low ethylene. In the BCP_2 population, the number of melons with NFS and FS were 12 and 14, respectively, which also fit the expected ratio of 1:1 (χ^2 = 0.15, *df* = 1, and *P* = 0.70). However, in the BCP_1 population, all 22 melon fruits were FS.

Discussion

The results from this study not only demonstrated the genetic inheritance of the RFLPs and confirmed our early hypothesis of their linkage to ethylene genes (Zheng and

Wolff 2000), but also mapped the RFLP-MEACS1 into a putative QTL of ethylene production in melon. The segregation of A_0 and B_0 fragments of the RFLP-MEL1 closely fit an expected ratio for two alleles from a single locus. Results from the single-copy-reconstruction assay provided strong evidence that the ACC oxidase gene exists as a single copy in the melon genome. Together, these data strongly support the concept that the RFLP-MEL1 fragments are ACC oxidase gene polymorphisms (J. Giovannoni, Department of Horticultural Sciences and Crop Biotechnology Center, Texas A&M University, College Station, USA, personal communication). The segregation analysis also suggests that the B and C fragments of the RFLP-MEACS1 are the two alleles (i.e., *B* and *C*) from a single locus. The evidence from the single-copy-reconstruction assay indicates the possibility that the ACC synthase gene used in this study was from a multigene family.

The linkage analysis of the RFLP-MEL1 revealed that melons with the heterozygous genotype A_0B_0 produced an ethylene rate between the median value of the homozygous genotypes (A_0A_0 and B_0B_0) and the high ethylene producing genotype B_0B_0 in the F_2 population, suggesting partial dominance of the B_0 allele over the A_0 allele. The data supported our earlier results generated from diverse melon genotypes (Zheng and Wolff 2000). In contrast, the linkage analysis of the RFLP-MEACS1 indicated that melons with the heterozygous genotype *BC* produced an ethylene rate close to the median value of the homozygous *BB* (low ethylene-producing genotype) and *CC* (high ethylene-producing genotype) in the F_2 population, suggesting that the RFLPs map to a QTL for ethylene production. The ethylene bioassays in the backcrossing populations (BCP_1 and BCP_2) also supported this hypothesis. This is not surprising because, in tomato, 32 independent loci were mapped to genes known or hypothesized to influence ripening and/or ethylene response. The ethylene response genes were reported to be involved in either ethylene biosynthesis or perception, while additional ripening-related genes included those involved in cell-wall metabolism and pigment biosynthesis (Giovannoni et al. 1999).

The linkage analysis also suggested that the RFLP-MEL1 and the RFLP-MEACS1 were not in the same linkage group. The former is an independent locus away from the complex ethylene production trait as revealed by the latter. When the ethylene production rate in the F_2 population was averaged over different genotypes combined from both the RFLP-MEL1 and the RFLP-MEACS1, melons with the high ethylene-producing genotype (*CC*) of the RFLP-MEACS1 always had the highest ethylene production rates, whereas melons with the low ethylene-producing genotype (*BB*) had the lowest ethylene production rates, suggesting the predominance of the RFLP-MEACS1 over the RFLP-MEL1 in determination of ethylene biosynthesis. This implied that ACC synthase was more important than ACC oxidase in its contribution to ethylene production, and supports the conclusion that ACC synthase is the rate-limiting enzyme in the last two steps of ethylene biosynthesis (Yang and Hoffman 1984; Zarembinski and Theologis 1994). Moreover, within the low ethylene production-linked homozygous genotype *BB* of RFLP-MEACS1, melons with the high ethylene production-linked genotype B_0B_0 of RFLP-MEL1 had a significantly higher ethylene production than those with the low ethylene production-linked genotype A_0A_0 These data further supported our previous conclusion (Zheng and Wolff 2000) 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) from a multigene family existed and encoded additional ACC synthase. The hypothesis that the ACC synthase gene is part of a multigene family was also indicated by the single-copy-reconstruction assay.

It should be pointed out that the ethylene production rates in F_1 melons varied noticeably when different pollen sources were used. This phenomenon is called xenia (Riley 1948). It has never been reported in melon. Further experiments are needed to confirm it. Differences in ethylene production in the same lines between years might be attributed to fluctuations of sunlight, temperature and other environmental factors beyond the control of this experiment. No effort to quantify these G × E interactions was undertaken.

Summary

The data of this investigation, together with our early report, were the first attempts towards elucidating the molecular genetics of ethylene production in melon, an additional climacteric fruit other than the model plant tomato (Giovannoni et al. 1999). It revealed strong evidence for linkage of RFLPs of the ACC oxidase gene and the ACC synthase gene to ethylene production in melon. More importantly, the RFLP-MEACS1 seems to map to the complex trait of ethylene biosynthesis. This will ultimately facilitate the effort toward fine mapping of the QTLs. The RFLP markers will be useful in marker-assisted selection to develop melons with low ethylene production for prolonging postharvest storage life. Finally, it was found that the ripening and ethylene production-related fruit development phenotype, fruit abscission, seemed to be controlled by an independent single locus with full-slip allele dominance over the non-full-slip allele.

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