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Identification and molecular mapping of loci controlling fruit ripening time in tomato

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Abstract Using RAPD marker analysis, two quantitative trait loci (QTLs) associated with earliness due to reduced fruit-ripening time (days from anthesis to ripening = DTR) were identified and mapped in an F_2 population derived from a cross between *Lycopersicon esculentum* 'E6203' (normal ripening) and *Lycopersicon esculentum* 'Early Cherry' (early ripening). One QTL, on chromosome 5, was associated with a reduction in both ripening time (5 days) and fruit weight (29.3%) and explained 15.8 and 13% of the total phenotypic variation for DTR and fruit weight, respectively. The other QTL, on chromosome 12, was primarily associated with a reduction only in ripening time (7 days) and explained 12.3% of the total phenotypic variation for DTR. The gene action at this QTL was found to be partially dominant (d/a=0.41). Together, these two QTLs explained 25.1% of the total phenotypic variation for DTR. Additionally, two QTLs associated with fruit weight were identified in the same F_2 population and mapped to chromosomes 4 and 6, respectively. Together, these two QTLs explained 30.9% of the total phenotypc variation for fruit weight. For all QTLs, the 'Early Cherry' alleles caused reductions in both ripening time and fruit weight. The polymorphic band for the most significant RAPD marker (OPAB-06), linked to the reduced ripening time QTL on chromosome 12, was converted to a cleaved amplified polymorphism (CAP) assay for marker-aided selection and further introgression of early ripening time (DTR) into cultivated tomato.

Key words QTL · Earliness · CAP · RAPD · *Lycopersicon esculentum*

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Introduction

Earliness is an economically important trait for both processing and fresh-market tomatoes. Early ripening is crucial for regions with short growing seasons. The trait is also desirable for taking advantage of high prices during the early season and for staggering tomato production throughout the growing season. In addition, earliness can reduce the heating and lighting expenses of greenhouse-grown tomatoes. Earliness is a quantitatively inherited trait (Peirce and Currence 1959) with significant additive, partial or complete dominance and epistatic gene effects (Poysa 1991). The expression of early maturity is also significantly affected by environmental factors such as temperature and light intensity (Kerr 1955).

The stages/components of earliness have been defined (Powers and Lyon 1941) and extensively studied (Corbeil and Butler 1965; Mihalska and Kubicki 1978; Gibrel et al. 1982). Based on these studies, the major determinants of earliness can be divided into four different components: (1) days from sowing/transplanting to the first flowering (anthesis), (2) days from anthesis to the first fruit set, (3) days from the first fruit set to the first ripe fruit, (4) days from the first ripe fruit to the end of ripening. Each of these components of earliness was shown to be a unique and heritable trait (Powers and Lyon 1941). Earliness due to an earlier switch from vegetative to reproductive growth, which is measured by determining the number of days from sowing to the appearance of the first ripe fruit (Kemple and Gardner 1992), is correlated with a decrease in fruit size presumably because plants do not have enough time and biomass to produce and support a full load of high-quality fruits (Khalf-Allah and Peirce 1963). As a result, it has been very difficult to develop cultivars that are early ripening and bear large fruit (Banerjee and Kalloo 1989).

In the 1980s, a novel form of earliness was identified in the open-pollinated variety 'Early Cherry', a cherry tomato line derived from crosses between *Lycopersicon esculentum* and *Lycopersicon pimpinellifolium* (M.

Mutschler, unpublished data). This type of earliness is the result of a decreased length of time between anthesis and the first ripe fruit. Kemple and Gardner (1992) reported that an inbred (Cornell 871213-1) derived from 'Early Cherry' produced ripe fruits 9 days earlier than a normal cherry inbred (NC21C-1) in the greenhouse and 17 days earlier than a large-fruited inbred (NC84173) in the field. Interestingly, this form of earliness was not accompanied by a decrease in fruit weight. Instead, 'Early Cherry' inbred lines were able to produce more and heavier fruits than the highest-yielding commercial cherry lines (M. Mutschler, unpublished data). However, the introgression of this new form of earliness into superior lines has been hampered because screening populations for earliness is very labor intensive. The plants must be synchronized for flowering and then carefully monitored for the first ripe fruit. In addition, this type of screening can result in false positives (identifying normal plants as being early). The development of a quick, cost-effective screen for earliness using molecular markers would greatly facilitate the incorporation of earliness into superior tomato lines.

A number of QTL studies involving interspecific crosses have been conducted in tomato and have allowed many of the loci controlling the different components of earliness to be identified (Weller et al. 1988; de Vicente et al. 1989; Lindhout et al. 1994; Grandillo and Tanksley 1996). Numerous QTLs for days to first flower have been identified in populations derived from crosses between *L. esculentum* and *L. pimpinellifolium* (Weller et al. 1988; Lindhout et al. 1994; Grandillo and Tanksley 1996). In most of these cases, alleles from the wild parent decreased the number of days to flowering. QTLs have also been identified for the number of days to first ripe fruit in populations derived from crosses between cultivated tomato and *L. pimpinellifolium* (Grandillo and Tanksley 1996; Doganlar et al., in preparation), *Lycopersicon cheesmanii* (Monforte et al. 1997) and *Lycopersicon peruvianum* (Fulton et al. 1997). Interestingly, most of the *L. pimpinellifolium* and *L. peruvianum* alleles at these loci decreased days to ripening, whereas the *L. cheesmanii* alleles increased the period of time between sowing to first ripe fruit.

The goals of the current research were to identify and map QTLs controlling early ripening (DTR = days from anthesis to ripening) and fruit weight (FW) in an F_2 population derived from *L. esculentum* 'E6203' × *L. esculentum* 'Early Cherry', and to develop a PCR-based assay for the early ripening QTLs.

Materials and methods

Plant materials

The source of the earliness in this study is the very early ripening line 'Early Cherry'.'Early Cherry' was developed from a cross of 'Valiant' × 'Farthest North', a determinate extremely early tomato variety with small fruit. 'Farthest North' was developed from a cross between an extreme early 'Red Currant' (*L. piminellifolium*) and a determinate type 'Bison' (*L. esculentum*) (Yaeger and Meander 1937). Since the *L. pimpinellifolium* parent of 'Farthest North' was chosen for its earliness, it is likely that the earliness of 'Early Cherry' originated from the *L. pimpinellifolium* progenitor. The open-pollinated variety 'Early Cherry' tomato was crossed to *L. esculentum* cv E6203, and the resulting F_1 was selfed to produce F_2 seed. A total of 104 F_2 progeny (referred to as EC-F_2) were evaluated for earliness. A sub population of 76 EC-F_2 plants was used for RAPD marker analysis as described below.

Sixty seven F_2 plants derived from the cross *L. esculentum* × *L. pennellii* (Tanksley et al. 1992) were used to map the polymorphic DNA fragments that were found to be linked to early ripening and fruit weight in the EC-F_2 population. Fifty introgression lines (ILs) (Eshed and Zamir 1995) were also used to confirm the map position of each significant marker linked to DTR and FW.

Phenotypic analysis

Earliness was measured by determining the number of days from anthesis to ripe fruit (DTR). The flowering of the plants was synchronized by waiting until nearly all plants had open flowers on the first and second clusters. At that time, all open and fading flowers and developing fruits were removed from all of the plants on a single day. The day after synchronization was considered day 1 for fruit development. The first ripe fruit dates were determined by monitoring the plants every other day starting at 30 days after synchronization until most of the plants had ripe fruit (around 58 days after synchronization). Fruit weight (FW) was evaluated by weighing ten representative fruits from each individual plant.

RAPD analysis

DNA samples from the early and late ripening parents were amplified using 530 random RAPD primers (Operon Technologies, California) to select those that generated polymorphic bands. DNA was isolated as described by Bernatzky and Tanksley (1986). PCR reactions and amplification conditions were performed as described by Martin et al. (1991) using a MJ Research PTC100 Programmable Thermal Controller. Amplification products were analysed by electrophoresis through 2% agarose followed by ethidium bromide staining. RAPD primers that gave polymorphic bands for the two parents were used to analyze a population of 76 F_2 plants.

Selected fragments were excised from the agarose gel and reamplified using the same primers and conditions. Subsequently, single-band RAPD products were purified and ligated into plasmid PCRII using the TA Cloning kit (Invitrogen) and then transformed into the *Escherichia coli* strain DH5α as recommended by the manufacturer (Stratagene).

Conversion of RAPD markers to cleaved amplified polymorphisms (CAPs)

Both ends of selected cloned genomic sequences (derived from RAPD analysis with markers OPAB-06, OPC-09, OPT-06, OPAH-19) were sequenced and PCR primer pairs were designed based on these sequences. CAPs were identified as described by Konieczny and Ausubel (1993). PCR reaction and amplification conditions were performed with a Perkin Elmer Cetus Thermocycler using the following profile: 94°C, 1 min; 55°C, 1 min; and 72°C, 2 min for 35 cycles followed by 72°C for 5 min and a hold at 4°C. Because the primers that were developed only amplified bands from either the *L. esculentum* or the *L. pennellii* parent of the F_2 mapping population, no restriction enzyme step was needed for use of these primers on the *L. esculentum* × *L. pennellii* F_2 mapping population (Tanksley et al. 1992). However, use of the CAP primer (OPAB-06 F/R) with the EC-F_2 population required restriction digestion with *Taq*I to generate polymorphic bands. Following PCR, the products were resolved on 2% agarose electrophoresis gels in 1× TAE buffer (40 mM Tris-acetate/1 mM EDTA, pH 8.0).

Molecular mapping of CAP and RAPD markers

The CAP primers (OPAB-06 F/R, OPC-09 F/R, OPT-06 F/R, OPAH-19 F/R) were used to amplify DNA from 67 plants of an *L. esculentum* × *L. pennellii* mapping population (Tanksley et al. 1992) in order to locate polymorphic bands on the tomato high-density linkage map (Pillen et al. 1996). Additionally, RAPD primers were used to amplify DNA from 50 plants of introgression lines (Eshed and Zamir 1995) in order to verify the location of polymorphic bands. PCR reaction and amplification conditions were as described above.

Linkage analysis

Linkage analysis and ordering of the RAPD loci were done using the MAPMAKER computer program (Landers et al. 1987). Chi-square goodness-of-fit tests were performed on putative loci by using the STATVIEW program (Abacus 1992) and those deviating from expected 3:1 segregation ratios at $P < 0.05$ were excluded from further analysis. Putative linkage groups were inititally determined by comparisons of pairwise combinations of markers using the group command for MAPMAKER with a minimum LOD linkage score for 3.0 for statistical acceptance and a maximum recombination fraction of 0.3. The most-likely order for the linkage group was tested with the ripple command. Kosambi's mapping function was used to estimate the map distance between markers (Kosambi 1944).

QTL analysis

Single-point regressions were used to determine the association between markers and each trait using the qGENE program (Nelson 1997). Significant ($P < 0.001$) differences in marker class means were interpreted as a likely linkage of DTR and/or FW to the marker locus. The percentage of the total phenotypic variation explained by QTLs identified for DTR and FW was estimated by the R^2 value. A multiple regression analysis was employed to determine the effects of two QTLs on total phenotypic variation for ripening time and fruit weight. Two-way analysis of variance was used to test for epistatic interactions between markers significantly associated with DTR and FW.

Results and discussion

Phenotypic analysis

The average DTR for 'Early Cherry' was 36 days, with a standard deviation of ±2.5 days, whereas the average DTR for 'E6203' was 56±6.9 days. Therefore, fruit development of 'Early Cherry' was approximately 20 days faster than that of 'E6203'. The mean DTRs of the F_1 and the EC-F_2 population were 43±4.5 and 42±6.3 days, respectively, and were skewed toward the mean DTR on the 'Early Cherry' variety. DTRs ranged from 31 to 54 days in the EC-F_2 population. A histogram of DTR in the EC-F_2 population (Fig. 1) showed a clear bimodal shape distribution, suggesting the segregation of at least one gene with major effects, rather than strict quantitatve control. Results from the phenotypic analysis of this material indicated that the gene action of DTR is partially dominant, although a high level of dominance for DTR was previously reported (Kemple and Gardner 1992). A positive correlation was also found between FW and DTR ($r = 0.44$, $P < 0.01$), suggesting that increasing

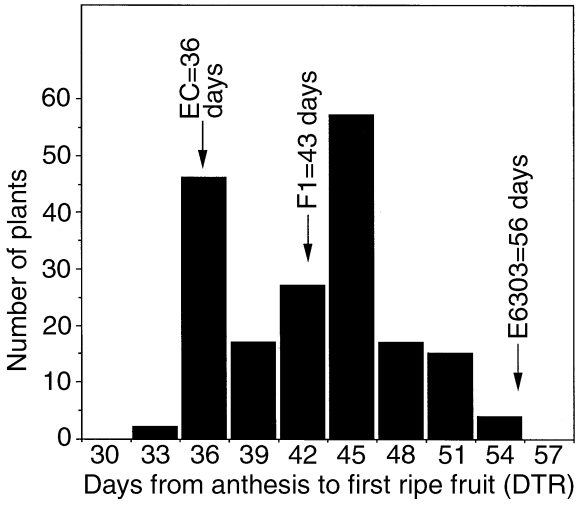

Fig. 1 Frequency distribution of 104 F_2 plants for days from anthesis to the first ripe fruit (DTR). The F_2 generation displayed a bimodal shape distribution. Mean DTRs of the early and late parents are 36 and 56 days, respectively

DTR is associated with larger fruits and indicating that some common gene(s) may control both traits. Similar results have been found in earlier studies (Banerjee and Kalloo 1989; Kemple and Gardner 1992).

RAPD analysis

DNA from both the early ('Early Cherry') and late ('E6203') parents were screened with a total of 530 RAPD primers. Of these, 480 primers yielded a total of 2440 scorable bands, corresponding to an average of 5.9 fragments (1–13 bands) per primer. A total of 102 primers revealed polymorphism between the parents. However, when DNA from the EC-F_2 plants was analyzed, only 42 primers yielded clear segregation for polymorphic bands. These 42 primers generated a total of 45 segregating bands (Table 1) and showed the expected (3:1) presence/absence segregation ratio. These primers were used to construct a linkage map for this population and for QTL analysis. A total of six putative linkage groups (Table 1) were formed by 35 polymorphic RAPD bands (data not shown) with a LOD score greater than 3 using the group command of the MAPMAKER program. An additional ten primers segregated independently of any other primers.

QTL analysis

Based on the marker-trait analysis, two significant QTLs ($P < 0.001$) associated with DTR were identified in the EC-F_2 population by two different clusters of RAPD primers (linkage groups A and F, Table 1), represented by bands amplified by primers OPC-09 and OPAB-06. One QTL (identified with OPC-09) was associated with

Table 1 Polymorphic RAPD markers used for QTL analysis and map construction

Operon primers	Primer sequences	Molecular weight of segregating fragment (bp)	Linkage group	Chr.	Traits[a]	Proposed QTL name
OPC-09	5′-ctcaccgtcc-3′	1200	A	5	FW/ER	*er5.1, fw5.1*
OPAK-05	5′gatggcagtc-3′	1600	A	5	FW	
OPAA-12.A	5′-ggacctcttg-3′	1250	A	5	ns	
OPJ-13.A	5′-ccacactacc-3′	1250	A	5	ns	
OPJ-13.B	5′-ccacactacc-3′	1100	A	5	ns	
OPJ-04	5′-ccgaacacgg-3′	350	A	5	ns	
OPG-16	5′-agcgtcctcc-3′	700	B	?	ns	
OPAM-02	5′-acttgacggg-3′	650	B	?	ns	
OPAH-19	5′-ggcagttctc-3′	1100	C	4	FW	*fw4.1*
OPW-13	5′cacagcgaca-3′	900	C	4	ns	
OPZ-18	5′-agggtctgtg-3′	1630	C	4	ns	
OPAJ-11.A	5′gaacgctgcc-3′	1100	C	4	FW	
OPAJ-11.B	5′-gaacgctgcc-3′	850	C	4	FW	
OPAL-13	5′-gaatggcacc-3′	1200	C	4	FW	
OPK-12	5′-tggccctcac-3′	600	C	4	FW	
OPAA-12.B	5′-ggacctcttg-3′	350	C	4	FW	
OPB-16	5′-tttgcccgga-3′	1150	C	4	FW	
OPB-18	5′ccacagcagt-3′	900	C	4	ns	
OPH-01	5′-ggtcggagaa-3′	1550	C	4	FW	
OPU-03	5′-ctatgccgac-3′	1400	C	4	FW	
OPM-04	5′-ggcggttgtc-3′	1450	C	4	FW	
OPAM-07	5′-aaccgcggca-3′	650	C	4	ns	
OPAD-02	5′-ctgaaccgct-3′	700	C	4	ns	
OPT-06	5′-caagggcaga-3′	500	D	6	FW	*fw6.1*
OPY-16	5′-gggccaatgt-3′	750	D	6	ns	
OPU-08	5′-ggcgaaggtt-3′	1950	D	6	FW	
OPB-12	5′-ccttgacgca-3′	500	D	6	FW	
OPAD-19	5′-tcgcttctcc-3′	800	D	6	ns	
OPAK-08	5′-ccgaagggtg-3′	850	D	6	FW	
OPAN-20	5′-gagtcctcac-3′	1650	E	?	ns	
OPB-08	5′-gtccacacgg-3′	1600	E	?	ns	
OPG-02	5′-ggcactgagg-3′	1200	E	?	ns	
OPAK-04	5′-agggtcggtc-3′	250	E	?	ns	
OPAB-06	5′-gtggcttgga-3′	1650	F	12	ER	*er12.1*
OPS-14	5′-aaaggggtcc-3′	1400	F	12	ns	
OPE-09	5′-cttcacccga-3′	650	*	?	ns	
OPAJ-08	5′-gtgctccctc-3′	900	*	?	ns	
OPAL-01	5′tgtgacgagg-3′	850	*	?	ns	
OPI-06	5′-aaggcggcag-3′	1650	*	?	ns	
OPV-17	5′-accggcttgt-3′	800	*	?	FW	
OPAB-01	5‘-ccgtcggtag-3′	700	*	?	ns	
OPAL-09	5′-cagcgagtag-3′	1000	*	?	ns	
OPAC-13	5′-gacccgattg-3′	1800	*	?	ns	
OPZ-19	5′-gtgcgagcaa-3′	1200	*	?	ns	
OPA-02	5′-tgccgagctg-3′	500	*		ns	

[a] Trait for which markers showed a significant association ($P < 0.01$)
(*) Independently segregating markers; (?) not mapped, (ns) not significantly associated with any trait

a reduction in both DTR and FW and explained 15.8 and 13% of the total phenotype variations for each trait. Plants homozygous for the ‘Early Cherry‘ allele at this QTL showed 5-day and 29.3% reductions in DTR and FW, respectively. The other QTL (detected by OPAB-06) was primarily associated with DTR and explained 12.3% the total phenotypic variation for this trait. Plants homozygous for the ‘Early Cherry‘ allele at this QTL showed a 7-day reduction in DTR. As determined by multiple regression analysis, jointly, these two QTLs explained 25.1% of the total phenotypic variation for DTR. Additionally, two significant QTLs ($P < 0.001$) associated with only FW were identified in the same F_2 population in two additional linkage groups (C and D) of primers (Table 1). These two FW-related QTLs were detected by OPAH-19 and OPT-06 and explained 22 and 13.7% of the total phenotypic variation for FW in the EC-F_2 population, respectively. Together, these two QTLs explained 30.9% of the total phenotypic variation for FW. For all QTLs, the ‘Early Cherry‘ alleles caused reductions in both DTR and FW. Two-way ANOVA tests for epistasis between these significant marker loci revealed no significant epistatic interactions for either DTR or FW.

Chromosome mapping of polymorphic RAPD bands

The four most-significant polymorphic RAPD fragments that were found to be associated with ripening time and fruit weight QTLs were subsequently cloned and mapped as CAPs (OPAB-06, OPC-09 for DTR; OPAH-19 for FW) and an RFLP (OPT-06 for FW) on the toma-

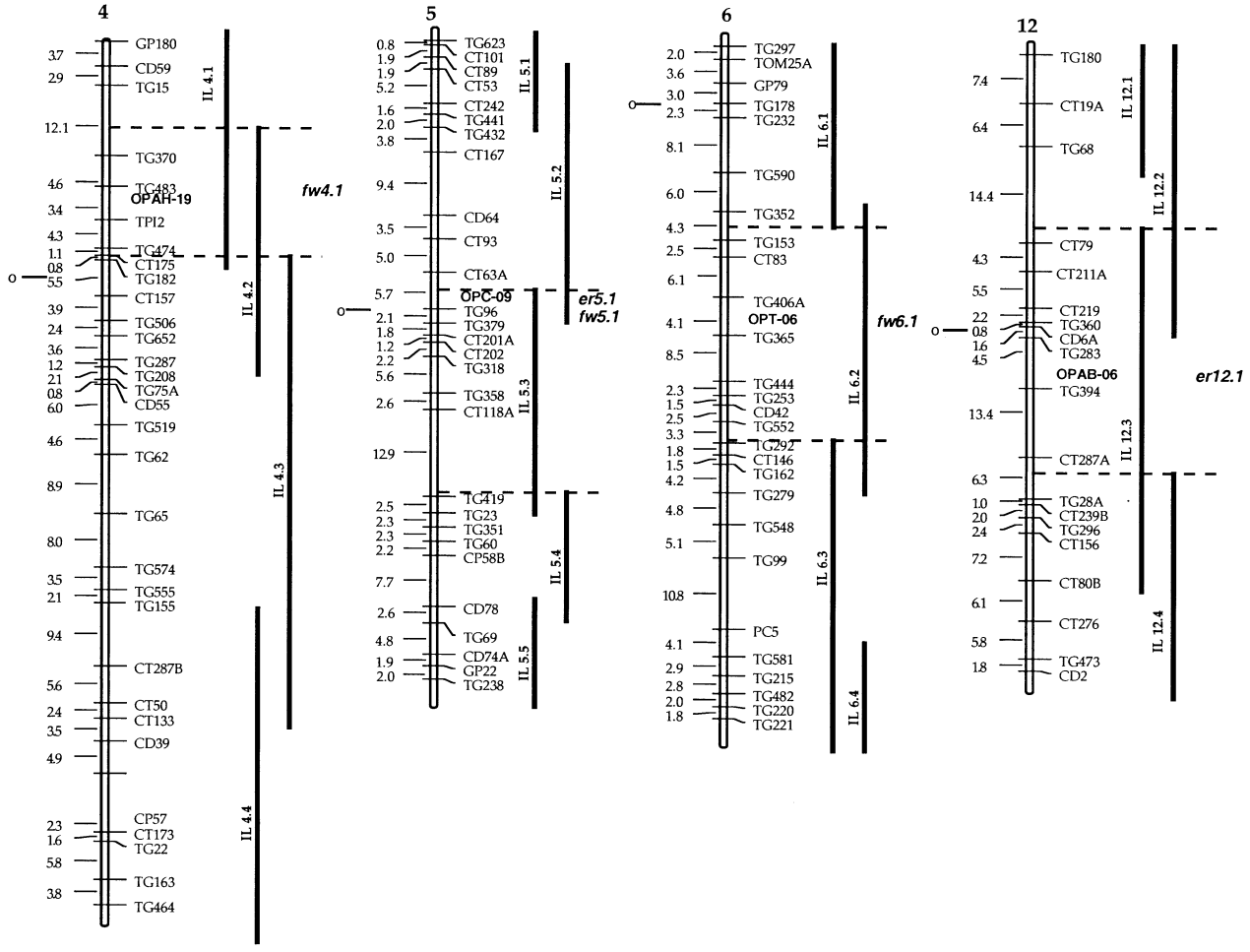

Fig. 2 Linkage map of tomato chromosomes 4, 5, 6, and 12 (Tanksley et al. 1992) showing positions of the *fw4.1*, *fw6.1*, *er5.1*, *fw5.1* and *er12.1* genes; RAPD markers that are linked to FW and DTR are shown in *bold*; *bars* to the right side of the chromosomes indicate the introgressed region in the ILs (Eshed and Zamir 1995); *o* = centromeric region

Table 2 The *er12.1* CAP assay. Primer designation, sequences, molecular size of the PCR product and the restriction enzyme that cleaves the PCR product

OP number	Primer designation	Primer sequences	Genomic PCR product size (bp)	Enzyme
OPAB-06	OPAB-06 F/R	5′-d[GCTTGGAGAGGATGTATAGGC]-3′5′-d[CCCATAAACTACTCACAGAC]-3′	1000	*Taq*I[a]
OPC-09	OPC-09 F/R	5′-d[AAAGAGATGGTGGCAGGAG]-3′5′-d[TCCAACGGCTAATTCTTCG]-3′	1300	nf[b]
OPT-06	OPT-06 F/R	5′-d[TGACGATCTGCTGAAGCTGC]-3′5′-d[CCGTCTTTTGCTTTTGTGTG]-3′	500	nf
OPAH-19	OPAH-19 F/R	5′-d[ACAGCCAGTGTTGTGGATGC]-3′5′-d[GTGGAGTAGCAAAGTTGTCG]-3′	700	nf

[a] Restriction enzyme step (*Taq*I) was required to detected polymorphisms in the EC-F_2 population
[b] nf = no restriction enzyme was found to detect polymorphisms in the mapping population (Tanksley et al. 1992)

to high-density linkage map (Pillen et al. 1996). In most instances, the cloned RAPD bands hybridized to a few or multiple-copy fragments and, therefore, could not be used for RFLP markers. As an alternative, polymorphic RAPD bands were converted into a cleaved amplified polymorphism (CAP) as described by Konieczny and Ausubel (1993). For this, the cloned RAPD bands were end-sequenced and primer pairs (OPAB-06 F/R, OPC-09 F/R OPAH-19 F/R and OPT-06 F/R) were designed (Table 2).

Using the CAP primer pairs, DNA from the 67 individuals of the F_2 mapping population (Tanksley et al. 1992) was amplified. PCR primer pairs OPAB-06 F/R, OPC-09 F/R and OPAH-19 F/R amplified 1000-bp, 1200-bp and 800-bp products, respectively, in the *L. esculentum* parent. None of these primers amplified DNA

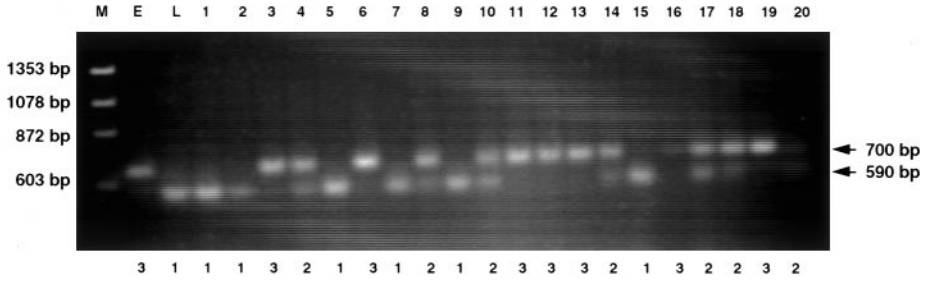

Fig. 3 Segregation of the co-dominant CAP marker OPAB-06 F/R in 20 plants selected from the F_2 population derived from the cross between *L. esculentum* cv E6203 × *L. esculentum* cv Early Cherry. When amplified DNA was digested with the restriction enzyme *Taq*I, the early parent yielded a 700-bp fragment and the late parent produced a 590-bp fragment. *M* = molecular weight standard; *E* = early parent (Early Cherry); *L*=late parnt (E6203); *1—20* F_2 segregating population. *3* = horn E/E; *2* = het E/L; *1* = hom L/L

of *L. pennellii*. The polymorphisms were scored as presence/absence and mapped using the MAPMAKER program.

The DTR-associated CAP marker OPAB-06 was mapped to the long arm of chromosome 12 between TG283 and TG394 (Fig. 3). This QTL was referred to as *er12.1*. The other DTR-related CAP marker OPC-09 was mapped to the short arm of chromosome 5 between TG96 and CT63 close to the *rin* gene (Fig. 2). This QTL was referred to as *er.51* and *fw5.1*. The FW-related CAP marker OPAH-19 was associated with fruit weight and was mapped to the short arm of chromosome 4 between TG483 and TG474 (Fig. 2). This QTL was referred to as *fw4.1*. The other FW-related CAP marker (OPT-06) amplified a 600-bp product in both parents but none of the 30 restriction enzymes tested gave a polymorphism. This marker was eventually mapped as a RFLP. One copy of this marker mapped to the long arm of chromosome 6 between TG406A and TG365 (Fig. 2). This QTL was referred to as *fw6.1*. The map position of each polymorphic RAPD band (OPAB-06, OPAH-19, OPC-09 and OPT-06) was further verified by locating these bands on overlapping introgression lines (Eshed and Zamir 1995). DNA from 50 ILs was amplified with each of these RAPD primers. Primer OPAB-06 amplified the DNA of IL12-2 and IL12-3, but no amplification was obtained from IL12-1 and IL12-4. Thus, we concluded that OPAB-06 maped between CT79 and TG565. This result verified the map position of OPAB-06 that had been previously determined with the CAP marker. Similarly, OPAH-19 amplified the DNA of IL4-2 and IL4-3, suggesting that OPAH-19 mapped on chromosome 4 between TG182 and TG155 confirming the results from the CAP marker. OPT-06 amplified DNA of only IL6-1, which spans the interval from TG612 to TG352. This was consistent with the results obtained with OPT-6 was used as a RFLP marker as one of the hybridizing fragments mapped to the same section of chromosome 6 (Fig. 2).

To develop a CAP assay which could differentiate between ‘Early Cherry‘ and ‘E6203‘ alleles for screening populations for early ripening and for introgression of the trait (DTR) into cultivars, we chose OPAB-06 because the other DTP-related marker (OPC-09) also showed a highly significant association with FW. Table 2 summarizes the PCR conditions for the *er12.1* assay which is able to differentiate the ‘Early Cherry‘ and ‘E6203‘ alleles. When used to amplify DNA from the ‘Early Cherry‘ and ‘E6203‘ parents, OPAB-06 F/R yielded a 1000-bp product. The restriction enzyme *Taq*I digested the PCR product of both parents and gave 700-bp and 590-bp fragments for the ‘Early Cherry‘ and ‘E6203‘ alleles, respectively (Fig. 3). This CAP assay cosegregated with early ripening (DTR) ($P < 0.002$), but not fruit weight ($P > 0.06$), in the EC-F_2 population. DTR for plants with the ‘Early Cherry‘ and ‘E6203‘ alleles in the homozygous state as determined by the OPAB-06 CAP marker were 39 days and 46 days, respectively. Therefore, the plants with the ‘Early Cherry‘ alleles ripened 7 days earlier than those with ‘E6203‘ alleles. The FW for plants with the ‘Early Cherry‘ and ‘E6203‘ alleles in the homozyous state, as determined by the OPAB-06 marker, were 32 g and 44 g, respectively. This was not a statistically significant difference in fruit weight ($P > 0.06$). The degree of dominance (d/a=0.41) for this CAP marker (OPAB-06 F/R) indicated that this locus is partially by dominant. Together these two data indicated that the assay is useful for detecting the earliness gene.

Earliness in tomato is a very desirable trait but is very difficult to breed for because of the slow and tedious progress of phenotypic selection. However, this process can now be expediated by the use of a marker-assisted approach for selection of the ‘Early Cherry‘ allele at *er12.1*, which reduces ripening time without significantly reducing fruit size. For this purpose, RAPD markers linked to early ripening were identified and one marker was subsequently converted into a co-dominant CAP assay for further introgression of earliness into superior tomato lines. Eventual cloning of *er12.1* will shed light on the molecular controls of the ripening process.

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