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Genetic mapping and QTL analysis of horticultural traits in cucumber (*Cucumis sativus* L.) using recombinant inbred lines

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Abstract A set of 171 recombinant inbred lines (RIL) were developed from a narrow cross in cucumber (*Cucumis sativus* L.; 2n = 2*x* = 14) using the determinate (*de*), gynoecious (*F*), standard-sized leaf line G421 and the indeterminate, monoecious, little-leaf (*ll*) line H-19. A 131-point genetic map was constructed using these RILs and 216 F_2 individuals to include 14 SSRs, 24 SCARs, 27 AFLPs, 62 RAPDs, 1 SNP, and three economically important morphological [*F* (gynoecy), *de* (determinate habit), *ll* (little leaf)] markers. Seven linkage groups spanned 706 cM with a mean marker interval of 5.6 cM. The location of *F* and *de* was defined by genetic linkage and quantitative trait locus (QTL) analysis to be associated with SSR loci CSWCT28 and CSWCTT14 at 5.0 cM and 0.8 cM, respectively. RIL-based QTL analysis of the number of lateral branches in three environments revealed four location-independent factors that cumulatively explained 42% of the observed phenotypic variation. QTLs conditioning lateral branching (mlb1.1), fruit length/diameter ratio (ldr1.2) and sex expression (sex1.2) were associated with *de*. Sex expression was influenced by three genomic regions corresponding to *F* and *de* both on linkage Group 1, and a third locus (sex6.1) on linkage Group 6. QTLs conditioning the number of fruit per plant (fpl1.2), the number of lateral branches (mlb1.4) and fruit length/diameter ratio (ldr1.3) were associated with *ll*. The potential value of these marker-trait associations (i.e., yield components) for plant improvement is portended by the relatively high LOD scores (2.6 to 13.0) and associated R^2 values (1.5% to 32.4%) that are affiliated with comparatively few genetic factors (perhaps 3 to 10).

Keywords Linkage Analysis · Yield Components · Linkage Map · Composite Interval Mapping · Pleiotropic Effects

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Introduction

A moderately saturated genetic map that defines major genes and quantitative trait loci (QTLs) is a prerequisite for implementing molecular marker technologies for selection and species synteny comparisons. The dissection of quantitative traits into their component Mendelian factors is of particular importance for genetic analysis. Recent technological advances in biotechnology and increased computational speed have facilitated decisive detection of economically important QTLs in several crop species to include organoleptic quality in tomato (*Lycopersicon esculentum* L.) (Saliba-Colombani et al. 2001), resistance to bacterial leaf streak (*Xanthomonas oryzae* pv *oryzicola*) in rice (*Oryza sativa* L.) (Tang et al. 2000), fruit texture in apple (*Malus* × *domestica* Borkh.) (King et al. 2001) and malting quality in barley (*Hordeum vulgare* L.) (Marquez-Cedillo et al. 2000). The detection and characterization of QTLs has traditionally been performed using backcross or F_2/F_3 populations (Austin and Lee 1996; Fulton et al. 1997; Serquen et al. 1997a). In these cross-progeny types, linkage disequilibrium (caused by physical linkage between loci) is used to detect and map polygenic traits (Tanksley 1993).

In species where inbreeding is possible (e.g., cucumber), mapping populations can be derived by self-polli-

nating F_2 progeny to yield lines that are essentially homozygous at all loci [i.e., recombinant inbred lines (>F_8) or RILs]. RILs have been successfully employed for mapping quantitative traits (Carrillo et al. 1990; Goldman et al. 1995; Paran et al. 1995, 1997) and offer several advantages over other mapping populations for detecting QTLs (Burr et al. 1988; Cowen 1988; Simpson 1989; Reiter et al. 1992). Although codominant markers are preferred over dominant markers for genetic mapping using F_2 plants, dominant and codominant marker systems provide equivalent information in RIL analysis (Reiter et al. 1992; Staub et al. 1996a). Moreover, RILs allow for replication and evaluation over space and time, reducing experimental error (Knapp and Bridges 1990), and increasing the precision and accuracy for quantification of genetic × environment interactions (Mansur et al. 1996).

The estimation of QTLs is, however, often cross specific (i.e., only segregating factors are detected), subject to environmental and sampling effects (Beavis 1998), potentially biased (Melchinger et al. 1998), and subject to inaccurate placement (Utz et al. 2000). Despite these potential detriments, the validation and potential utility of QTL information is being clarified by comparative analyses of marker-assisted (MAS) and phenotypic (PS) selection. For instance, MAS of metric traits has been shown to be as effective as phenotypic selection in barley (Romagosa et al. 1999), sweet corn (*Zea mays* L.) (Yousef and Juvik 2001), and cucumber (*Cucumis sativus* L.) (Fazio 2001; Fazio and Staub 2003).

Improved cultural practices, the accumulation of specific yield genes, disease resistances and gynoecy in elite breeding lines have been the source of yield-increases in cucumber hybrids (Cargill 1962; Peterson 1960, 1978; personal communication, T. C. Wehner, Raleigh, North Carolina, 1996). These significant factors have contributed to early yield and yield stability in cucumber. However, the yield (MT/ha) in U.S. processing cucumber reached a plateau in the 1980s, and has remained generally unchanged during the 1990s (USDA-NASS 1999). Incorporating such plant habit genes as a determinate (*de*) character and the quantitatively inherited multiple lateral-branching trait will most likely be a source of future yield improvements in processing cucumber (Serquen et al. 1997b; Cramer and Wehner 2000). Thus, understanding these and other factors underlying yield in cucumber is crucial for the development of cultivars that will eclipse this plateau (Staub et al. 1996a). The F_2:F_3 RAPD analysis and mapping of yield components in cucumber by Serquen et al. (1997a) resulted in the identification of QTLs describing mainstem length, days to anthesis, sex expression, multiple lateral branching, fruit number and weight, and length/diameter ratio. However, the presence and strength of QTLs were underestimated by these RAPD marker loci (Serquen and Staub 1996). The recent development of codominant SSR (Katzir et al. 1996; Danin Poleg et al. 2000, 2001; Fazio and Staub 2000), AFLP (Bradeen et al. 2001), as well as SCAR and SNP markers (Fazio et al. 2002) should allow for increased map saturation and more precise QTL estimation in cucumber. Therefore, a study was designed to employ these markers in the analysis of a unique array of RILs derived from F_2 progeny used by Serquen et al. (1997a) to characterize more completely yield component-associated QTLs in cucumber.

Materials and methods

Germplasm and population development

The monoecious, indeterminate, little-leaf (40 cm^2) line H-19 (University of Arkansas-Fayetteville 1993) was crossed with the gynoecious, determinate cucumber experimental line G421 (breeding line GY7; University of Wisconsin-Madison 1997) possessing standard-sized leaves. H-19 plants normally have 5 to 15 primary lateral branches depending on the environment, and possess a sequential fruiting habit (i.e., several fruits enlarge on a branch). In contrast, G421 has relatively few branches (1 to 3), and exhibits a strong crown set and sequential fruit inhibition (Fuller and Leopold 1977). An F_1 plant resulting from a G421 × H-19 mating was self-pollinated to produce 250 F_2 progeny which were then self-pollinated by single-seed descent to obtain 171 F_2S_6 recombinant inbred lines (RILs). The sex expression of plants exhibiting the gynoecious character during the formation of RILs was modified by treatment with silver thiosulfate (Beyer 1976) to allow for self-pollination. The RILs and 216 F_2 individuals were used in map construction and the assessment of linkage information.

Marker analysis

Four leaves (<1 cm^2) from 2-week-old RILs (171), F_2 (216), F_1 and parental seedlings were collected and introduced in a unique well-position of a 0.5-ml polypropylene microtiter plate (Fisher Scientific, Pittsburgh, Pa.). The tissue was then immediately lyophilized in the preparation of DNA extraction using the Aquapure genomic DNA extraction kit (Bio-Rad Laboratories, Hecules, Calif.) methodology.

RAPD and SCAR markers from previous mapping studies (Serquen et al. 1997a; Horejsi et al. 1999, 2000) and more recently developed SSR, SCAR and SNP markers (Fazio 2001; Fazio et al. 2002) were selected for genome analysis (fragment sizes and sequences are delineated therein). The markers used segregate in a Mendelian manner. The SSR markers employed were codominant (Fazio et al. 2002) and the remaining markers acted in a dominant fashion (Serquen et al. 1997a; Horejsi et al. 2000). In addition, DNA of H-19 and G421 were used in an annealing temperature gradient PCR (ATG-PCR) to evaluate currently available *Cucumis* SSR markers to identify polymorphisms specific to the mapping population (Katzir et al. 1996; Danin Poleg et al. 2000, 2001; Fazio 2001). Those that were polymorphic and reliable were used in the genomic analysis described herein.

PCR reactions for RAPD, SCAR, SNP and SSR markers were performed in 15-μl volumes of a uniform reaction mixture [3 mM of MgCl, 0.2 mM of dNTPs, 15–20 ng of DNA, 0.4 μM of each primer, *Taq* polymerase and commercial buffer (Promega, Madison, Wis.)] incorporating 10 μl of a light-weight mineral oil overlay (Fisher Scientific, Pittsburg, Pa.). The PCR reactions were resolved in 1.6% agarose (3% for SSR) (Life Technologies, Gaithersburg, Md.) by staining with ethidium bromide (0.5 μg/ml). Gels were visualized with the Dark Reader trans illuminator (Clare Chemical Inc., Denver, Colo.) and a closed circuit digital (CCD) camera.

Five *Eco*RI/*Mse*I primer combinations (E11M60, E14M62, E13M50, EACMCAC, ETGMCAA) exhibiting dispersed, region-specific polymorphism in the cucumber genome were selected from a previously developed AFLP-based map (Bradeen et al. 2001). AFLP methodology was applied to 171 RILs, parents and the F_1 for

genotyping according to the protocol outlined in the manufacturer's AFLP Analysis System II and the AFLP Small Genome Primer Kit (Life Technologies, Rockville, Md.).

Field evaluation

Recombinant inbred lines, parents and F_1 were evaluated in two locations (University of Wisconsin Experiment Station, Hancock, Wisconsin, and Brigham Young University Agricultural Experiment Station, Spanish Fork, Utah) in 1999, and in one location (Hancock, Wis.) in 2000. These test environments are hereafter referred to by location, abbreviation and year (i.e., WI 1999, UT 1999, and WI 2000). RILs were arranged in a randomized complete block design with three replications per location. Each replication had 12 plants and consisted of single rows with plants spaced 13-cm apart in rows, to include edge borders positioned on 1.5-m centers corresponding to a plant density of approximately 51,000 plants/ha.

Characters examined

Data were collected on plant habit, days to anthesis, sex expression, leaf type, number of lateral branches originating from the mainstem, and fruit number and fruit length/diameter ratio in WI 1999 and WI 2000. Lateral branch-number data were collected in UT 1999. Data were collected in 1999 and 2000 unless specified as follows.

(1) Determinate (*de*) plant habit was determined by classifying plants having between 7 to 10 nodes. Plants that possessed short vines ($\leq$0.6 m) with the main stem terminating in a flower cluster were classified as determinate (Pierce and Whener 1990).

(2) Days to anthesis were recorded on individual plots as the number of days from planting to the time the corolla of at least three fully expanded flowers were present on 50% of the plants in each plot.

(3) Two sex-expression assessments were made (WI 1999 only). Sex expression was recorded as the number of pistillate nodes in ten nodes of the mainstem starting with the first flowering node in each of three consecutive plants in a plot in all replications. Plants were also classified by recording the number of female nodes in the first ten nodes of primary lateral branches in each of three plants per plot.

(4) Plants were classified as little (30 to 40 cm^2) or standard (80 to 100 cm^2) leaf types by visual examination at least 1 week before first harvest (Staub et al. 1992).

(5) The total number of primary lateral branches (25 cm or longer) was recorded for each plant within a plot at or before anthesis.

(6) Fruit number per plant was obtained in WI 1999 and WI 2000 in each of three harvests. In WI 1999 fruit was harvested on an individual plot basis at defined intervals. In WI 2000, plots were harvested 49, 58 and 69 days after sowing. Determination of harvest interval was similar in both years. The first harvest interval of each plot occurred, as two to three fruits >51-mm in diameter (oversized) were observed within a plot (Wehner 1989), such that all immature fruits >20 mm in diameter were taken for analysis. The second harvest interval occurred (before 5 days after the first harvest) when three mature fruits were observed to be oversized within a plot. A third harvest (at least 11 days after the second harvest) was performed by counting all fruits greater than 20 mm in diameter.

(7) After each harvest in Wisconsin 1999 and 2000, five commercially mature fruits (2B–3A grade; 25 to 30 mm in diameter) from each plot were selected randomly and their lengths and diameters averaged. The mean fruit length:diameter ratio (L:D) for each plot at each harvest was calculated.

Linkage analysis

Map construction

Genotype data for 171 RILs and 216 F_2 plants were used to perform linkage analyses using Joinmap 3.0 software for Windows (Van Ooijen and Voorrips 2001). A minimum LOD of 3.5 was set as a threshold to religate marker loci into linkage groups, to order markers, and to estimate interval distances (Kosambi function). A graphic representation of the linkage groups was created using MapChart software for Windows (Voorrips 2001).

Segregating but previously unmapped markers (Fazio 2001; Fazio et al. 2002) were placed on the map in relation to more recently published maps (Fig. 1). The Roman numerals and letters under the linkage-group heading correspond to linkage groups identified by Serquen et al. (1997a) and Bradeen et al. (2000), respectively. RAPDs are identified by OP and BC according to Serquen et al. (1997a), SSR by CS, CM and NR, AFLP by E_M_, and SCARs by the designation SCAR (Fazio 2001; Fazio et al. 2002). Morphological markers are in italic.

QTL analysis

Least-square means for traits were calculated by location and obtained using the mixed-models procedure (PROC MIXED) in SAS (Littell et al. 1996). RILs were considered fixed effects, while blocks and interactions were considered as random effects. Trait distributions were examined for fit to normality using the QSTATS function of QTL Cartographer 1.21 for Windows (Basten et al. 2001).

Morphological marker data for the *F* and *de* loci were not employed in the genetic map used for QTL analysis. Their effect(s) was measured and analyzed as quantitative characters, and are therefore not suitable for inclusion in the linkage analysis of this RIL population. Composite interval mapping (CIM) was performed using Model 6 of QTL Cartographer (version 1.21) with a walking speed of 1 cM, a window size parameter of 3 cM, and the inclusion of 15 maximum background marker loci in a stepwise forward-regression procedure. The inclusion of background marker loci allowed for greater sensitivity in QTL detection and a conservative estimation of their effects (R^2). The significance of each QTL interval was tested by a likelihood-ratio statistic (LOD). The LOD threshold for significance at $p = 0.05$ was determined using 1,000 permutations.

Results

Linkage map development

The addition of 28 previously unmapped markers (14 SSRs, 4 SCARs, 1 SNP, 4 RAPDs and 5 AFLPs) to 103 markers selected from the published G421 × H19-based cucumber maps (Serquen et al. 1997a; Bradeen et al. 2001) resulted in a genetic map with seven linkage groups (LG 1–7; Fig. 1). This map spans 706 cM consisting of 131 markers (14 SSRs, 24 SCARs, 27 AFLPs, 62 RAPDs, 1 SNP, and three morphological markers). While the mean marker interval was 5.6 cM, the largest interval between any two markers was 29.5 cM (ETGMCAA-205 and OP-AI4; LG 5). Two relatively small linkage groups were identified; LG 3 spanning 16 cM with an average interval distance of 1.5 cM among 11 markers, and LG 7 spanning 30.6 cM with an average interval distance among nine markers of 3.3 cM. Five linkage groups designated as 1, 2, 4, 5 and 7 spanned 121, 133, 152, 117

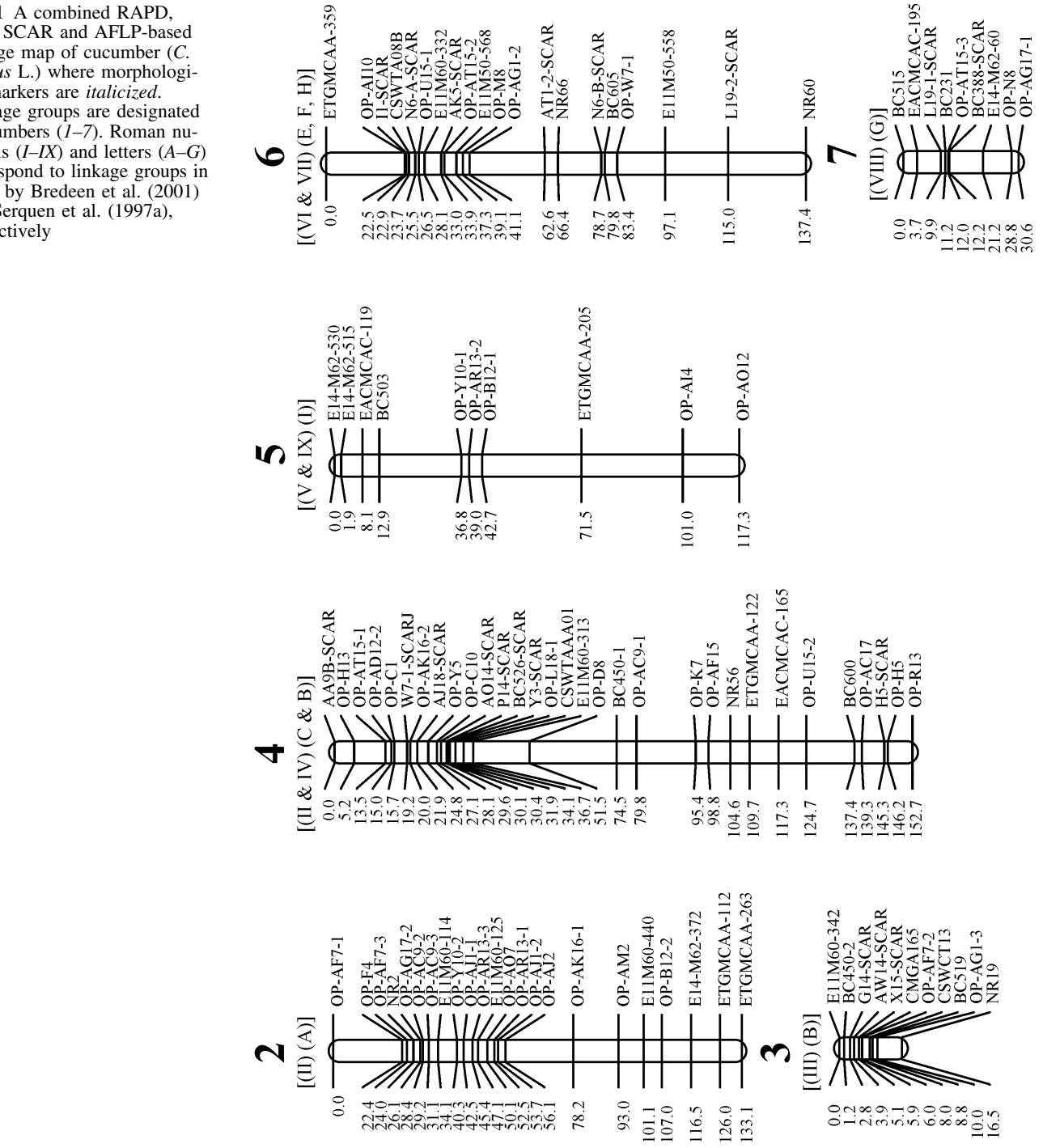

Fig. 1 A combined RAPD, SSR, SCAR and AFLP-based linkage map of cucumber (*C. sativus* L.) where morphological markers are *italicized*. Linkage groups are designated by numbers (*1–7*). Roman numerals (*I–IX*) and letters (*A–G*) correspond to linkage groups in maps by Bredeen et al. (2001) and Serquen et al. (1997a), respectively

Table 1 Position on linkage group (LG), log of likelihood ratio (LOD), percentage of the phenotypic variation explained (R^2), and effect (calculated by QTL Cartographer version 1.21) of QTLs associated with days to anthesis, lateral branch number and mean length/diameter ratio in cucumber in three locations (Wisconsin and Utah)

Trait/QTL	LG marker	Position	WI 1999			WI 2000			UT 1999		
			LOD	Effect	R^2	LOD	Effect	R^2	LOD	Effect	R^2
Days to anthesis											
ant1.1	1 *ll*	79.3	3.0	–0.60	6.8%	3.5	0.82	6.4%			
ant2.1	2 E11M60-114	33.8	nd	nd	nd	2.7	–0.66	4.4%			
ant5.1	5 ETGMCAA-205	85.1	2.6	0.74	14.2%	nd	nd	nd			
ant6.1	6 AT1-2-SCAR	62.3	2.7	–0.54	5.4%	4.7	–0.83	8.2%			
Lateral branch number											
mlb1.1	1 E14M62-273	25.7	11.6	0.36	9.1%	8.2	0.24	10.6%	6.8	0.33	11.5%
mlb1.2	1 CSWCT16	40.9	3.7	–0.19	2.3%	nd	nd	nd	nd	nd	nd
mlb1.3	1 OP-AJ6	56.2	3.1	0.16	1.8%	nd	nd	nd	nd	nd	nd
mlb1.4	1 *ll*	79.3	32.9	0.63	32.4%	7.0	0.23	8.1%	9.8	0.42	17.2%
mlb3.1	3 G14-SCAR	2.4	nd	nd	nd	3.5	0.14	3.5%	nd	nd	nd
mlb4.1	4 AA9B-SCAR	0.0	nd	nd	nd	5.3	–0.20	5.4%	nd	nd	nd
mlb4.2	4 OP-H13	10.9	nd	nd	nd	2.9	0.20	4.2%	nd	nd	nd
mlb4.3	4 AJ18-SCAR	21.7	nd	nd	nd	2.8	–0.19	2.8%	nd	nd	nd
mlb4.4	4 CSWTAAA01	36.4	3.0	0.17	1.7%	4.6	0.37	4.6%	2.7	0.20	3.3%
mlb5.1	5 E14M62-515	6.1	nd	nd	nd	6.1	0.21	7.2%	nd	nd	nd
mlb6.1	6 E11M50-568	37.1	2.7	0.11	1.5%	3.0	0.17	2.9%	nd	nd	nd
mlb6.2	6 L19-2-SCAR	121.3	4.2	–0.17	3.7%	3.7	–0.15	4.3%	2.7	–0.18	4.9%
mlb7.1	7 EACMCAC-195	6.1	3.8	–0.23	2.8%	nd	nd	nd	nd	nd	nd
Cumulative fruits per plant over three harvests											
fpl1.1	1 I1B-SCAR	55.7	3.4	0.49	3.9%	nd	nd	nd			
fpl1.2	1 *ll*	79.3	15.2	0.76	22.2%	2.9	0.56	5.0%			
fpl4.1	4 AO14-SCAR	27.9	3.3	–0.40	3.3%	2.7	–0.43	4.1%			
fpl4.2	4 CSWTAAA01	36.4	7.2	0.56	7.8%	nd	nd	nd			
fpl5.1	5 E14M62-515	1.6	nd	nd	nd	3.6	0.49	5.4%			
fpl6.1	6 OP-AG1-2	40.8	nd	nd	nd	7.4	0.71	11.6%			
fpl7.1	7 OP-AG17-1	25.0	2.6	–0.23	2.6%	nd	nd	nd			
Mean length:diameter ratio over three harvests											
ldr1.1	1 L18-2H19A	3.6	3.6	0.05	4.8%	5.6	0.06	5.0%			
ldr1.2	1 E14M62-273	25.7	4.6	0.06	4.5%	nd	nd	nd			
ldr1.3	1 *ll*	77.8	8.6	–0.09	11.6%	6.0	–0.09	9.7%			
ldr4.1	4 OP-L18-1	33.1	nd	nd	nd	5.4	0.13	5.3%			
ldr4.2	4 E11M60-313	37.9	nd	nd	nd	4.7	–0.11	6.1%			
ldr4.3	4 OP-AF15	100.0	nd	nd	nd	2.8	–0.05	2.7%			
ldr4.4	4 H5-SCAR	151.9	nd	nd	nd	3.6	0.05	3.3%			
ldr6.1	6 I1-SCAR	22.7	4.2	–0.05	5.3%	7.3	–0.08	6.3%			
ldr6.2	6 OP-U15-1	27.8	nd	nd	nd	6.1	–0.07	5.5%			
ldr6.3	6 OP-AG1-2	60.3	2.6	0.05	4.0%	3.2	0.07	5.9%			
ldr6.4	6 E11M50–558	101.3	nd	nd	nd	3.1	0.05	4.1%			
ldr7.1	7 BC515	0.0	4.2	0.05	5.4%	3.7	0.04	3.0%			

and 137 cM, respectively. The SSR markers analyzed were distributed relatively evenly on five linkage groups (LG 1, 2, 3, 4 and 6), and their residual heterozygosity in the RILs was less than 1%. Linkage Groups 2, 4 and 6 had contrasting regions of high and low marker densities.

All morphological markers mapped to linkage Group 1. The SSR marker CSWCT28 mapped 5 cM from the *F* locus on a distal end of linkage Group 1. This linkage was confirmed independently by F_2 progeny segregation (LOD = 6.0). Furthermore, QTL mapping of gynoecious sex expression (*F*) identified a QTL (*sex1.1*) tightly linked (LOD = 13.0) to CSWCT28. The determinate (*de*) gene mapped 0.8 cM from the SSR marker CSWCT14 and 26.1 cM from *F*. The little-leaf trait (*ll*) mapped 57 cM proximal to *de*, and is flanked by E14-M62-224 (2.6 cM) and OP-W7-2 (4.8 cM).

QTL analysis

Days to anthesis

Four QTLs were detected for days to anthesis (Table 1). Only ant6.1 was consistent in its effect over the environments tested, explaining 5.4% and 8.2% of the phenotypic variation observed in the WI 1999 and WI 2000 environments, respectively. The effect of QTL ant1.1 was detected in both environments with opposite action. Another QTL, ant 5.1, explained 14.2% of the observed phenotypic variation, but was detected only in WI 1999.

Table 2 Position on linkage group (LG), log of likelihood ratio (LOD), percentage of the phenotypic variation explained (R^2) and effect (calculated by QTL Cartographer version 1.21) of QTLs associated with sex expression traits of cucumber plants grown in Wisconsin 1999

Trait/QTL	LG	Marker[c]	Position	LOD	Effect	R^2
Number of female nodes on mainstem[a]						
sex1.1	1	*F*	1.5	13.0	–1.88	16.4%
sex1.2	1	*de*	25.7	7.3	–1.63	9.4%
sex6.1	6	AT1-2-SCAR	63.8	5.0	–1.05	5.5%
Number of female nodes on primary lateral branches[a]						
fnl1.1	1	*F*	1.5	3.8	–0.25	5.3%
fnl1.2	1	E13M50-454	18.0	2.6	–0.26	5.0%
fnl1.3	1	CSWCTT14	22.3	3.3	–0.26	5.0%
fnl1.4	1	*ll*	79.3	3.7	0.25	5.3%
Earliness[b]						
ear1.2	1	CSWCT28	3.6	7.1	1.59	6.6%
ear1.3	1	OP-AG1-1	28.0	6.4	2.37	7.9%
ear1.4	1	OP-AJ6	57.7	2.7	–1.08	2.7%
ear1.5	1	*ll*	77.8	4.1	1.18	3.5%

[a] On the first ten nodes
[b] Days to first harvest when at least two fruits per plot were of USDA grade 3 (>30 mm) or higher
[c] *F* = gynoecious, *de* = determinate, and *ll* = little-leaf

Number of lateral branches per plant

Analysis of MLB-number data in each of three environments resulted in the detection of 13 QTLs (Table 1). Four QTLs were detected consistently in all environments (WI 1999, UT 1999 and WI 2000), and two of these four QTLs were placed on linkage Group 1 and defined relatively large combined effects (R^2 = 9.1 to 32.4%). The largest effect was exhibited by mlb1.4, which was associated with the little-leaf (*ll*) character. This QTL explained 32% and 17% of the observed phenotypic variation in WI 1999 and UT 1999, respectively. The QTL mlb1.1, spanned an interval between markers E14M62-273 and CSWCT14, which was associated with the position of the determinate character (*de*). The effect of this QTL was consistent over all environments, and explained 9.1%, 10.6% and 11.5% of the observed phenotypic variation in WI 1999, WI 2000 and UT 1999, respectively. QTLs that mapped to linkage Group 4 (mlb4.4 linked to CSWTAAA01) and Group 6 (mlb6.2 linked to L19-2-SCAR) exhibited consistent but comparatively smaller effects. The latter QTL demonstrated a consistent adverse effect (i.e., an allele associated with a reduced number of branches) common to the high-parent (H-19) allele in all environments. In contrast, the QTL mlb6.1 also provided a relatively small positive effect (alleles for high branch number), but this effect was consistent in two environments (WI 1999 and WI 2000).

Number of pistillate (female) nodes on mainstem

Two QTLs, sex1.1 and sex1.2, were detected on linkage Group 1 (Table 2). These QTLs are associated with the positions of *F* and *de*, and explained 16.4% and 9.4% of the observed phenotypic variation, respectively. A third QTL mapped to linkage Group 6 (sex6.1) and explained 5.5% of the observed variation. The H-19 allele demonstrated a negative (i.e., >staminate flowers) effect in all three QTLs.

Number of pistillate nodes on primary lateral branches

Four QTLs, each explaining about 5% of observed phenotypic variation were detected and associated with linkage Group 1 (Table 2). Three of these QTLs (fnl1.1, fnl1.2 and fnl 1.3) were associated with either the *F* locus or the *de* locus. In each case the H-19 allele had a negative effect on trait expression (more staminate flowers). Another QTL (fnl1.4), associated with the H-19 allele *ll*, resulted in a positive effect (more pistillate flowers).

Earliness

Four QTLs for earliness (days to first harvest) were detected and associated with linkage Group 1 (Table 2). These QTLs explained 22% of the observed phenotypic variation. In all but one QTL (ear1.4), the H-19 allele consistently delayed female flower development, and thus first harvest date.

Cumulative number of fruits per plant

Seven QTLs were detected, which had an effect on the total number of fruit per plant, in WI 1999 and WI 2000 (Table 1). However, only in two QTLs, fpl1.2 and fpl4.1, were these effects consistent in both environments. The QTL fpl1.2 was associated with *ll* and explained 22.2% and 5% of the observed phenotypic variance in WI 1999 and WI 2000, respectively. The effect of the H-19 allele at fpl4.1 was negative (i.e., decreased the total number of fruits per plant) and of the same magnitude in both environments. Although the effect of QTL fpl6.1 associ-

Table 3 Position on linkage group (LG), log of likelihood ratio (LOD), percentage of the phenotypic variation explained (R^2) and effect (as calculated by QTL Cartographer version 1.21) of QTLs associated with the number of fruits per plant for three sequential harvests (Wisconsin 2000)

Trait/QTL	LG marker	Position	Harvest 1			Harvest 2			Harvest 3		
			LOD	Effect	R^2	LOD	Effect	R^2	LOD	Effect	R^2
Number of fruits per plant											
nfp1.1	1 J5-SCAR	10.4	2.7	–0.41	5.8%	nd	nd	nd	nd	nd	nd
nfp1.2	1 CSWCT16	43.9	4.1	0.65	7.3%	nd	nd	nd	nd	nd	nd
nfp1.3	1 E14M62-214	50.3	2.8	–0.54	5.6%	4.9	–0.67	7.8%	nd	nd	nd
nfp1.4	1 OP-AJ6	56.2	3.0	0.44	5.3%	4.1	0.64	5.7%	4.5	0.43	4.7%
nfp1.5	1 EACMCAC-107	60.9	4.4	–1.03	11.1%	nd	nd	nd	nd	nd	nd
nfp2.1	2 OP-AF7-1	21.0	4.1	–0.86	9.0%	nd	nd	nd	nd	nd	nd
nfp2.2	2 NR2	25.8	7.2	1.11	13.3%	nd	nd	nd	nd	nd	nd
nfp3.1	3 CMGA165	5.3	nd	nd	nd	5.1	–0.84	6.4%	nd	nd	nd
nfp4.1	4 AJ18-SCAR	21.7	3.0	–0.29	4.0%	5.5	–0.44	7.2%		nd	nd
nfp5.1	5 EACMCAC-119	7.2	nd	nd	nd	3.8	0.35	4.9%	nd	nd	nd
nfp6.1	6 CSWTA08B	23.4	2.9	–0.47	5.0%	nd	nd	nd	nd	nd	nd
nfp6.2	6 AK5-SCAR	38.6	2.6	0.58	6.2%	3.5	0.34	4.7%	5.5	0.33	10.9%
nfp6.3	6 BC605	79.5	nd	nd	nd	4.0	0.40	5.2%	nd	nd	nd

ated with linkage Group 6 was detected only in WI 2000, it exhibited a LOD of 7.4 and explained 11.6% of the observed phenotypic variation. Another QTL (LOD = 7.2) with a significant effect on linkage Group 4 was defined by CSWTAAA01, and explained 7.8% of the observed variation in WI 1999.

Number of fruits per plant in three harvests

A total of 13 QTLs were detected that affected the number of fruits per plant in each of three harvests in WI 2000 (Table 3). Two QTLs, nfp1.4 and nfp6.2, were associated with significant (LOD > 3.5), consistent effects in each harvest. The effects of two other QTLs, nfp1.3 and nfp4.1, were detectable in the first two harvests. Likewise, six QTLs (nfp1.1, nfp1.2, nfp1.5, nfp2.1, nfp2.2 and nfp6.1) were identified as unique to harvest one, and three (nfp3.1, nfp5.1 and nfp6.3) were unique to harvest two. Taken cumulatively, QTLs with significant effects in the first harvest explained about 70% of the total observed phenotypic variation for this yield component compared to the description of 40% and 15% of the variation in the second and third harvest, respectively.

Mean fruit length/diameter ratio

Analysis of the mean fruit length/diameter (LD) ratio over three harvests and two locations (WI 1999 and WI 2000) resulted in the detection of 12 QTLs (Table 1) associated with linkage Groups 1, 4, 6 and 7. In general, more QTLs were detected in WI 2000 than in WI 1999. Most of these QTLs had LOD scores of 3.5 or higher. Five QTLs, ldr1.1, ldr1.3, ldr6.1, ldr6.3 and ldr7.1, demonstrated consistent response in magnitude and effect in each test environment. The QTL, ldr1.3, was associated with *ll*, and provided an informative trait description to explain 11.6% and 9.7% of the observed phenotypic variation in WI 1999 and WI 2000, respectively. The QTL ldr6.1 had, on average, the second largest effect on trait expression. The effect of the H-19 parental allele was negative (i.e., a decreased L:D ratio) at five QTLs (ldr1.3, ldr4.2, ldr4.3, ldr6.1 and ldr6.2).

Discussion

The cucumber genome (750–1,000 cM in length) is predicted to have seven linkage groups (Staub and Meglic 1993). Knerr and Staub (1992) assigned 12 of the 14 isozyme loci in cucumber to four linkage groups spanning about 215 cM. Meglic and Staub (1996) identified 21 polymorphic and 17 monomorphic cucumber isozyme loci in 15 enzyme systems. Nine morphological markers were linked to isozyme loci and were integrated to form a map containing four linkage groups spanning 584 cM with a mean linkage distance of about 19 cM.

Kennard et al. (1994) used RFLP, RAPD, isozyme, morphological and disease resistance markers to identify ten linkage groups in cucumber. They constructed a 58-point and a 70-point map using a narrow (*C. sativus* L. var. *sativus* × var. *sativus*) and a wide [*C. sativus* var. *sativus* × var. *hardwickii* (R.) Alef.] cross, respectively. Although ten linkage groups were identified in each map, markers in the narrow cross spanned 766 cM (mean marker interval = 13 cM) and in the wide cross markers spanned 480 cM (mean marker interval = 7 cM).

An 83-point RAPD and morphological map was constructed in cucumber which spans 630 cM (mean marker interval = 7.6 cM) in a relatively wide *C. sativus* var. *sativus* cross (line G421 × line H-19) (Serquen et al. 1997a). This map included *F, de, ll* and yield component QTLs. Of the 78 dominant mapped RAPDs, 47 were converted to SCARs for increased marker stability (Horejsi et al. 1999), and were then used in this study.

Bradeen et al. (2001) used AFLP markers to merge the location of markers mapped in narrow and wide crosses in

cucumber (Kennard et al. 1994; Serquen et al. 1997a), and markers linked to disease resistance (Horejsi et al. 2000) to produce a consensus map in cucumber. The AFLP markers allowed for the joining of several previously characterized linkage groups and the creation of new linkage groups not present in the map of Serquen et al. (1997a), resulting in a map with ten linkage groups.

The use of previously unmapped markers (14 SSRs, 4 SCARs, 1 SNP, 4 RAPDs, and 5 AFLPs) in our study allowed the construction of a map with seven linkage groups predictive of the cucumber genome. This map placed *F, de* and *ll* on the same linkage group (LG 1), and defined the linkage distance between *F* and *de* to be about 26 cM, which is in agreement with published morphological maps (Walters et al. 2001). There are disparities between the location (genetic distances) of markers in Fig. 1 and Table 2. The location (cM) of a particular QTL (Table 2) represents the highest peak of the curve derived from Composite Interval Mapping function in the QTL Cartographer. The marker listed on the table is the closest marker to that peak. The apparent disparities are due to this difference.

Sex expression in the recombinant inbred lines evaluated, ranged from gynoecious to andromonoecious (data not presented). Results of this analysis, which considered sex expression as a quantitative trait support the principal control of sex expression at the *F* locus (Kennard et al. 1994) and the presence of sex modifying genes (Serquen et al. 1997a), the likely involvement of the determinate (*de*) character in gynoecy (Lower and Nijs 1979) and the presence of QTL sex6.1 on linkage Group 6, which has a demonstrable effect on gynoecy (Table 2).

The estimation of effects (R^2 values) of the *F* locus in this study (16%) is considerably lower than that reported by Serquen et al. (1997a) (67 to 74% depending on location). The R^2 values obtained by QTL Cartographer are inherently conservative. These values result from an estimation of the variance explained by the QTLs based on background markers and other explanatory variables (e.g., *de*). Sex expression in the population examined (taken as a function of the number of female flowers on the first ten nodes of the mainstem) is partially a function of *F* and *de* which do not act independently of each other. The estimation of the effects of the variables are a function of QTLs specfic to the model and are not given as a proportion of the total variance.

Environment is known to play a major role in sex expression in cucumber (Cantliffe 1981; Zhang et al. 1992; Dijkhuizen and Staub 2003). This QTL is on the same linkage group as a sex expression-associated QTL previously detected by interval mapping in $F_{2:3}$ families (Serquen et al. 1997a), and has now been identified as an additional unique sex expression modifier on this linkage group. While RILs were strongly gynoecious when alleles at these three loci came from the G421 parent, RILs possessing H-19 alleles at these loci were monoecious to andromonoecious.

All four QTLs associated with days to anthesis in this study (Table 1) were different than those described by Serquen et al. (1997a). Two of the four QTLs (ant2.1, ant5.1) were detectable in only one location and another (ant1.1) had an opposite effect in the two locations examined, indicating the importance of genotype × environment interactions in the timing of cucumber flower development. This finding supports and extends the findings of Serquen et al. (1997a, b) and Dijkhuizen and Staub (2003) regarding environmental effects on the map placement of QTLs. Moreover, it is of importance that a QTL for days to anthesis (ant6.1) and the QTL (sex6.1) for sex expression were defined by the same marker interval, and that days to anthesis is independent of *F* and *de*. These observations indicate a possible association of this trait with sex expression, as suggested by Lower and Nijs (1979). In contrast, earliness (days to first harvest) is affected by genomic regions (ear1.2, ear1.4), which are associated with *F* and *de*. G421 is determinate and gynoecious and thus, predictably, G421 alleles at these loci decreased the days to first harvest in the RILs examined. This is in agreement with observations that gynoecious cultivars flower earlier and are generally harvested earlier than monoecious ones (Peterson 1960; Lower and Edwards 1986; Wehner 1989).

The region of the determinate character (*de*) demonstrated large pleiotropic effects on multiple lateral branching (MLB) and sex expression, and comparatively smaller effects on mean length/diameter ratio (Table 1). Similarly, in tomato, segregation of the *sp* (self-pruning) gene (i.e., similar to *de* in cucumber) in mapping populations resulted in the detection of several QTLs surrounding *sp* (Paterson et al. 1991; Goldman et al. 1995). These associations most likely reflect the pleiotropic effect of the *sp* locus itself (Saliba-Colombani et al. 2001). Similarly, the pleiotropic effects recorded on MLB and sex expression in this study are likely due to *de*.

Mainstem length was determined to be under the influence of the *F-de* genomic region and, similar to MLB, has exhibited a mostly additive genetic variance in trait expression (Serquen et al. 1997a, b). These findings may explain the difficulty encountered in the development of gynoecious, short determinate, multiple lateral-branched genotypes (Staub et al. 1995), and may provide insights into the use of determinate × indeterminate hybrids to achieve such plant types using MAS. For instance, when using H-19 as a donor parent for MLB in backcrossing to G421 (recurrent parent), plants heterozygous for H-19 alleles conditioning MLB located in the same genomic region as *de* were highly branched and gynoecious (data not presented).

Differences in the detection and characterization of QTLs across environments in Wisconsin may be due to climatic differences. July and August 1999 were characterized by heavy precipitation (40 cm), compared to the year 2000 precipitation (17 cm) and the five year mean (23.8 cm) for the same months. The 1999 growing season also was characterized by higher mean temperature (22 °C) and heat units (1,013) during the first 40 days of culture followed by a period of cool weather beginning at anthesis and continuing through the second harvest. In

contrast, the 2000 growing season had higher mean temperatures and heat units following anthesis. Environmental stress suppresses fruit enlargement and mitigates L:D ratio differences among developing fruits. In WI 1999, the magnitude of documented genotype × environment interactions may have been increased because of higher than normal precipitation and temperatures, resulting in the detection of fewer QTLs describing fruit-size characteristics (i.e., the L:D ratio) than those detected in 2000. Such effects on QTL detection and placement have been observed in a multiple branching populations (BC and F_2S_1) derived from a *C. sativus* var. *sativus* × var. *hardwickii* (R.) mating when grown at varying plant densities (Dijkhuizen and Staub 2003).

The use of a more comprehensive genetic map and composite interval mapping in this study allowed for more precision in the placement of the QTLs identified by Serquen et al. (1997a). For instance, the two L:D ratio QTL detected by Serquen et al. (1997a) at the same growing location were also detected in this study (ldr1.1, ldr1.3), along with ten additionals on other linkage groups (WI 2000; Table 1). The number and cumulative predictive power (R^2 = 50%) of these markers suggest their candidacy for use in MAS to improve cucumber quality (i.e., greater fruit length).

Increasing the number of primary lateral branches in processing cucumber theoretically can increase its total yield (Staub et al. 1995; Cramer and Wehner 2000). Path analysis of the correlation between fruit number per plant and several plant traits (e.g., branches per plant, nodes per branch, pistillate nodes and fruit set) in cucumber indicates that MLB provides the highest correlation (r = 0.82) with the number of fruits per plant (Cramer and Wehner 2000). Moreover, MLB is controlled by at least five effective factors and has a narrow-sense heritability between 0.4 to 0.8, depending on growing environment and the populations employed (Wehner et al. 1987; Serquen et al. 1997a, b; Dijkhiuzen and Staub 2003). These characters make MLB an attractive candidate trait for indirect selection for yield via marker-assisted selection.

Serquen et al. (1997a) identified three QTLs associated with MLB that explained about 60% of the phenotypic variance observed under Wisconsin growing conditions. These, along with ten additional QTLs for MLB, were identified in this study. The detection of additional QTLs for this, and other traits, through our RIL analysis might be partially due to the reduced error variances associated with inbred-line evaluation when compared to the $F_{2:3}$ family analysis and to the increased number of experimental units (171 RIL vs 103 $F_{2:3}$ families) than those used by Serquen et al. (1997a, b). In contrast to the findings by Serquen et al. (1997a), the QTL mlb1.1 associated with determinate (*de*) explained less variation (R^2 = about 10%) than mlb1.4 associated with little-leaf (*ll*) (R^2 = about 20%). This is likely due to differences in the type of populations used (RIL vs $F_{2:3}$ families), and upward biases (i.e., relatively strong linkage effect) derived from using *de* (a morphological character associated with MLB) in QTL mapping by Serquen et al. (1997a).

Some QTLs for MLB reported herein were placed relatively close to each other (e.g., mlb4.1 and 4.2, and nfp2.1 and nfp2.2; Table 1). This may be due to imprecision in the localization of these QTLs. The mapping precision is dependent on several programing factors that can be choosen during the operation of QTL Cartographer (i.e., options window). A walking speed of 1 cM and a window of 3 cM was used in this analysis which is considered relatively stringent. Moreover, these closely associated QTLs differ in the direction of their effects (positive versus negative), suggesting that they are unique.

The effectiveness of PCR markers associated with mlb1.1, mlb1.4, mlb3.1 and mlb4.4 was examined by MAS during backcrossing (Fazio 2001; Fazio and Staub 2003). These markers were used to select for the MLB trait in BC_1 and BC_2 populations derived from H-19 × G421 matings. MAS was as effective as phenotypic selection, and thus establishes the potential utility of QTLs associated with this trait. Furthermore, four MLB QTL regions (mlb1.3, mlb1.4, mlb4.4 and mlb6.1) were equivalent in location to that of QTLs (fpl1.1, fpl1.2, fpl4.2 and fpl6.1) associated with total fruit number per plant (total yield; Table 1). The H-19 parental allele was associated with a positive effect in both traits. Therefore, MAS in breeding for yield components with relatively high heritability (h^2 = 0.4 to 0.8) such as MLB may provide small, but economically significant increases in fruit number per plant.

Comparison of fruits per plant QTLs in three harvests (Table 3) reveals that only two QTLs (nfp1.4 and nfp6.2) were detected consistently in each of three harvests in WI 2000. These QTLs were placed in the same genomic regions as two QTLs (fpl.1.1 and fpl6.1) associated with total fruit per plant. The fact that proportionately more fruit per plant QTLs were detected at the first harvest than in successive harvests may indicate that the genes segregating for this trait in this cross had their greatest demonstrable physiological effect during early fruit development. One QTL consistently detected only at the first harvest (nfp.2.2, LOD = 7.2) explained 13.3% of the observed phenotypic variation. In this case the H-19 parental allele increased the number of fruits per plant in the RIL examined by 1.1 units over the G421 parental allele. The molecular markers associated with these QTLs may prove useful in breeding to increase early, concentrated, first-harvest yield.

DNA sequence and location information for molecular markers characterized in Fazio et al. (2002) has proven useful not only in marker-assisted breeding for MLB in cucumber (Fazio and Staub 2003), but also in defining QTL effects in this study. When multiple trait interactions are known and this information is used strategically in combination with well-documented, reliable, QTL-marker-associated MAS, gain from selection can be realized. The strategic combination of MAS and phenotypic selection cycles should allow for increased efficiency in

the release of high yielding-processing cucumber cultivars. Likewise, the predictability and discriminatory nature of molecular markers defined herein will allow for their use in cultivar identification and in plant variety protection (Staub and Meglic 1993; Staub et al. 1996b; Staub 1999).

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