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Molecular Markers to Select for the *j-2*–mediated Jointless Pedicel in Tomato

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Abstract. **The jointless pedicel trait of tomato conferred by the *j-2* gene is widely used in processing markets for stem-free removal of fruit to accommodate mechanized harvest. Although current utilization of *j-2* for fresh-market tomato breeding is limited, interest in this trait may increase as breeders seek to address high labor costs through the development of mechanically harvestable cultivars for the fresh market. Yet, the introduction of this trait into new market classes heavily relies on phenotypic selection because there are presently no high-throughput methods available to genotype *j-2*. Reliable, high-throughput molecular markers to genotype the presence/absence of *j-2* for selective breeding were developed. The molecular markers described here use the high-resolution DNA melting analysis (HRM) genotyping with single-nucleotide polymorphism (SNP) and derived cleaved amplified polymorphic sequence (dCAPS)–based genotyping. Two separate HRM-based markers target the *j-2* on chromosome 12 or a linked sequence region 3.5 Mbp apart from the gene, and a dCAPS marker resides on the latter. We demonstrate the association between each marker and the jointless pedicel phenotype using segregating populations of diverse filial generations in multiple genetic backgrounds. These markers provide a useful resource for marker-assisted selection of *j-2* in breeding populations.**

Tomato (*Solanum lycopersicum* L.) is the most valuable horticultural crop worldwide (Food and Agriculture Organization of the United Nations; http://www.fao.org/faostat/en/#data/QC/metadata). Fresh-market and processing tomatoes, the two most commonly consumed types of tomatoes, are economically important in many countries, including the United States [United States Department of Agriculture Economic Research Service (USDA ERS); www.ers.usda.gov/topics/crops/vegetables-pulses/tomatoes]. Nonetheless, further improvement in horticultural performance is necessary to achieve future productivity gains, especially given the rapidly growing labor costs and recent trends in uncertainty about trained labors (California Tomato Growers Association; www.ctga.org, Florida Tomato Committee; www.floridatomatoes.org, USDA ERS).

A major change among agricultural industries has been a shift toward farm machinery to achieve higher levels of productivity and market value (Pardey et al., 2016; Zahara and Johnson, 1981). Unlike processing tomatoes that have been successfully adapted for mechanized harvest, production of fresh-market tomatoes continues to rely on manual labor for harvesting and other common cultural practices (such as staking, tying, and pruning), which can account for as much as a half of the total production cost (Davis and Estes, 1993; USDA ERS). Thus, there is a significant need to research traits which will facilitate a transition to broader mechanization in fresh-market tomato production.

Tomato inflorescences typically have an abscission zone (joint) in the pedicel of each flower. Detachment of the fruit at this joint at harvest results in the calyx and stem remaining attached to the fruit, which can in turn puncture or otherwise damage neighboring fruit. The jointless pedicel trait was first reported by Butler (1936). Because jointless tomatoes lack an abscission zone in the pedicel, the calyx and stem remain attached to the plant, enabling of stem-free harvest of fruit. Hand harvesting of jointed pedicel tomatoes involves the manual removal of any attached stems from fruit, but jointless pedicels are an essential component for maintaining fruit quality and marketability in cultivars intended for mechanical harvest (Scott et al., 2013; Zahara and Scheuerman, 1988).

Two recessive genes known to mediate the jointless pedicels in tomato have been identified. The first gene, *jointless* (*j*), is located on chromosome 11 and was identified from *S. lycopersicum* accession LA624 (Rick, 1980; Wing et al., 1994). Mao et al. (2000) determined that *j* was a MADS-box gene controlling tomato flower abscission zone development. Later, an alternative allele, *jointless-2* (*j-2*; Rick, 1956) was identified in *S. cheesmanii* accession LA166 and also as a spontaneous mutation in cultivated tomato (Reynard, 1961). *j-2* was mapped to an ≈6-Mbp interval in the centromeric region on chromosome 12 (Budiman et al., 2004; Yang et al., 2005; Zhang et al., 2000). Recent progress in understanding the molecular characteristics of the jointless trait has revealed the underlying gene, a MADS-box transcription factor 11 gene (Solyc12g038510) of *S. lycopersicum* (the jointed pedicel trait-derived allele) (Soyk et al., 2017), and determined that loss of function mutations in this gene resulted in the jointless inflorescence. Hence, mutated versions of Solyc12g038510 are referred to as *j-2* in the present study.

j-2 has been broadly used by tomato breeding programs in the United States and around the world. However, current selection methods rely predominantly on phenotypic expression at flowering or thereafter. Marker resources to aid in selection efforts are limited. In the late 1990s, Zhang et al. (2000) developed a random amplified polymorphic DNA (RAPD) marker for *j-2* which has been used in independent research efforts (Budiman et al., 2004; Yang et al., 2005). However, the marker system is not fully feasible because of the cumbersome process of the RAPD system and difficulty in its reproduction. The CAPS marker tagging *j-2* alleles (Soyk et al., 2017) is detected via a gel-based polymerase chain reaction (PCR) image and is not immediately useful for high-throughput genotyping. Thus, a practical means to select for this trait could be very helpful for introducing and selecting the jointless trait in tomato germplasm, especially for fresh-market backgrounds which are predominantly jointed.

The objective of this project was to develop molecular markers linked to the *j-2* locus that can be useful for marker-assisted selection (MAS). We used whole-genome

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Table 1. Markers used for detection of the jointless pedicel trait.

Marker designation	Marker type[z]	Position on Chr. 12 (bp), SL3.0 assembly	Position on Chr. 12 (bp), SL2.5 assembly	Sequence polymorphism	Strand	5′–3′ sequence	Size of fragment(s) (bp)[y]
j2-dCAPS	dCAPS	54,951,734	43,866,871	C | A[x]	Forward	TCAGCGGTCAGCAAGAAAATAGCTC	153, 124 + 29
					Reverse	CCAAGGCTACGAGAGTTGAAGTTCC	
j2-HRM1	HRM	54,951,734	43,866,871	C | A	Forward	CAGCGGTCAGCAAGAAAAT	53, 53
					Reverse	CACTCCTTTGAAGGGATAATAAACA	
j2-HRM2	HRM	51,393,057	47,428,799	C | A	Forward	GGATAGCTTTAAAACATGAC	44, 44
					Reverse	TTAAGAAATATATGAGAAAACT	

[z]dCAPS = derived cleaved amplified polymorphic sequences; HRM = high-resolution DNA melting analysis.
[y]Jointless pedicel genotype, jointed; obtained two fragments subjected to restriction enzyme DdeI in the jointed genotype.
[x]Jointless pedicel genotype | jointed.

Fig. 1. Identification of single-nucleotide polymorphisms (SNPs) near the jointless pedicel locus (*j-2*) in 19 tomato lines. Boxes indicate the SNPs used for the parker development in this study. SNPs in the 1-kb region surrounding each box are shown. Nucleotide positions are from the SL3.0 version of Heinz 1706 reference. Positions in the *J-2* gene (Solyc12g038510) are marked.

Fig. 2. Results of dCAPS analysis using an SNP located near the *j-2*. In homozygous jointed pedicel or heterozygous genotypes, a 20-bp fragment by restriction enzyme digestion is not captured in this figure. "H jointless" and "H jointed" represent homozygous jointless pedicel and homozygous jointed pedicel, respectively.

sequencing of jointless and jointed tomato lines to identify SNPs proximal to the locus.

Materials and Methods

Plant material. Nineteen inbred lines from the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) tomato breeding program were selected for whole-genome sequencing to identify polymorphisms near the *j-2* locus. These included four jointless inbreds, Fla. 8383, Fla. 8916, Fla. 8924, and Fla. 8932; and 15 jointed inbreds, Fla. 630, Fla. 645, Fla. 7781, Fla. 8000, Fla. 8111B, Fla. 8297, Fla. 8344, Fla. 8516, Fla. 8517, Fla. 8570, Fla. 8624, Fla. 8638, Fla. 8820, Fla. 8872B, and Fla. 8925. Among these, Fla. 630, Fla. 645, and Fla. 8344 are plum fruit types and all others are large-round fruit types. The phenotype of each line was confirmed before sequencing. Molecular marker–phenotypic trait cosegregation was evaluated among 41 lines/populations segregating for *j-2* and among 11 lines that were fixed for the trait. Pedigrees of these materials, including the source of *j-2* in each, are described in the marker evaluation tables.

Whole-genome shotgun sequencing and SNP detection. We performed whole-genome shotgun sequencing of the 19 lines using Illumina technology. Genomic DNA extraction was performed as described in Li et al. (2018). Genomic DNA was sequenced using the Illumina HiSeq. 2000 at the Cornell

University Life Sciences Core Laboratories Center. For each line, Bowtie 2 (Langmead and Salzberg, 2012) was used to align the reads to the Heinz 1706 reference sequence. SNPs were predicted from aligned read data using FreeBayes (version 0.9.16-1-gf46d24f; Garrison and Marth, 2012). For marker development, only SNPs that were monomorphic among jointless lines, monomorphic among jointed lines, and polymorphic between these phenotypic classes were considered. SNPs in close proximity to a gene Solyc12g038510 (Soyk et al., 2017) and to BAC clone 121H12 (Yang et al., 2005) were used to develop HRM and dCAPs markers.

Marker development and testing. HRM markers were developed according to the manufacturer's instructions (Roche, Indianapolis, IN). Fluorescence signals were measured using the Roche LightCycler 480 system (Roche) with AccuStart II PCR ToughMix (Quantabio, Beverly, MA) according to the manufacturer's instructions. Amplification of HRM markers used the following temperatures and durations: 95 °C for 3 min, followed by 42 cycles of 95 °C for 20 s; 57 °C for 20 s; 72 °C for 15 s; and a 10-min extension at 72 °C. The products from a jointed and a jointless line were sequenced by the Sanger method to validate single-product amplification of the target region.

Two software packages were used for dCAPS primer development: dCAPS Finder 2.0 (Neff et al., 2002; http://helix.wustl.edu/dcaps/dcaps.html) and Primer-BLAST (Ye et al., 2012; https://www.ncbi.nlm.nih.gov/tools/primer-blast). The marker was PCR amplified using Phire Hot Start DNA polymerase (TaKaRa, Tokyo, Japan) as per the manufacturer's instructions. Single-product amplification of the target region was validated for a jointed and a jointless line using Sanger sequencing. PCR amplicons were digested using the *Dde* I restriction enzyme (New England Biolabs, Beverly, MA) according to the manufacturer's instructions, and the digested products were visualized on a 3.0% agarose gel strained with ethidium bromide (0.05 $\mu L \cdot mL^{-1}$).

Two HRM markers and one dCAPS marker were used to investigate marker–trait cosegregation among a diverse set of tomato lines fixed or segregating for *j-2* (Table 1). Leaf discs (6 mm diameter) were individually collected from 3-week-old seedlings grown in a greenhouse. Genomic DNA was extracted using a rapid NaOH-based extraction method as described in Xin et al. (2003), and genotypes were determined as described previously. Plants were grouped according to genotype and then planted in the field and subsequently phenotyped for the jointless trait.

Results

Whole-genome sequencing was used to discover SNPs between jointless pedicel and jointed pedicel genotypes. Because BAC clone 121H12 was reported to contain candidate gene(s) for *j-2* (Yang et al., 2005), nucleotide positions that harbored polymorphic SNPs between two different phenotypic classes and which were near this BAC were selected for molecular marker development (Fig. 1). Two useful SNPs were identified near 54.95 Mbp on chromosome 12, and a primer set for one of these gave robust PCR amplification (hereafter, this marker is termed j2-dCAPS) (Table 1; Fig. 2). To determine the utility of the j2-dCAPS marker

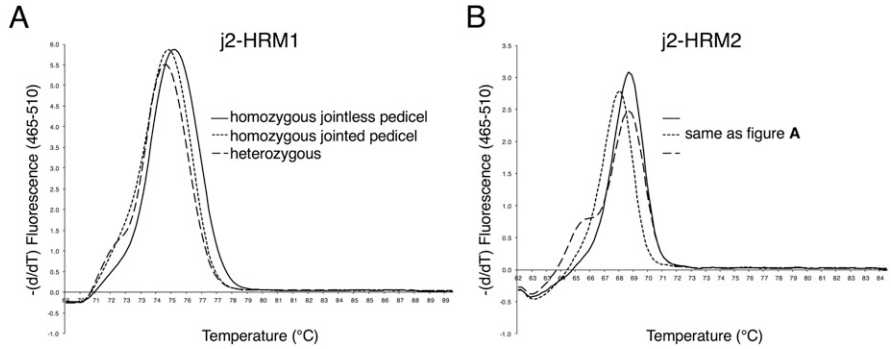

Fig. 3. SNP genotyping of the jointless pedicel trait. For both analyses, a clearly separated curve is observed in the homozygous jointless pedicel genotype, which melted at higher temperatures than the homozygous jointed one. Heterozygous genotype has a different shape than homozygotes in the low melting region. At least three independent experiments per analysis are performed showing similar results. In all three PCR products, data from a single genotype are presented. The LightCycler system was used for melting curve acquisition.

Table 2. Sequence polymorphisms in the 200-kb region across the j2-HRM2 marker.

Marker	Position on Chr. 12 (bp), SL3.0 assembly	Position on Chr. 12 (bp), SL2.5 assembly	Genotype[z]	
			Jointless	Jointed
	51,284,392	47,537,462	T	C
	51,286,001	47,535,853	T	C
	51,287,399	47,534,455	A	C
	51,289,461	47,532,393	C	T
	51,292,281	47,529,573	G	A
	51,295,006	47,526,848	T	A
	51,309,258	47,512,597	A	G
solcap_snp_sl_18995[y]	51,311,102	47,510,753	A	T
	51,328,794	47,493,061	T	C
	51,337,139	47,484,717	A	T
	51,344,173	47,477,683	A	G
	51,350,358	47,471,498	A	T
	51,356,208	47,465,648	A	G
	51,390,786[x]	**47,431,070**	**T**	**C**
	51,391,262	**47,430,594**	**A**	**G**
j2-HRM2	**51,393,057**	**47,428,799**	**C**	**A**
	51,394,868	**47,426,988**	**T**	**C**
	51,395,999	**47,425,857**	**C**	**A**
	51,397,210	47,424,646	C	T
	51,401,085	47,420,770	A	C
	51,411,471	47,410,385	C	T
	51,417,314	47,404,542	A	G
	51,419,916	47,401,940	A	T
	51,427,293	47,394,563	T	C
	51,433,514	47,388,342	G	A
	51,433,782	47,388,074	T	C
	51,438,275	47,383,581	A	G
	51,443,687	47,378,169	T	G
	51,445,435	47,376,420	C	T
	51,445,851	47,376,005	A	G
	51,447,572	47,374,283	C	A
	51,447,602	47,374,254	C	T
	51,447,843	47,374,013	T	G
	51,450,639	47,371,217	T	C
	51,454,036	47,367,820	G	A
	51,457,258	47,364,598	T	C
	51,459,743	47,362,113	T	G
	51,463,629	47,358,227	A	G
	51,463,922	47,357,934	A	T
	51,465,764	47,356,092	T	A

[z]SNPs that were monomorphic among jointless lines, monomorphic among jointed lines, and polymorphic between these phenotypic classes were considered.
[y]A marker identification incorporated in the tomato SolCAP infinium array platform (http://solcap.msu.edu/tomato_genotype_data.shtml).
[x]SNPs in the gene Solyc12g038510 were bolded.

Table 3. Evaluation for the jointless or jointed pedicel phenotypes and a segregating dCAPS marker.

Source of jointless allele	Population designation[z]	Generation tested	Pedigree[y]	Number of lines/plants[x]					
					Phenotype		Genotype[w]		
				Total	Jointless	Jointed	H jointless	H jointed	Heterozygous
	Fixed lines								
Fla. 8208	15016a	F_8	Fla. 8208 × (Fla. 8879 × Fla. 8000)	2	2	0	2	0	0
Fla. 8651	150198	F_4	Fla. 8111B × Fla. 8651	1	1	0	1	0	0
Fla. 8652	150149	F_5	Fla. 8044 × Fla. 8652	1	1	0	1	0	0
	150155	F_4	Fla. 8044 × Fla. 8652	1	1	0	1	0	0
Fla. 8652	150151	F_6	Fla. 8652 × Fla. 8874	1	1	0	1	0	0
	150218	F_3	(Fla. 7781 × NC1CS) × Fla. 8652	1	1	0	1	0	0
Fla. 8653	150142	F_8	Fla. 8869 × Fla. 8653	1	1	0	1	0	0
	150172	F_6	Fla. 8626 × Fla. 8653	1	0	1	0	1	0
Fla. 8812	150056	F_6	Fla. 8812 × Fla. 8874	1	1	0	1	0	0
Fla. 8917	150694	F_3	Fla. 7907B × Fla. 8917	1	1	0	1	0	0
	150695	F_4	Fla. 8917 × Fla. 7804	1	1	0	1	0	0
	Segregating lines								
	150234	F_2	Fla. 7781 × (Fla. 8293 × Fla. 8208)	20	4	16	4	4	12
	150447	F_2	(Fla. 8293 × Fla. 8208) × (Fla. 7987 × Fla. 7781)	20	8	12	8	1	11
Fla. 8651	150235	F_2	Fla. 7781 × Fla. 8651	20	2	18	2	8	10
	150252	F_2	Fla. 8111B × Fla. 8651	20	6	14	6	4	10
Fla. 8834	150761	F_5	Fla. 8834 × Fla. 8355	8	1	7	1	1	6
Fla. 8914	15058a	F_3	Fla. 8914 × (Fla. 8000 × Fla. 8589)	72	33	39	33	13	26
	15059a	F_3	Fla. 8914 × (Fla. 8044 × Fla. 8027)	72	31	41	31	15	26
Fla. 8915	150580	F_3	(Fla. 8059 × Fla. 8044) × Fla. 8915	12	7	5	7	0	5
	150597	F_3	Fla. 8915 × (Fla. 8000 × Fla. 8589)	12	10	2	10	1	1
	150a	F_3	Fla. 8915 × (Fla. 8044 × Fla. 8027)	48	29	19	29	5	14
Fla. 8916	15057a	F_4	Fla. 8044 × Fla. 8916	16	13	3	13	1	2
	150603	F_3	Fla. 8916 × (Fla. 8000 × Fla. 8589)	15	8	7	8	0	7
	150623	F_2	(Fla. 8811 × Fla. 8590) × Fla. 8916	20	6	14	6	1	13
Fla. 8919	150572	F_4	Fla. 8919 × Fla. 8869	8	6	2	6	0	2
	150571	F_5	Fla. 8919 × (Fla. 8059 × Fla. 8044)	8	5	3	5	1	2
	1506a	F_3	Fla. 8919 × (Fla. 8044 × Fla. 8027)	60	27	33	27	14	19
	150612	F_3	Fla. 8919 × Fla. 8921	12	9	3	9	0	3
Fla. 8924	150618	F_2	Fla. 8924 × Fla. Fla. 8925	20	5	15	5	0	15
	15061a	F_3	(Fla. 7781 × NC1CS) × Fla. 8924	33	22	11	22	2	9
	150617	F_2	Fla. 8924 × Fla. 8044	16	6	10	6	1	9
	150622	F_2	Fla. 8924 × (Fla. 8822 × Fla. 8589)	8	5	3	5	0	3
	150624	F_2	(Fla. 8811 × Fla. 8590) × Fla. 8924	20	11	9	11	2	7
	150625	F_2	Fla. 7781 × Fla. 8924	20	9	11	9	2	9
Fla. 8932	15015a	F_3	Fla. 8925 × Fla. 8932	14	17	7	17	0	7
	150236	F_2	Fla. 7781 × Fla. 8932	6	1	5	1	2	3
	150246	F_2	(Fla. 8810 × Fla. 7770) × Fla. 8932	20	6	14	6	5	9
	150249	F_2	(Fla. 8811 × Fla. 8590) × Fla. 8932	20	4	16	4	4	12
Fla. 8933	150250	F_2	(Fla. 8811 × Fla. 8590) × Fla. 8933	20	5	15	5	4	11
Fla. 8934	15016b	F_3	Fla. 8934 × (Fla. 8044 × Fla. 8027)	42	22	20	22	12	8
	150247	F_2	(Fla. 8810 × Fla. 7770) × Fla. 8934	20	7	13	7	4	9
	150251	F_2	(Fla. 8811 × Fla. 8590) × Fla. 8934	20	7	13	7	0	13
Fla. 8935	150160	F_3	Fla. 8925 × Fla. 8935	12	5	7	5	0	7
Fla. 8936	150248	F_2	(Fla. 8810 × Fla. 7770) × Fla. 8936	20	4	16	4	5	11
	150268	F_2	Fla. 8949 × Fla. 8936	20	2	18	2	9	9
Fla. 8960	150245	F_2	(8436B × Fla. 7781) × Fla. 8960	6	3	3	3	0	3
B3485	150450	F_2	Fla. 8811 × B3485	20	3	17	3	6	11
	150451	F_2	B3485 × (Fla. 7987 × Fla. 7781)	20	3	17	3	4	13
Fla. 8538, Fla. 8848	150641	F_2	(Fla. 8538 × Fla. 8848) × (Fla. 7987 × Fla. 7781)	20	5	15	5	7	8
	150644	F_2	(Fla. 8538 × Fla. 8848) × Fla. 8937B	20	6	14	6	5	9
	150647	F_2	(Fla. 8538 × Fla. 8848) × Fla. 8949	20	3	17	3	6	11
Fla. 8651, Fla. 8652	150157	F_3	(Fla. 8651 × Fla. 8652) × (Fla. 8044 × Fla. 8027)	12	9	3	9	0	3
Total				904	386	528	386	150	378

[z]Populations with the same core crossing and same filial generation were grouped together. Populations genotyped by all three markers, j2-dCAPS, j2-HRM1, and j2-HRM2, are underlined.
[y]Pedigrees indicate the inbred parents used in crosses; where breeding lines were used in crosses, the parents of these are described within parentheses. Jointless parents are underlined.
[x]Correspond to number of individual lines where genotype/phenotype are fixed and to number of individual plants for segregating lines.
[w]H jointless: homozygous jointless pedicel genotype, H jointed: homozygous jointed.

for genotyping, we tested the association of the marker and the trait in segregating populations of diverse filial generations (Table 3). A total of 11 fixed and 41 segregating lines (904 plants) were phenotyped and genotyped in Spring 2015. In all cases, plants with the jointless pedicel phenotype were homozygous for the single larger fragment, and plants with jointed pedicels were either heterozygous or homozygous for the smaller fragment (Fig. 2).

Concurrently, the SNP on which the j2-dCAPS marker is based was used for development of a HRM marker (Table 1; Fig. 3A). None of the genotypes with single base A at 54,951,734 bp have shown jointless pedicel phenotype (referred to henceforth as j2-HRM1) (Table 4).

We originally initiated the development of a *j-2* marker system based on the location of a BAC clone reported by Yang et al. (2005). Recently, however, *j-2* was attributed to mutations in a gene Solyc12g038510 (Soyk et al., 2017), which is located ≈3.5

Table 4. Evaluation for the jointless or jointed pedicel phenotypes and segregating HRM markers.

Source of jointless allele	Population designation[z]	Generation developed	Pedigree[y]	Total number of plants	Phenotype		Genotype[x]		
					Jointless	Jointed	H jointless	H jointed	Heterozygous
Fla. 8651	150235	F_2	Fla. 7781 × Fla. 8651B	128	26	102	26	43	59
Fla. 8917	150694	F_3	Fla. 7907B × Fla. 8917	128	25	103	25	44	59
Fla. 8919	150572	F_4	Fla. 8919 × Fla. 8869	101	25	76	25	23	53
Fla. 8924	150618	F_2	Fla. 8924 × Fla. 8925	114	25	89	25	36	53
Fla. 8932	144890	F_2	Fla. 8932 × Fla. 8872B	128	32	96	32	39	57
Total				599	133	466	133	185	281

[z]Populations that were also genotyped using the j2-dCAPs marker are underlined.
[y]Donor and recipient parents in an initial breeding cross are listed unless otherwise described. Jointless parents are underlined.
[x]Genotypes were determined using the j2-HRM1 and j2-HRM2 markers, which consistently gave similar results. H jointless: homozygous jointless pedicel genotype, H jointed: homozygous jointed.

Mbp apart from the BAC. In light of this, we searched for additional polymorphisms near this gene for use in further marker development (Tables 1 and 2; Fig. 1). An [A/C] (adenine to cytosine) SNP located in gene Solyc12g038510 at 51,393,057 bp was successfully converted to a HRM marker and distinguished among jointed and jointless genotypes (Fig. 3B; hereafter, this marker is termed j2-HRM2). Homozygotes had similar shapes in melting curves but their T_ms differed by up to 1 °C. Heteroduplex products were likewise distinguishable from either homozygote. Both j2-HRM1 (described previously) and j2-HRM2 were used to genotype five segregating populations in Spring 2017 (nearly 600 plants, total) (Table 4). Genotypes for j2-HRM2 showed a perfect link to the jointless pedicel phenotype, and no discordant genotypes between j2-HRM1 and j2-HRM2 were identified.

Discussion

In this study, molecular markers were developed that can accurately genotype the *j-2* jointless pedicel locus in tomato. Each of the markers consistently distinguished jointed genotypes from jointless genotypes among the range of breeding materials tested, which represents a large portion of U.S. fresh-market tomato germplasm. The two HRM markers developed in this study are ≈3.5 Mbp apart, one marker (j2-HRM2) located at the *j-2* homolog and the other (j2-HRM1) located in the telomere-proximal to that gene. Distinguishable DNA sequence polymorphisms were acquired from whole-genome sequencing data. The sequencing data, which are increasingly available for major crop plants, provided adequate sequence resources to saturate the locus, with the advantage of no ascertainment bias from the analyzed *j-2* region. The advantages of these markers over presently existing phenotyping efforts are a) genotyping does not require mature (flowering) plants and thus the jointless pedicel phenotype can be screened in seedlings, b) the use of codominant markers will enable more rapid, efficient backcrossing of *j-2* as opposed to a modified backcrossing approach where selection for the trait is only possible in an F_2, and c) the ability to distinguish the heterozygous *J-2*/*j-2* plants from homozygous jointed plants may increase the probability of identifying horticulturally superior plants while ensuring that the jointless trait is maintained, allowing a more judicious allocation of resources for achieving breeding objectives. In addition, the HRM marker system is one that does not rely on variations in the electrophoretic mobility of PCR amplicons for detection of DNA variation, and the SNPs identified in this study can be easily converted to other detection platforms.

Given the high production and labor costs and uncertainties about future availability of labor resources, as well as increasing imports of product from competing markets, the fresh-market tomato industry is under significant pressure to reduce inputs to maintain viability/profitability (McAvoy and Ozores-Hampton, 2011; Scott et al., 2010; USDA ERS; www.ers.usda.gov/topics/crops/vegetables-pulses/trade.aspx). Such circumstances force the industry to seek new production systems to reduce both dependence on farm labor and cost. A transition to mechanical harvest is one potential solution to this challenge, and the availability of improved jointless pedicel cultivars is a necessary component to this solution as stem-free harvest of fruit is essential. Our markers should contribute to increased use of the *j-2* gene in fresh-market breeding programs, as the locus can now be selected with greater efficiency.

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