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The first genetic map of the American cranberry: exploration of synteny conservation and quantitative trait loci

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Abstract The first genetic map of cranberry (*Vaccinium macrocarpon)* has been constructed, comprising 14 linkage groups totaling 879.9 cM with an estimated coverage of 82.2 %. This map, based on four mapping populations segregating for field fruit-rot resistance, contains 136 distinct loci. Mapped markers include blueberry-derived simple sequence repeat (SSR) and cranberry-derived sequence-characterized amplified region markers previously used for fingerprinting cranberry cultivars. In addition, SSR markers were developed near cranberry sequences resembling genes involved in flavonoid biosynthesis or defense against necrotrophic pathogens, or conserved orthologous set (COS) sequences. The cranberry SSRs were developed from next-generation cranberry genomic sequence assemblies; thus, the positions of these SSRs on the genomic map provide information about the genomic location of the sequence scaffold from which they were derived. The use of SSR markers near COS and other functional sequences, plus 33 SSR markers from blueberry, facilitates comparisons of this map with maps of other plant species. Regions of the cranberry map were identified that showed conservation of synteny with *Vitis vinifera* and *Arabidopsis thaliana*. Positioned on this map are quantitative trait loci (QTL) for field fruit-rot resistance (FFRR), fruit weight, titratable acidity, and sound fruit yield (SFY). The SFY QTL is adjacent to one of the fruit weight QTL and may reflect pleiotropy. Two of the FFRR QTL are in regions of conserved synteny with grape and span defense gene markers, and the third FFRR QTL spans a flavonoid biosynthetic gene.

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Abbreviations

FFRR	Field fruit-rot resistance
QTL	Quantitative trait locus/loci

SSR	Simple sequence repeat
SCAR	Sequence-characterized amplified region
COS	Conserved orthologous set
TAcy	Total anthocyanin
PAC	Proanthocyanidin
KW	Kruskal–Wallis
IM	Interval mapping
MQM	Multiple QTL mapping
MRR	Mean rot rating
RR	Rot rating
MFW	Mean fruit weight
SFY	Sound fruit yield
GAIIx	Genome Analyzer IIx

Introduction

Cranberries are a rich source of an array of dietary phytochemicals that are believed to have beneficial effects for human health, including lowering the risk of cardiovascular disease and cancer, among others (Neto 2007). Reports continue to accrue from both epidemiological studies and clinical trials of the potential benefits of consumption of cranberry products. Consumption of low-energy cranberry juice was reported to increase plasma antioxidant capacity and lipid oxidation in pre-diabetic women (Basu et al. 2011). Improved insulin resistance and plasma lipid profile in obese mice on diets supplemented with cranberry flavonoids was associated with alterations in the adiponectin and AMPK signaling pathway (Shabrova et al. 2011). Cranberry proanthocyanidins reduced dental caries development (Koo et al. 2010) and inhibited osteoclast development and activity (Tanabe et al. 2011). Cranberry proanthocyanidins were also shown to induce apoptosis in cancer cells (Singh et al. 2009, 2012; Kresty et al. 2011). Ursolic acid and esters, abundant in cranberries, inhibited growth of prostate tumor cells and reduced activity of matrix metalloproteinases associated with tumor invasiveness (Kondo et al. 2011).

The American cranberry, *Vaccinium macrocarpon* Ait., is a self-fertile diploid ($2n = 2x = 24$) woody perennial trailing shrub native to North America. The karyotype of cranberry consists of 12 metacentric or submetacentric chromosomes (Hall and Galletta 1971). Although separated from the native cranberry germplasm by only a few breeding and selection cycles, it already appears that varieties selected for high yield or other traits, such as early flowering or early ripening, may be compromised in defenses against insect herbivores (Rodriguez-Saona et al. 2011). Nonetheless, the introgression of desired traits such as field fruit-rot resistance (FFRR) into highly productive genetic backgrounds is being attempted. In common with other woody perennial crops, the long generation interval (5–6 years) to assess traits such as yield, the limited field space, and the expense in maintaining field plots hamper genetic study of cranberry. To identify useful parental material in the germplasm collection, current cranberry-breeding programs typically utilize many crosses, often with a common parent (e.g., 'Stevens'), thus generating many half-sib populations, but with limited progeny population sizes. Approaches that exploit existing breeding populations, albeit with limited numbers, will therefore be most valuable in the genetic mapping of cranberry. Managing multiple traits, and occasionally undesirable linkages, in a breeding program requires a thorough understanding of the genetic basis for these traits and of the genotypes and phenotypes of the available germplasm (Tester and Langridge 2010). Molecular genetic markers and genetic maps are essential tools for investigating the genetic basis for traits conditioned by multiple genes, and for manipulating them efficiently.

Field fruit rot is among the most serious threats to cranberry production. Without fungicide applications, the entire crop may be unsalable. Multiple species of fungi have been associated with cranberry fruit rot, including *Phyllosticta vaccinii*, *Physalospora vaccinii*, *Phomopsis vaccinii, Coleophoma empetri* and *Colletotrichum gloeosporioides,* and comparisons of fungi isolated from ten different cranberry cultivars provided no evidence for fungal species-specific resistance (Oudemans et al. 1998; Stiles and Oudemans 1998). Screening of a cranberry germplasm collection revealed variation in fruit-rot resistance among the accessions, and initial progeny tests indicated that the differences were moderately heritable (Johnson-Cicalese et al. 2009).

This paper presents construction of the first genetic map for cranberry, comparisons of genomic organization with *Arabidopsis thaliana* and *Vitis vinifera*, and identification of quantitative trait loci (QTL) for FFRR, fruit weight, titratable acidity, and sound fruit yield (SFY). The map includes markers previously used for clone (genotype) identification and for investigating relationships among germplasm accessions (Bassil et al. 2009; Polashock and Vorsa 2002), as well as SSR markers transferred from blueberry (*V. corymbosum*) (Boches et al. 2005; Rowland et al. 2010) and cranberry SSRs mined from large scaffolds assembled from SOLiD (Applied Biosystems) short-read sequence data (Georgi et al. 2012). It also includes newly developed cranberry SSR markers from scaffolds [assembled using SOLiD or Genome Analyzer IIx (GAIIx, Illumina) sequence data] that contain putative conserved orthologous set (COS) sequences (Wu et al. 2006; Cabrera et al. 2009), or defense-related (Chen et al. 2006; Laluk and Mengiste 2010) or flavonoid biosynthetic pathway genes (Winkel-Shirley 2001; Polashock et al. 2002; Owens et al. 2008; Preuß et al. 2009; Jaakola et al. 2010).

Materials and methods

Plant materials

The following cranberry genotypes were used for genetic mapping: three accessions exhibiting FFRR (US88-70, US88-1 and US89-3), three susceptible cultivars or accessions ('Stevens', #35, and US88-81), and a total of 182 progeny from four crosses between the resistant and susceptible cranberry parents (Johnson-Cicalese et al. 2009). At the time the crosses were made, the resistance levels of the parents were not known. Based on SCAR fingerprinting (Johnson-Cicalese et al. 2009), two of the resistant accessions, US88-70 and US88-1, are genetically very similar, whereas the third resistant accession (US89-3) and the susceptible accessions/cultivars appear not to be closely related to US88-70/US88-1 or each other. US88-70 was a parent in two of the crosses: CNJ98-153 (US88-70 × 'Stevens'), with 64 progeny; and CNJ98-154 (US88-70 × #35) with 60 progeny. US88-1 was the resistant parent of the third cross, CNJ98-164 (US88-81 × US88-1), with 48 progeny. The fourth cross, CNJ97-86 (US89-3 × 'Stevens'), originally had 48 progeny, but had been reduced to 20 individuals before the present study, preserving the range of rot-resistance phenotypes. While these are not large mapping populations, particularly for the mapping of QTL, the cloned progeny were well-established in experimental plots from which data had been collected over a number of years, as described below. Because two parents, US88-70 and 'Stevens', are represented in more than one cross, three of the populations constitute a single-round-robin partial diallel design. The combined analysis of such populations can increase the power to detect QTL (Verhoeven et al. 2006). Investigating traits in more than one population also provides useful information about the general applicability or family specificity of QTL detected (Verhoeven et al. 2006). Concerning the parents of the four mapping populations, 'Stevens' is a widely adapted, productive cultivar, #35 also is high-yielding and has resistance to the false blossom disease vector (blunt-nosed leafhopper), US88-81 produces fruit with high proanthocyanidin content, and US89-3 is of interest, because its fruit has high total phenolics (Johnson-Cicalese et al. 2009; Vorsa and Johnson-Cicalese 2011). CNJ99-125-1, a fifth-generation inbred derived by self-pollination cycles from the cultivar Ben Lear, was the source of DNA and mRNA for genomic and transcript sequencing on the Illumina GAIIx platform; this is the same genotype used previously for genomic sequencing on the ABI SOLiD platform (Georgi et al. 2012).

Nucleic acid extraction and sequence analysis

DNA for genotyping and sequencing was extracted from greenhouse-grown cranberry leaves as described previously (Stewart and Via 1993; Georgi et al. 2012). RNA was extracted from greenhouse-grown tender shoot tips with leaves using the RNAqueous kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. An Illumina (San Diego, CA, USA) mRNA Seq kit was used to convert the total RNA into a cDNA fragment library (average insert size 354 bp) for sequencing one flowcell lane of 100 × 100 bp paired-end reads on the Illumina Genome Analyzer (GA) IIx. Nuclear genomic DNA was also sequenced using one flowcell lane on the Illumina GA IIx (150 × 150 bp reads); the paired end library insert size averaged 430 bp. Transcript sequences were assembled with ABySS using a range of k-mer values (Simpson et al. 2009; Robertson et al. 2010) and with CLC Genomics Workbench (Aarhus, Denmark). The resulting assemblies were merged using BLAT/CD-HIT-EST (Kent 2002; Li and Godzik 2006) and mapped to the draft cranberry genomic assembly using GMAP (Wu and Watanabe 2005). Genomic sequences were assembled with CLC Genomics Workbench.

Phenotypic evaluation

Phenotypic data, including fruit-rot ratings (Johnson-Cicalese et al. 2009), bloom date, and measures of fruit yield and quality, were collected as indicated in Table 1, from progeny of the four crosses planted in May 2000 in 2.25-m^2 plots (without replication) in a 2-ha bed at the Marucci Center in Chatsworth, NJ, USA. The plots received standard maintenance in terms of harvesting, water management (irrigation and flooding), fertilization, and pesticide applications, except that fungicide applications were withheld in 2005–2007 and 2009. Visual fruit-rot ratings were based on a scale of 1–5, with 1 corresponding to "no rot" and 5 corresponding to "all fruit severely rotten". Fractional ratings were used, particularly at assessment times when the entire phenotypic range was not present. Quantitative trait mapping methods have been found to work reasonably well on categorical data with at least five categories (Li et al. 2006), as in the rating scale described here. These ratings reflect broad-spectrum rot resistance or susceptibility only, and may fail to identify differences in resistance to particular species of rot fungi. Ratings in years in which fungicides were applied (2004 and 2011) were averaged separately from ratings in years without fungicide applications, as ratings under the two regimes are less-strongly correlated. Where more than one rating was

Table 1 Means/standard deviations and ranges (minimum–maximum) of phenotypic data collected for four full-sib cranberry families

Trait	Mapping population			
	CNJ98-153	CNJ98-154	CNJ98-164	CNJ97-86
Field fruit rot				
7 Oct 2004 rot rating[a]	1.92/0.10 (1–4)	2.12/0.09 (1–4)	2.11/0.11 (1–4)	1.85/0.18 (1–3)
30 Aug 2005 rot rating	3.30/0.10 (2–5)	3.50/0.10 (2–5)	3.94/0.10 (3–5)	2.75/0.22 (1–4)
6 Oct 2005 rot rating	4.42/0.07 (3–5)	4.37/0.09 (3–5)	4.70/0.08 (3–5)	3.88/0.20 (2.5–5)
21 Aug 2006 rot rating	3.12/0.08 (2–4)	3.07/0.07 (2–4)	2.99/0.07 (2–4)	2.48/0.19 (1–4)
12 Sept 2006 rot rating	4.38/0.07 (3–5)	4.35/0.08 (3–5)	4.74/0.05 (4–5)	3.25/0.23 (1.5–5)
Mean Aug 2005–2006 rot rating	–	–	–	2.61/0.18 (1.25–4)
20 Aug 2007 rot rating	4.15/0.11 (2.5–5)	4.27/0.09 (2.5–5)	4.38/0.10 (2.5–5)	–
Mean Aug 2005–2007 rot rating	–	–	3.77/0.07 (2.83–4.67)	–
4 Sept 2009 rot rating	3.66/0.11 (2–5)	3.88/0.10 (2–5)	–	–
Mean Aug–Sept 2005–07 and 2009 rot rating	3.55/0.07 (2.38–4.75)	3.68/0.07 (2.5–4.75)	–	–
17 Sept 2011 rot rating[a]	4.33/0.08 (3–5)	3.93/0.10 (2–5)	–	–
Mean 2004 and 2011 rot rating[a]	3.13/0.07 (2–4)	3.03/0.06 (2–4)	–	–
Bloom date				
First bloom 2008 (Julian date)	155.6/0.18 (155–163)	155.8/0.17 (155–158)	158.1/0.40 (155–163)	–
Last bloom 2008 (Julian date)	189.4/0.55 (179–198)	193.2/0.54 (185–198)	197.3/0.37 (185–198)	–
Fruit yield and quality				
13 Sept 2010 total yield (g/0.09 m^2)	96.9/8.3 (7.4–343.3)	165.0/13.2 (14.6–424.8)	–	–
13 Sept 2010 sound fruit yield (g/0.09 m^2)	70.6/6.6 (3.1–275.1)	139.6/11.7 (7.8–387.0)	–	–
13 Sept 2010 % rot[b]	25.63/0.04 (6.7–66.9)	14.31/0.04 (0–48.4)	–	–
13 Sept 2010 Total anthocyanin (mg/100 g fruit)	12.86/0.75 (5–32)	8.45/0.51 (2–19)	–	–
13 Sept 2010 Brix (% solids)[b]	8.86/0.0002 (7.26–10.12)	8.92/0.0002 (7.48–10.12)	–	–
13 Sept 2010 % titratable acidity[b]	2.5/0.00004 (2.11–2.82)	2.6/0.00006) (2.19–2.90)	–	–
13 Sept 2010 proanthocyanidin	1.00/0.02 (0.59–1.48)	1.05/0.02 (0.73–1.50)	–	–
13 Sept 2010 fruit weight (g/berry)	1.69/0.04 (0.93–2.47)	1.73/0.04 (1.08–2.75)	–	–
4 Aug 2011 fruit weight (g/berry)	1.55/0.03 (1.04–2.20)	1.37/0.03 (0.87–2.04)	–	–
12 Sept 2011 fruit weight (g/berry)	1.79/0.04 (1.06–2.46)	1.77/0.03 (1.18–2.38)	–	–
Mean Sept 2010–2011 fruit weight (g/berry)	1.73/0.04 (1.07–2.33)	1.75/0.03 (1.24–2.50)	–	–
Inoculation studies				
2010 percent rot[b]	–	40.98/0.05 (11.2–72.5)	–	–
2010 rot diameter	–	19.84/1.01 (5.3–35.9)	–	–
2010 berry diameter	–	14.74/0.14 (12.4–17.3)	–	–
2011 percent rot[b]	36.60/0.03 (9.4–77.5)	45.02/0.02 (23.2–74.5)	–	–
2011 rot diameter	15.83/0.61 (7.3–27.2)	17.50/0.59 (9.3–34.1)	–	–
2011 berry diameter	14.81/0.14 (12.3–17.5)	14.03/0.16 (10.0–16.5)	–	–
Mean 2010–2011 percent rot[b]	–	42.42/0.02 (11.7–65.2)	–	–
Mean 2010–2011 rot diameter	–	18.47/0.64 (5.3–29.3)	–	–
Mean 2010–2011 berry diameter	–	14.39/0.13 (11.9–16.7)	–	–

"–" denotes not done

[a] With fungicide applications

[b] Back-transformed from arcsin $\sqrt{p}$ transformed data

performed in a season, the earlier rating was included in the average. Averaging ratings from different years produces trait data with a more continuous distribution, which are thus more appropriate for QTL analysis, and may increase the significance of marker–trait associations (Simko and Piepho 2011). First and last bloom dates were based on visual examination of plots for the first flower undergoing anthesis, and the completion of petal senescence (all petals dropped). Yield was determined by harvesting and weighing all fruit within a 0.09 m^2 square, sorting the harvested fruit to obtain SFY, and counting 50 (in 2010) or 25 (in 2011, due to lack of fruit) berries and weighing to determine average berry weight. Fruit chemistry traits were analyzed using standard industry methods: total anthocyanin (TAcy) was measured spectrophotometrically in an acidic environment at 515 nm; Brix was measured as percent soluble solids using a refractometer; and titratable acidity (expressed as milliequivalents of citric acid) was determined using 0.1 N NaOH and an endpoint of pH 8.1 (Deubert 1978; Sapers et al. 1983). Proanthocyanidin (PAC) was measured spectrophotometrically using 4-dimethylaminocinnamaldehyde (DAC) as a reagent, at 640 nm (essentially as described in Vorsa and Johnson-Cicalese 2005). Percentage traits (percent rot, Brix, titratable acidity) were subjected to arcsin $\sqrt{p}$ transformation prior to analysis.

Inoculation studies

Experimental inoculations with *Phyllosticta vaccinii* were performed in 2010 on population CNJ98-154, and in 2011 on populations CNJ98-153 and CNJ98-154. Inoculum was prepared by placing the tips of wooden toothpicks ($\sim$1-cm long) in 20 ml of potato dextrose broth in baby-food jars. Plugs of potato dextrose agar-grown *P. vaccinii* were added and the jars were incubated on a rotary shaker for 7 days. Control toothpicks were incubated in the liquid medium without *P. vaccinii*. Twenty fruits of each genotype were inoculated by stabbing once with a fungus-colonized toothpick tip, leaving the toothpick in the fruit. In 2010, berries harvested from each of the CNJ98-154 progeny were inoculated on October 8, placed in covered plastic boxes on the benchtop for 11 days, and evaluated on October 19. In 2011, the berries were inoculated in the field while still on the plant, and after 14 days were harvested and brought into the laboratory for assessment; CNJ98-154 was inoculated on August 10 and evaluated on August 24, and CNJ98-153 was inoculated on August 5 and evaluated on August 19. Diameters (equatorial) of the fruit and of the rotten area were measured, and percent rot was estimated visually.

Markers and genotyping

Sequence-characterized amplified region (SCAR) genotypes of the progenies were obtained using multiplex PCR reactions as described by Polashock and Vorsa (2002). To determine the source of bands segregating in the progenies, individual SCAR primer pairs were used in PCR reactions on the parental DNAs. The resulting markers were designated "SCAR" followed by numbers corresponding to the published primer numbers; thus, a marker amplified with SCAR primers 9 and 10 was designated SCAR0910. All other mapped markers are simple sequence repeat (SSR) markers.

Several categories of SSR markers were used for genotyping: initially, markers developed for the congeneric highbush blueberry (*V. corymbosum*), and subsequently, cranberry SSRs when cranberry sequences became available for marker development. Some of the blueberry SSRs had been published before the start of the present project (Boches et al. 2005; Rowland et al. 2010; Supplemental Table 1), and some (Table 2) are reported here for the first time. Initially, cranberry SSRs were mined from large genomic scaffolds selected solely on the basis of their size (Georgi et al. 2012; Table 3 and Supplemental Table 1). Subsequently, scaffolds were selected on the basis of putative gene sequences they contained: BLASTN searches (Zhang et al. 2000) were performed using transcript sequences from *A. thaliana* to identify matching regions in cranberry genomic scaffolds. These scaffolds were retained only if the matching regions are proved to be BLASTN reciprocal best matches, when used to query the *A. thaliana* transcript database. Searches were performed at the nucleotide rather than the amino-acid sequence level, because the cranberry SOLiD sequence scaffolds contained many gaps. Three categories of *A. thaliana* transcripts were used to query the cranberry genomic sequences: a collection of 87 genes (the "defense" set, Table 4) associated with responses to necrotrophic pathogens (Laluk and Mengiste 2010), ten genes encoding enzymes in the flavonoid biosynthetic pathway (Winkel-Shirley 2001; Owens et al. 2008; Preuß et al. 2009; Table 5), and 967 conserved orthologous set (COS) sequences (Table 6) common to sets compiled for both the Solanaceae/Rubiaceae (Wu et al. 2006) and the Rosaceae (Cabrera et al. 2009). The best hit cranberry SOLiD scaffolds for the COS sequences were sorted by size and the largest were tested for reciprocal best matches. One gene (*rph1*) appeared in both the defense and COS gene lists. Scaffold 2505 was retained in spite of failing the reciprocal-best-match test: it was initially identified using the mlo2 transcript sequence, but its reciprocal best-match proved to be mlo12, which is defense-related

Table 2 Newly developed blueberry microsatellite markers used for cranberry genetic map

Marker	T_a (°C)[a]	Forward and reverse primer sequences
2ms2a02	60	ACCGCAAGAGAGAGATTCCA GTTTGATGATCACGGTGGTG
2ms2g09	62	GGGGAACTCAGATGGGTTTT GCTGTCATTTTTCGGAGAGC
2ms4d10b	52	GGAAACGATGCCGTTTTCTA CAACCCTTCCAGGTCAAAAA
3ms2g09	63	CCTAAATTGCAGCCACTGGT ACGGCAAGACAACGTTCATT
5ms2b12	52	AAAACTGCAACTGGAATCGG GTCTGCAGGTCACAGGTTCA
6ms4e4b	63	GGCCAAGGTTCTACCCTTTC CAACTACCCACCACCACCAT
CA187F	54	TGCAGAGAGAGTGCGAAAAA TTTGCAGCTGATCTGGTTTG
CA325	64	ACCACCCTCCCATTTCAAAC AGGCGAAAAAGGTGTTGATG
CA933	64	TCCCTCGTACAAATTGAGGAA GATCAGGTGAAGAGCTTGGC
CA1413	58	GCGATCCTTCAACTCCTTCA ATACCCCAGGAGGAAAGGAA
Contig130Fb	61	GAGATTCTCGCTTTTTCCCC ATGCACAGCTGCAACAAAAG
Contig259Fb	59	TTGCTGAAGCCCTAAGCAGT AAACCAGATCTGTTGGACGC
Contig428	63	TTGGCCAGAACAACCAAAGT CGTCGTGTTCCTCTTGTTCA
Contig480Fb	56	GATGATGTGGGGCCTAAGAA CGCATTCGACTCAATGTTGT
Contig600	56	GCCAAAGCTGGAGAGAGAAA GACTTCAGCAGCCAACATCA
Contig652	53	AAAACTGTCGGCAGATCCTC GGGATACCAATGTGGGTCAG
Contig704	53	AAATGGCAGGAATCATGGAC CTGTTGATCAGCACCACCAC
NA172	62	CCTCGTCCTCCTCTTCCTCT GTTTGACTTTGGAGAAGGCGAAG
NA619	62	TCACACTACAGGCAGGAGAGA GAAGCCCCAGTTCTCACAAG
NA1713	52	ATTCGCGTATGGAAGGTGAC CTCACACCACTGTGGCTCAT
NA1792	58	GCATCATCGCCGTCAAG TTGACTTCATCGAAAGCACG

[a] Annealing temperature

Table 3 Newly developed cranberry microsatellite markers designed from large genomic sequence scaffolds assembled from SOLiD data

Marker	Forward and reverse primer sequences
scf6i	TTGTTTGGTGCTACGAGTGC GGCCTGAACTTTCCTGACTG
scf9e	TCACAGCGGAGAAGTTGATG ATTTGCGAATCAACCCAAAC
scf11l	TAATGAGTGCTGGTTCTGCG TTCAAATCCACGTCAGCAAA
scf15b	CTGCCTTGTTTCCCTCTCTG GGATTGGTTTGTTGGTCGTC
scf17d	TCGCTTGAAGCTTACCGAAT AGAACGAACACCTCGGTCAC
scf19e	AGGGTGTCCTGAACAACTGG TTGGGGACTAAATGGGTTATG
scf20g	TGAGTGCCGATGAGGTATTG AGAGGAGGAGACGTGCATTG
scf21n	ACCAATTCCCTCCCAAGTTC CCCTGGATATTTGCTTGCAT
scf28l	AACTCTTCGCTTTGGTTGGA TCGGTCGTAGAGACGAGGAT
scf31h	TGGAACTCCAAATGTGCGTA TGGCACCATAAATAGCACGA
scf35k	TCACCTTAAACCCTGGCTTG GTGGAGATGGATAGCTTGGG

(Chen et al. 2006) but not included in Laluk and Mengiste's (2010) list. Table 5 contains two markers from scaffolds not identified as containing reciprocal best matches with *A. thaliana* transcripts. One was developed from a scaffold containing a putative homolog of a bilberry (*Vaccinium myrtillus*) gene encoding a VmTDR4, a transcription factor regulating anthocyanin accumulation in fruit (Jaakola et al. 2010; Table 5). The second was developed from scaffold 3514, which appears to encode a UDP-glycosyl transferase with an asparagine in a conserved position at the end of the Plant Secondary Product Glycosyltransferase motif (PSPG box) that has been associated with sugar substrate specificity (Gachon et al. 2005). *Arabidopsis thaliana* protein UGT78D1, which also has an asparagine residue at this position, encodes a flavonol UDP-rhamnosyltransferase (Jones et al. 2003). Thus, the cranberry sequence may encode an enzyme involved in the biosynthesis of the quercitin rhamnoside found in cranberry fruit (Vvedenskaya and Vorsa 2004). The DFR gene sequence in scaffold 9025 (Table 5) corresponds to DFR2 in Polashock et al. (2002).

For comparative mapping, the genomic locations of grape sequences (Jaillon et al. 2007) corresponding to the *A. thaliana* loci listed in Tables 4, 5, 6 were determined by BLASTN searches on the website http://www.plantgdb.org/VvGDB/cgi-bin/blastGDB.pl, and TBLASTX searches on the website http://www.genoscope.cns.fr/cgi-bin/blast_

Table 4 Cranberry microsatellite markers designed from sequence scaffolds containing putative defense-related genes

Marker	Forward and reverse primer sequences	*A. thaliana*		*E* value
		Gene	Locus	
Ig51a	TTGGTGCAAGATCACCACAT GCACAAACGGATGTAGCAGA	IRX5	AT5G44030	0
Ig729b	GAAAGAAAGGTAAAGGGCCG ATCGAAGGCATTTCCATGAG	ZFAR1	AT2G40140	9.00E−40
Ig1296a	CCCCTGAATTCTTGTTCCAA GAGTGGAAAACGCAGTGGAT	ACD1	AT3G44880	0
Ig6523b	CCATCTACCACGGCAGAGAT GCATATTTTGGTTGGATCGG	BDG1	AT1G64670	2.00E−112
Ig9279a	CCACTCATTGCCATCAAGTC ACTGGCTCTGAATGCCATCT	CERK1	AT3G21630	2.00E−102
Ig15420a	TGGGGGATTTCTCACAAGAG AATCCCACTTGATTAGGCCC	PGIP2	AT5G06860	5.00E−42
Ig16780a	GTGAGGGGTGCCAAGTAGTC CCAAAATTGGTGACCCTTTC	GAI	AT1G14920	2.00E−161
Ig13662a	CATCTAGCCATGCACCATTG CCAAGTTCGACATTTTCCGT	AXR2	AT3G23050	6.00E−106
Ig21768b	AGGGGGCAGAGGAAAGTTTA AGGAGGAGGAAGAAGGTGGA	BAK1	AT4G33430	4.00E−73
Ig28559a	CAAGAGTCGCAAATTCCACA CCTCCTTCTAGAGAGGGCCA	BBD1	AT1G75380	5.00E−99
scf191a	TCTCTTCCTCCACTTTCCCC CAACTTGCACTGCTACCCAA	RPH1[a]	AT2G48070	7.70E−27
scf248	CAACTGGAGGCAAAACAACA CACGCATTGCAATTATACCG	AXR6	AT4G02570	4.50E−39
scf439	TTGTGTGATCCGCTACTTGG ATCGTTCAAAACGAAGGGTG	MED21	AT4G04780	1.60E−14
scf511	CTCCCTCCTTCCGATGAAGT CACAAAGTTCCACGCAGAAA	MKK2	AT4G29810	2.50E−13
scf1172	GGGGTTTGTGTGTTTATCGC GTATGCGAATTCAAAGCCGT	MPK6	AT2G43790	1.70E−77
scf1594	ATGCGAATGGAGAAATCTGG ATACCGCAAATGGAGTCTGC	JAR1	AT2G46370	5.70E−23
scf1655c	CATCTATTGATCAGCCGCAA ACGACCATATGAGCCGAGTT	SYD	AT2G28290	4.80E−39
scf2000b	GGCCCTTTTTATCCCCAATA AATCAAAAGCTGCGAGGAAA	COI1	AT2G39940	1.90E−41
scf2177	TCCGGCAACCTACAATCTTC AACCACCGGAACCCATTATT	ERF1	AT3G23240	4.60E−25
scf2505a	CCAGAGAGAAGGGGGAAATC TTATCCCGCCGCTTAGTAGA	MLO12	AT2G39200	3.00E−13
scf2882	CGCTACCATTGTCAGCTTCA ACACTCAAAAGCAGGTGGCT	SLY1	AT4G24210	1.00E−07
scf3072b	AGTTTAAGCGGAGCGAATGA TTTGGCGACATTTTTCTTCC	AUX1	AT1G05180	5.00E−23
scf3298	CAAAACATCATCCGCATCTG ATTACACCCTCCCGACACAT	BOS1	AT3G06490	1.10E−30
scf4860	TTCGCTCAAGTCAACTGTGG CCTTGGACATTTTTCTGGGA	LSD1	AT4G20380	1.40E−07
scf5304	TACACAGCTTCATTCGGCAA AAGCTCACCCAATCGAAAGA	SHMT1	AT4G37930	5.70E−41
scf6213	GCTCGCTCTCGCATATTTTC CCTAGCCCGTTCATCATTGT	DND1	AT5G15410	7.50E−63
scf6341b	AAACCCAGAAATGTTGGCTG TGGTGGTGTGTGTCTGTGTG	FOU2	AT4G03560	2.10E−19
scf6355	ACAATGTTGTCATTCCGACG CTAGACTCGTCCAAAAGCCC	PLP2	AT2G26560	3.00E−20
scf6955c	ATGCCTGCCATTCATCATTT TTTCCGTCATTTCGTCCTTC	IRX1	AT4G18780	1.10E−69

[a] Also a conserved orthologous set sequence

Table 5 Cranberry microsatellite markers derived from sequence scaffolds containing putative flavonoid-biosynthetic pathway enzymes and transcription factors

Marker	Forward and reverse primer sequences	Gene	Locus or accession no.	*E* value
scf2001	CCCTACATTTCTTACCCGGA GGGGCCAGACCTCAATAAAT	BAN	AT1G61720	1.20E−11
scf2253d	TGGATTGTAACCAAGGGCTC GCCCATCAACACGTAAACCT	TT4, CHS	AT5G13930	7.20E−36
scf9025	TGGCTCCTATAGCGTGTCCT GCACACCAGGTTCCTTGATT	TT3, DFR	AT5G42800	2.40E−13
scf12916	GGAGATGGATTTGGCAAGAA ATCCATGTGGCAGCAGTGTA	TT5, CHI	AT3G55120	0.237
scf10688	TCACTTTTCTTTCATGCCCC GTGCTCCCACTAGCCCATAA	VmTDR4	FJ418852	1.00E−106
scf3514	TTGGAAAGGGAGTGTCTGCT TGTTGGCTGTACGATACGGA	UGT	–	–

server/projet_ML/blast.pl, for loci that failed to identify BLASTN grape matches at *E* value <E−4. To determine the frequency of random associations of genes and markers, 20 randomized orders of the comparatively mapped genes were generated for each species (cranberry, *A. thaliana* and grape), using the website http://www.randomizer.org (Urbaniak and Plous 2012) and assigned to the genetic map or genomic locations in the three species for analysis.

Cranberry sequences were submitted to the SSR tool on the Genome Database for Rosaceae website (http://www.

Table 6 Cranberry microsatellite markers designed from sequence scaffolds containing putative COS (conserved orthologous set) genes

Marker	Forward and reverse primer sequences	*A. thaliana* locus	*E* value
scf1p	AGAGTTGCCTCGAAGTAGCG	AT5G42850	1.50E−09
	TGGGTGTGCTGAGTTTCTTG	AT4G27700	9.70E−12
scf8l	CGAATCCGAAGATCAGAAGC	AT1G08550	5.30E−41
	GGGATACCAGAGATTTCCCG		
scf13a	TAGAGGGCGTTGAAAGGAGA	AT4G20130	2.20E−14
	CCCCAAATTTCTCCCCATTA	AT5G44710	1.10E−17
scf14j	CAGCAGAATTCAGGAAAGCC	AT1G67190	1.90E−47
	AGCTTTCCACACGCTCATTT		
scf23c	TCCTCAGCACACGTCAATTC	AT1G65020	2.80E−16
	GCAGTAAGGACCGAGATCCA		
scf26r	ATGATGTTGGATGTGCCTCA	AT2G17705	4.30E−32
	TTCCTCAACAAACCCTCCAC		
scf32j	ATCCACCAAACAAGCCACAT	AT5G48840	4.70E−55
	TCAATCAACGCGATTCCATA		
scf44a	ACAAAACCACTGGCGAAAAC	AT4G03280	9.90E−23
	GAGTGACCAGGGGAGATGAA		
scf45d	TTCTTGTGGTTGTGCTGCAT	AT2G47760	8.10E−21
	TAATGGCTGAAACGCTCACA	AT2G27490	6.40E−06
scf55c	AGCCATTGATCTCCAACCAC	AT1G03110	3.20E−09
	GCGTTTCAATCTTTGGCAAT		
scf72c	GAAGGAAGTGGCAATGATGG	AT5G26760	2.80E−07
	ACAGACAACGAATGCACAGC		
scf79c	GGTTCTTCGTGGCATGATAGT	AT5G03455	5.40E−05
	CCAAATAACCCAGGAGAGCA		
scf94a	ATGATTTCTTCGGTGCGACT	AT1G04200	5.40E−20
	GCATATCTGTCGCCATTGTG	AT5G51430	9.60E−48
scf105g	TCTGTACCTCCCCATTCCTG	AT3G06350	1.00E−24
	CCAAACACGCCGTTAATCTT	AT3G62450	1.20E−06
scf108b	ACATAAACGGCGATTCCAAC	AT4G26900	6.90E−15
	ATTGCTCGAGGATTGGACAC		
scf112c	ATGTGATTCGCGAAGGATTC	AT3G08780	3.00E−05
	GAAATCCGGGGGTGTAAACT		
scf137c	CTCCGGGAACTCTCCATACA	AT3G24090	1.30E−60
	CTTCGTTGTGAACGCAAAAG		
scf142e	CTACCGAGCTGGTTGAGGAG	AT4G02580	7.90E−35
	CGAGCGCATAATCATCTTCA	AT5G38880	1.90E−49
scf144d	TTGCAAATATCGGTAAGGGC	AT4G17620	2.10E−18
	TTTGTTTGTTTCTCCCACCC	AT5G47570	1.40E−20
scf171f	CTTCGCGCTGCTCTCTATCT	AT3G14910	2.90E−24
	ACAAGAGGAAAGCCCTTGGT		
scf203h	AAGTTACAACGGTTCGTGGC	AT4G38750	7.80E−16
	TGCAACATTGTGATGGTCCT		
scf207d	GACACACGTGGTGCACTGTT	AT3G46560	4.00E−09
	GGTTGATCTTAGGAGCTGCG		
scf210	TTTGATGGAGCCTAACAAGGA	AT1G05055	5.10E−48
	GCATATGCAGTTGTTGGTGG		
scf222a	CATGGAAAGGGCAAGTGATT	AT1G07910	3.30E−15
	GAGTCTGAATTCCAGCAGCC		

Table 6 continued

Marker	Forward and reverse primer sequences	*A. thaliana* locus	*E* value
scf239d	CCAAAGAACAGTCCCATGCT	AT1G48170	5.60E−06
	CAACTCCCTTCCAATAGCCA		
scf258d	GTAACGCATTGGTCGGCTAT	AT1G64430	1.10E−09
	TAAGCCAAACCCAATCCAAC		
scf262a	GAGGGGAAAGGAGAACAAGG	AT1G15140	6.90E−10
	CTAGATTGGGCCATGCAGAT		
scf275d	GCTTTTCTGAAGCGATTTGC	AT1G16740	1.70E−30
	CCGCATACACGGCGTACTA		
scf283b	CCCGATCGAAATAAGGAACA	AT3G22680	1.40E−09
	ATTGACGACCCAGACTCCAC		
scf300f	CCACACTTGGTGCATCTCAC	AT2G36895	8.50E−12
	AAAGGATGGGAGCTCATTTG		
scf303c	AACACCGGTCGATACACCAT	AT5G58920	1.50E−35
	TCCAAACGTGTGAAATGTCC		
scf306f	GGGCAAGGATAAAGGGTTGT	AT2G24395.1	7.50E−15
	TGCATGCAACTTCCTAGTCCT		

rosaceae.org; Jung et al. 2008) for SSR identification and primer generation. An 18-basepair M13 extension was added to the 5′ end of the sequence of each forward primer (Schuelke 2000). Primers were obtained from Integrated DNA Technologies (Coralville IA, USA). Cranberry SSRs from SOLiD scaffolds (Georgi et al. 2012) have the prefix "scf" and those from Illumina GAIIx-derived genomic scaffolds have the prefix "Ig".

SSR genotyping of the CNJ98-153 progeny set was performed as described previously (Georgi et al. 2012) with the exception of six primer sets (scf2s, scf4b, scf5k, scf8l, scf26r, and scf30g), for which the PCR reactions were performed using equimolar concentrations of the forward and reverse primers, without a fluorescently labeled M13 primer. These unlabeled PCR products were resolved and detected on an AdvanCE FS96 capillary electrophoresis apparatus (Advanced Analytical Technologies Inc, Ames, IA USA) using DNF-900 separation gel in 80 cm (total length) capillaries and electrophoresed for 90 min at −9 kV. For the remaining three progeny sets (CNJ98-154, CNJ98-164 and CNJ97-86), SSRs were genotyped as described previously (Honig et al. 2010). The annealing step was performed at the temperatures given in Table 2 and Supplemental Table 1 (blueberry SSRs) or at 52 °C (cranberry SSRs).

Map construction

Linkage maps were constructed for each parent and each cross, initially using the regression approach in JoinMap 4.0 (Van Ooijen 2006), and subsequently using the maximum likelihood (ML) method implemented for cross-pollinated populations in JoinMap 4.1 (Van Ooijen 2011). Only severely distorted markers ($p < 0.0005$) were excluded. Haldane's mapping function was used, predominantly with the program's default calculation options; on occasion, the default values for the spatial sampling threshold in ML interfered with mapping and needed to be reduced. For the parental maps in the complete populations (CNJ98-153, CNJ98-154, and CNJ98-164), the grouping tree was used to identify groups, generally using a minimum independence LOD score of 4.0, although individual markers (but not groups of markers) were on occasion added down to LOD 3.0. Groups were subsequently merged if they had at least two distinct strongest crosslinks at an LOD 3.0 or higher. The 'Stevens' map from CNJ98-153 was used for grouping 'Stevens' in the CNJ97-86 (US89-3 × 'Stevens') partial population, and the minimum LOD score was relaxed to 2.0 for grouping the other parent in the cross (and for the CNJ97-86 population as a whole for ML mapping in JoinMap 4.1) as this value appeared to be equivalent to the groupings obtained with the CNJ98-153 'Stevens' map. Nearest Neighbor fit values were used to identify problematic markers and groupings. In JoinMap 4.0, parental maps were compared, and shared markers used as fixed orders to produce integrated maps for the populations. Conflicting orders among markers spaced within ~2 cM (or 5 cM, in CNJ97-86) were disregarded, as such markers were considered to be essentially co-segregating. In such cases, the more informative marker was used to fix the order. Uniparental markers were added to the fixed orders as needed to maintain their original positions in the integrated maps. Only first- and second-round maps were used. A similar process was used to unify the maps of the four

populations in both JoinMap 4.0 and JoinMap 4.1. Marker order conflicts between JoinMap 4.0 maps were resolved by majority; in JoinMap 4.1, plausible positions were considered in choosing between conflicting marker orders. Map coverage was calculated by method four of Chakravarti et al. (1991), by inflating the total length in cM of each linkage group by a factor of $(m + 1)/(m - 1)$, where m is the number of mapped markers in the group; totaling these values for all groups in the map and dividing this total into the total map length.

Quantitative trait loci were mapped on the unified cranberry map with MapQTL 6.0 (Van Ooijen 2009) using the regression algorithm and default settings of the program. Traits were evaluated within populations and years, as well as across populations and years. The ability to combine QTL analysis across populations is a feature of MapQTL 6.0 not present in earlier versions of the program. As implemented in this latest version of the program, the analysis does not take into account the sharing of parents among families, which may result in reduced power to detect QTL (Bink et al. 2012). If, however, the families are not all segregating for the same QTL, separate analyses of the families can increase QTL detection power (Li et al. 2011). Since many of the traits were ratings (that is, categorical traits, not continuously variable quantitative traits), Kruskal–Wallis (KW) tests were performed prior to interval mapping (IM) and multiple QTL mapping (MQM). In view of the number of tests performed, the KW test statistic was considered significant at the 0.0005 level; for mapped markers, a gradient in the test statistic along the linkage group toward the marker in question was additionally required for significance. Significant QTL were declared in IM and MQM results if the LOD score exceeded the genome-wide 95th percentile (i.e., significant at the 0.05 level) calculated from 1,000 permutations. Due to the small sizes of the populations and consequent low available degrees of freedom for modeling, forward selection MQM was performed, using as cofactors those markers nearest QTL identified in IM, with additional rounds of MQM if any additional QTL were identified. MapChart 2.1 (Voorrips 2002) was used to produce the map figure.

Results

Transcript and genomic sequencing of cranberry

Illumina sequencing of transcripts from cranberry shoots and leaves yielded 63.6 million raw reads (31.8 million in each direction) for a total of 6.3 Gbp of sequence data. After quality trimming, assembly, merging and mapping, a total of 48,271 transcript sequences were obtained. Assembly of the 8.4 Gbp of Illumina genome sequence data yielded 231,033 contigs totaling 419,841,097 bp. These assemblies were used in the present work to generate ten SSR markers, described below and in Table 4. More detailed analyses of the cranberry transcript and genome sequence data will be presented elsewhere.

Phenotypic data

Means and standard deviations of the studied traits are presented in Table 1, and correlations among traits within populations appear in Table 7 Rot ratings tended to be negatively correlated with date of last bloom, total yield, and SFY (higher rot ratings correspond to more severe rot). Total yield was also negatively correlated with proanthocyanidin, and total anthocyanin was negatively correlated with titratable acidity. Principal components analyses (PCA) were performed on the rot ratings across years, both for each family separately, as well as combined across years and families. Uniformly, the first principal components, representing the largest proportion (approximately half) of the variation in the data, had eigenvectors consisting of factors of equal sign and approximately equal magnitude (results not shown), and thus are essentially equivalent to a mean of the rot ratings. In several, though not all, of the PCA, the eigenvectors of the second or third principal components showed factors of opposite sign for ratings from years with and without fungicide applications (results not shown), suggesting that some of the variation in the ratings can be attributed to fungicide applications in some of the years. Principal components have desirable attributes, notably statistical independence, and principal components, rather than the data from which they were calculated, could be used for QTL analysis, as Graham et al. (2009) did with principal coordinate analysis (PCO) of developmental traits in raspberry. However, as Graham et al. (2009) note, the interpretation of the derived (PCA or PCO) trait is less than straightforward. In the present case, means of rot ratings were used in QTL analysis, and ratings from years in which fungicide was or was not applied were averaged separately.

Genetic map construction

A total of 182 individual cranberry clones (progenies derived from four crosses) were genotyped with up to 129 SSR primer pairs, including 33 from blueberry and 96 from cranberry, plus the cranberry fingerprinting SCAR marker primers (nine primer pairs). Genotyping revealed four instances of duplicate genotypes, presumably resulting from overgrowth and invasion of neighboring clones: three in CNJ98-153, and the fourth in CNJ98-154, reducing the actual size of these mapping populations to 61 and 59 individuals, respectively. One individual in CNJ98-164

Table 7 Correlations between phenotypic traits within families

	04RR	05RR1	05RR2	06RR1	06RR2	07RR	09RR	NFRR1	11RR	0411RR	Fbloom	Lbloom	Yield	SYld	%rot	TAcy	Brix	Acid	PAC	10SepBwt	11AugBwt	11SepBwt	1011SepBwt	11rot	11Rdia	11Bdia	10rot	10Rdia	10Bdia	1011rot	1011Rdia
05RR1																															
CNJ9853	0.289																														
CNJ9854	0.347																														
CNJ9864	0.352																														
CNJ9386	0.101																														
05RR2																															
CNJ9853	0.553	0.491																													
CNJ9854	0.304	0.614																													
CNJ9864	0.407	0.569																													
CNJ9386	0.124	0.707																													
06RR1																															
CNJ9853	0.348	0.286	0.553																												
CNJ9854	0.171	0.503	0.428																												
CNJ9864	-0.089	0.126	0.029																												
CNJ9386	0.095	0.336	0.310																												
06RR2																															
CNJ9853	0.315	0.357	0.566	0.551																											
CNJ9854	0.369	0.592	0.718	0.621																											
CNJ9864	0.241	0.560	0.395	0.376																											
CNJ9386	0.051	0.518	0.515	0.696																											
07RR																															
CNJ9853	0.192	0.293	0.352	0.358	0.553																										
CNJ9854	0.238	0.325	0.464	0.452	0.567																										
CNJ9864	0.045	0.287	0.324	0.404	0.428																										
09RR																															
CNJ9853	0.276	0.147	0.172	0.153	0.280	0.396																									
CNJ9854	0.254	0.323	0.378	0.143	0.395	0.423																									
NFRR1																															
CNJ9853	0.399	0.644	0.558	0.610	0.635	0.780	0.658																								
CNJ9854	0.360	0.753	0.656	0.667	0.741	0.759	0.698																								
CNJ9864	0.172	0.704	0.470	0.625	0.641	0.808																									
CNJ9386	0.120	0.861	0.648	0.768	0.728																										
11RR																															
CNJ9853	0.265	0.232	0.238	0.211	0.341	0.152	0.361	0.350																							
CNJ9854	-0.083	0.085	-0.001	0.053	0.054	-0.044	0.321	0.155																							
0411RR																															
CNJ9853	0.827	0.320	0.516	0.356	0.408	0.216	0.384	0.461	0.764																						
CNJ9854	0.613	0.303	0.206	0.158	0.296	0.126	0.427	0.367	0.737																						

proved to be the product of self-pollination of US88-81, reducing this mapping population to 47 individuals. Most of the primer pairs amplified distinct single-locus markers. Four pairs produced more than one mappable marker: vccj1 yielded three distinct loci on as many linkage groups, and two distinct loci each were mapped for CA1413, NA800F, and scf210, the last pair (scf210s and scf201w, strong and weak, respectively) being linked. Scf15903c also amplified two loci that clearly cosegregated, so only one was mapped. NA800F amplified many fragments, most of which failed to map, possibly due to difficulties with scoring. Most of the markers amplified and segregated in at least two, and generally all four, of the families, the exceptions being 2ms2g09, ctg704, scf144d, and scf2001 (in CNJ98-153 only); SCAR5859 (in CNJ98-154 only); and NA800F and scf306f (in CNJ98-164 only). In all four crosses, a clear majority of the markers were heterozygous in both parents (Table 8).

In the CNJ98-153 family, segregation of the fragment from the 'Stevens' parent corresponding to vccj1b was severely distorted, with a better fit to a 3:1 than to the expected 1:1 segregation. Since the primer set clearly amplified more than one locus and it was possible that this 'Stevens' fragment represented two independent loci, the fragment was excluded from the CNJ98-153 maps. For consistency, all other similarly distorted markers ($p < 0.0005$) were also excluded from their respective maps: scf300f in CNJ98-153, and scf248 in CNJ98-154.

Marker order conflicts between parents within families were few and involved only closely spaced markers, very likely reflecting merely imprecision in mapping. This removes one possible concern about using JoinMap 4.1's maximum-likelihood method, which is subject to the constraint that marker order is the same in both parents. Marker order conflicts among the families were also negligible. The heterogeneity test failed to detect significant ($p < 0.005$) differences among families in recombination rates between pairs of markers on all linkage groups except Vm1, where most of the significant test statistics (six out of eight) were due to CNJ97-86. Only 20 progeny were genotyped in this family, and their selection was not entirely random, as progeny exhibiting higher levels of FFRR were preferentially retained. The remaining apparent heterogeneity may be attributable to difficulties in

Table 7 continued

	04RR	05RR1	05RR2	06RR1	06RR2	07RR	09RR	NFRR1	11RR	0411RR	Fbloom	Lbloom	Yield	SYld	%rot	TAcy	Brix	Acid	PAC	10SepBwt	11AugBwt	11SepBwt	1011SepBwt	11rot	11Rdia	11Bdia	10rot	10Rdia	10Bdia	1011rot	1011Rdia
Fbloom																															
CNJ9853	0.025	0.092	-0.136	-0.126	-0.208	-0.115	0.194	0.033	-0.239	-0.118																					
CNJ9854	0.122	0.247	0.036	0.213	0.057	-0.068	-0.055	0.104	0.052	0.124																					
CNJ9864	0.115	0.013	0.113	0.172	0.040	0.015		0.075																							
Lbloom																															
CNJ9853	-0.042	-0.039	0.072	-0.177	-0.158	-0.181	-0.294	-0.259	-0.367	-0.242	0.005																				
CNJ9854	-0.081	-0.034	-0.194	-0.301	-0.300	-0.206	-0.207	-0.244	-0.305	-0.297	0.074																				
CNJ9864	-0.194	-0.358	-0.160	-0.069	-0.218	-0.018		-0.216			0.085																				
Yield																															
CNJ9853	0.103	-0.224	0.107	0.008	-0.138	-0.346	-0.300	-0.350	-0.025	0.063	-0.098	0.238																			
CNJ9854	-0.107	-0.498	-0.268	-0.382	-0.349	-0.208	-0.282	-0.473	-0.159	-0.199	-0.164	0.225																			
SYld																															
CNJ9853	0.102	-0.234	0.039	-0.021	-0.225	-0.388	-0.309	-0.381	-0.041	0.053	-0.106	0.246	0.955																		
CNJ9854	-0.056	-0.477	-0.211	-0.352	-0.306	-0.159	-0.236	-0.421	-0.177	-0.178	-0.175	0.183	0.987																		
%rot																															
CNJ9853	0.128	0.101	0.301	0.221	0.320	0.252	0.183	0.280	0.183	0.196	-0.072	-0.178	-0.062	-0.295																	
CNJ9854	-0.228	0.012	-0.095	-0.073	-0.050	-0.215	-0.017	-0.097	0.318	0.097	-0.076	0.078	-0.113	-0.229																	
TAcy																															
CNJ9853	-0.040	0.084	-0.200	0.152	-0.030	0.106	0.190	0.188	0.160	0.067	0.097	-0.246	-0.082	-0.026	-0.162																
CNJ9854	0.051	-0.249	-0.136	0.034	-0.053	0.084	0.017	-0.050	0.148	0.152	-0.291	-0.223	0.143	0.151	-0.043																
Brix																															
CNJ9853	-0.149	-0.016	-0.206	-0.186	-0.281	-0.081	-0.162	-0.165	-0.118	-0.170	0.044	0.105	0.065	0.151	-0.201	0.181															
CNJ9854	-0.286	-0.463	-0.115	-0.068	-0.070	0.052	-0.265	-0.279	-0.206	-0.358	-0.077	-0.164	0.256	0.267	-0.321	0.091															
Acid																															
CNJ9853	0.220	-0.095	0.060	-0.084	-0.006	-0.088	0.297	0.015	0.119	0.220	0.044	-0.049	-0.019	0.011	0.013	-0.273	-0.185														
CNJ9854	-0.199	0.040	0.021	0.177	0.165	0.050	0.190	0.153	0.018	-0.121	0.178	0.229	-0.140	-0.171	0.131	-0.374	0.007														
PAC																															
CNJ9853	-0.034	0.205	-0.094	-0.080	0.107	0.113	0.007	0.118	0.026	-0.008	0.109	0.106	-0.390	-0.290	-0.310	0.020	0.134	-0.081													
CNJ9854	0.139	0.123	0.089	0.064	0.193	0.274	0.035	0.174	-0.178	-0.047	-0.023	-0.086	-0.353	-0.325	-0.181	0.159	-0.023	-0.191													
10SepBwt																															
CNJ9853	0.359	0.028	0.352	0.258	0.369	0.051	0.344	0.240	0.232	0.373	0.000	-0.115	0.179	0.105	0.249	-0.149	-0.288	0.252	-0.296												
CNJ9854	-0.001	-0.206	-0.158	-0.109	-0.231	-0.095	0.040	-0.125	0.134	0.105	-0.080	0.092	0.354	0.325	0.252	-0.020	-0.088	0.122	-0.456												
11AugBwt																															
CNJ9853	0.242	0.029	0.209	0.335	0.198	-0.118	0.043	0.084	0.178	0.267	0.086	-0.110	0.062	0.008	0.136	0.013	-0.227	0.058	-0.090	0.682											
CNJ9854	-0.105	-0.184	-0.191	-0.208	-0.193	-0.207	0.177	-0.128	0.315	0.179	-0.086	0.225	0.104	0.090	0.212	0.108	-0.175	-0.004	-0.359	0.304											
11SepBwt																															
CNJ9853	0.356	-0.057	0.149	0.275	0.148	-0.143	0.023	0.012	0.160	0.332	0.030	0.051	0.226	0.191	0.018	0.001	-0.198	0.034	-0.125	0.580	0.783										
CNJ9854	-0.027	-0.129	-0.232	0.006	-0.169	-0.067	0.154	-0.012	0.203	0.143	0.085	0.056	0.027	0.014	0.078	-0.061	-0.199	0.088	-0.226	0.596	0.526										
1011SepBwt																															
CNJ9853	0.374	-0.048	0.281	0.281	0.290	-0.058	0.207	0.123	0.223	0.381	0.022	-0.052	0.251	0.184	0.170	-0.084	-0.272	0.165	-0.247	0.908	0.815	0.868									
CNJ9854	-0.015	-0.190	-0.215	-0.063	-0.228	-0.092	0.104	-0.081	0.185	0.137	-0.004	0.084	0.227	0.203	0.192	-0.043	-0.156	0.119	-0.392	0.910	0.454	0.875									
11rot																															
CNJ9853	0.215	0.061	0.222	0.347	0.331	0.380	0.311	0.411	0.348	0.349	-0.222	-0.173	0.135	0.091	0.160	0.192	-0.055	-0.009	-0.139	0.293	0.119	0.125	0.242								
CNJ9854	0.275	0.079	0.151	-0.113	-0.031	-0.025	0.221	0.078	0.219	0.361	0.135	0.054	0.089	0.112	-0.119	-0.034	-0.183	-0.128	-0.025	-0.150	-0.018	-0.194	-0.190								
11Rdia																															
CNJ9853	0.178	0.095	0.208	0.362	0.379	0.415	0.330	0.455	0.466	0.393	-0.180	-0.209	0.141	0.068	0.248	0.149	-0.040	-0.051	-0.030	0.334	0.300	0.254	0.333	0.820							
CNJ9854	0.258	0.051	0.167	-0.150	-0.042	-0.077	0.411	0.114	0.301	0.415	0.056	0.091	0.138	0.164	-0.098	0.121	-0.247	-0.109	-0.101	0.045	0.242	0.064	0.060	0.816							
11Bdia																															
CNJ9853	0.036	-0.116	-0.069	-0.019	-0.089	-0.256	-0.245	-0.248	0.007	0.029	0.065	-0.135	-0.012	-0.042	0.082	-0.329	-0.131	0.050	-0.009	0.310	0.470	0.504	0.446	-0.345	-0.059						
CNJ9854	-0.091	-0.296	-0.391	-0.186	-0.288	-0.254	-0.065	-0.273	0.081	0.003	-0.042	-0.047	0.117	0.090	0.124	0.199	0.000	-0.097	-0.222	0.529	0.355	0.671	0.665	-0.486	-0.173						
10rot																															
CNJ9854	0.133	0.129	0.215	0.034	0.249	0.000	0.242	0.152	0.214	0.260	0.110	-0.315	-0.103	-0.093	-0.021	-0.120	-0.070	0.020	0.160	-0.061	-0.154	-0.073	-0.075	-0.053	-0.005	-0.002					
10Rdia																															
CNJ9854	0.098	0.125	0.179	0.056	0.212	-0.032	0.225	0.138	0.230	0.249	0.050	-0.313	-0.095	-0.092	0.068	-0.107	-0.144	-0.033	0.089	0.040	-0.065	0.033	0.041	-0.074	0.000	0.062	0.951				
10Bdia																															
CNJ9854	-0.104	-0.171	-0.207	-0.014	-0.311	-0.192	-0.015	-0.142	0.214	0.099	-0.213	-0.025	0.216	0.174	0.378	0.185	-0.119	-0.073	-0.403	0.672	0.323	0.512	0.669	-0.161	0.008	0.499	-0.219	-0.057			
1011rot																															
CNJ9854	0.272	0.131	0.219	-0.053	0.177	-0.050	0.318	0.142	0.299	0.422	0.179	-0.188	-0.029	-0.016	-0.037	-0.114	-0.204	-0.031	0.092	-0.104	-0.061	-0.115	-0.122	0.516	0.453	-0.274	0.839	0.792	-0.235		
1011Rdia																															
CNJ9854	0.228	0.122	0.207	-0.035	0.155	-0.088	0.381	0.154	0.339	0.423	0.089	-0.186	-0.011	-0.001	0.037	-0.033	-0.274	-0.060	0.012	0.064	0.101	0.078	0.079	0.356	0.513	-0.036	0.829	0.870	-0.032	0.919	
1011Bdia																															
CNJ9854	-0.098	-0.250	-0.320	-0.076	-0.310	-0.239	-0.064	-0.224	0.146	0.049	-0.146	-0.045	0.170	0.133	0.272	0.237	-0.061	-0.080	-0.344	0.690	0.355	0.665	0.759	-0.381	-0.100	0.877	-0.141	-0.011	0.861	-0.293	-0.055

See Table 1 and text for explanation of traits. In column 1, traits are in bold and family designations in plain text

04RR, 05RR, 06RR, 07RR, 09RR, 11RR, fruit-rot rating in 2004, 2005, 2006, 2007, 2009, or 2011; xxRR1, first rot rating of 20xx; xxrr2, second rot rating of 20xx. Scale of 1 (no rot) to 5 (100 % rot). NFRR, mean fruit-rot rating from years without fungicide (2005, 2006, 2007 and 2009 for CNJ98-153 and CNJ98-154; 2005-2007 for CNJ98-164, and 2005–2006 for CNJ197-86); 0411RR, mean of rot ratings from 2004 and 2011 (years with fungicide applications). Fbloom, Lbloom are the first and last bloom dates

10SepBwt, 11AugBwt, 11SepBwt, 1011SepBwt are the berry weights in September 2010, August 2011, September 2011, or the mean of September 2010 and 2011 berry weights, respectively

TAcy: fruit total anthocyanin; Acid: titratable acidity; PAC: fruit proanthocyanidin

10rot, 11rot, 1011 rot: percent rot of fruit inoculated with *Phyllosticta vaccinii* in 2010 or 2011, or the 2010/2011 average, respectively

10Rdia, 11Rdia, 1011Rdia: Rot diameter (mean of 20 fruit) in 2010 or 2011, or the 2010/2011 average, respectively, of fruit inoculated with *Phyllosticta vaccinii*

10Bdia, 11Bdia, 1011Bdia: berry diameter (mean of 20 fruit) in 2010 or 2011, or the 2010/2011 average, respectively, of fruit inoculated with *Phyllosticta vaccinii*

Syld sound fruit yield

Table 8 Numbers of loci exhibiting various segregation patterns (entries include fragments that did not map)

Family	Dominant		Co-dominant			Total
	Uniparental (1:1)	Biparental (3:1)	Two alleles (1:2:1)	Three alleles (1:1:1:1)	Four alleles (1:1:1:1)	
CNJ98-153	48 (18, 30)[a]	10	23	62	1	144
CNJ98-154	54 (25, 29)	6	8	22	48	138
CNJ98-164	60 (29, 31)	3	5	24	39	131
CNJ97-86	53 (11, 42)	3	4	22	49	131

[a] Female parent, male parent

positioning uniparental markers. Very similar maps were obtained with JoinMap 4.0 (using the regression algorithm) and with JoinMap 4.1 (using the maximum-likelihood algorithm), with a few trivial differences in marker order resulting from use of the plausible position information in JoinMap 4.1 for resolving conflicts. The JoinMap 4.1 mapping results are presented here (Fig 1). Summary information on the individual parental and population maps is presented in Supplemental Table 2.

The unified cranberry map contained 138 markers in 14 linkage groups. One small linkage group is drawn below group Vm7 and a second is placed above group Vm11 in Fig. 1; there was cross-link support connecting each of these pairs in at least one family, although not enough to unite them unequivocally. If these two pairings are accepted, the number of mapped groups is 12, corresponding to the haploid chromosome number in cranberry. The total map length was 879.9 cM, and the map coverage was estimated to be 82.2 %. Linkage groups ranged in size from 2.8 to 111.2 cM, with 3 to 22 markers per group at an average spacing of 8.6 cM; the largest gap was 27.7 cM. Although they are represented as separate loci in Fig. 1, markers vccj1a and vccj1b are likely allelic, and CA1413a and CA1413b may be allelic as well. Markers Ig16780a and scf16i remained ungrouped in all four crosses. SCAR0910 was not successfully positioned in the unified map in spite of mapping at a distance of 8.5 cM or less from scf28L in all four crosses. Segregation distortion appeared to be family specific: markers on the bottom of group Vm3 showed distorted segregation ($p < 0.01$) in CNJ98-164 (underlined), whereas segregation distortion on group Vm6 was seen in CNJ98-154 (italics), and on groups Vm7, Vm8, and Vm9 in CNJ98-153 (bold) (Fig. 1).

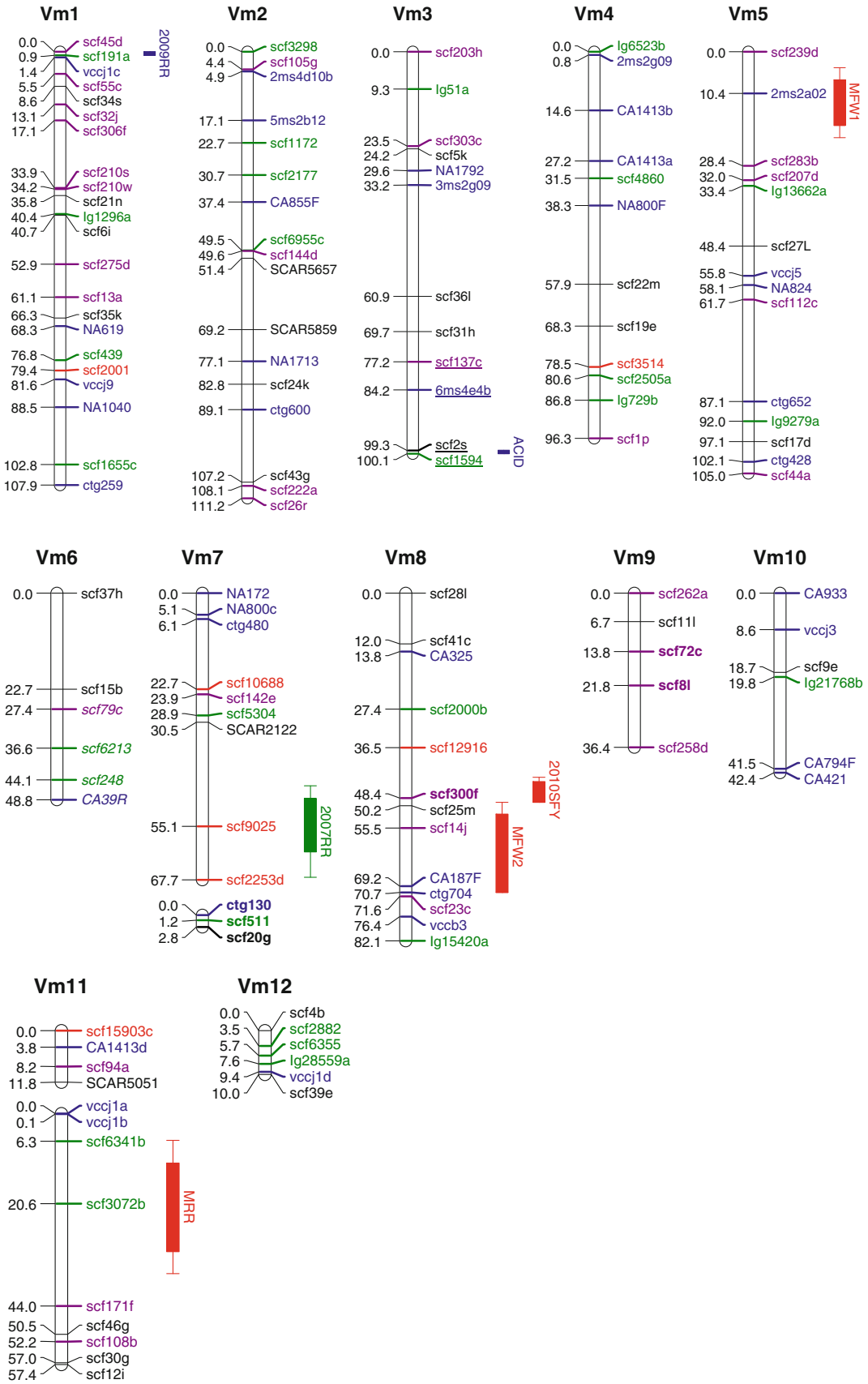
Comparative mapping

Conserved orthologous set sequences were used to develop markers for the cranberry map to facilitate comparisons with other plant species. Two caveats should be mentioned concerning these comparisons. First, a cranberry sequence identified as a reciprocal best match with a COS sequence may or may not be its ortholog. Second, the genetic map location of the putative cranberry COS was determined using an SSR on the same sequence scaffold, and thus is subject to errors in sequence assembly.

Even before the markers were mapped, the co-occurrence of putative COS sequences in cranberry genomic scaffolds (Table 6) provided a first glimpse at the extent of conservation of synteny between *A. thaliana* and cranberry on a small scale, with seven scaffolds containing two putative COS sequences each. Two of the scaffolds (45 and 105) could be said to show synteny conservation in the most general sense of the word: the two putative COS sequences in each corresponded to loci on the same *A. thaliana* chromosome (At2 and At3, respectively), although the pairs of loci are clearly much more distant from each other in *A. thaliana*. Four of the seven scaffolds (1, 13, 142 and 144) contained pairs of COS sequences, one of which corresponded to a locus on At4 and the other to a locus on At5. Numerous genome segments are duplicated between *A. thaliana* chromosomes, including between At4 and At5 (reviewed in Henry et al. 2006), so these four cranberry scaffolds might represent ancestral gene arrangements that in *A. thaliana* underwent selective and complementary gene loss following whole genome duplication.

Ordering the markers on a genetic map allowed synteny comparisons on a larger scale (on the order of linkage group rather than sequence scaffold), and of not merely COS sequences but also mapped defense-related and flavonoid biosynthetic pathway genes, for a total of 70 *A. thaliana* loci corresponding to 63 markers on the cranberry map. Three pairs of loci linked in cranberry (on Vm01 and Vm02) corresponded to pairs of loci that are also very close to each other (and not separated by another locus in the set) in *A. thaliana* (Table 9). This is, however, well within the number of pairs that might be expected to occur by chance, based on simulations (Fig. 2).

Of the available sequenced, BLAST-searchable plant genomes in addition to *A. thaliana,* grape was a logical choice for comparison, because both grape and cranberry are temperate-zone, long-lived woody perennials and occupy similarly basal positions in their respective Rosid and Asterid clades (Angiosperm Phylogeny Group 2003).


Vm1
0.0 scf45d
0.9 scf191a
1.4 vccj1c
5.5 scf55c
8.6 scf34s
13.1 scf32j
17.1 scf306f
33.9 scf210s
34.2 scf210w
35.8 scf21n
40.4 lg1296a
40.7 scf6i
52.9 scf275d
61.1 scf13a
66.3 scf35k
68.3 NA619
76.8 scf439
79.4 scf2001
81.6 vccj9
88.5 NA1040
102.8 scf1655c
107.9 ctg259
2009RR
Vm2
0.0 scf3298
4.4 scf105g
4.9 2ms4d10b
17.1 5ms2b12
22.7 scf1172
30.7 scf2177
37.4 CA855F
49.5 scf6955c
49.6 scf144d
51.4 SCAR5657
69.2 SCAR5859
77.1 NA1713
82.8 scf24k
89.1 ctg600
107.2 scf43g
108.1 scf222a
111.2 scf26r
Vm3
0.0 scf203h
9.3 lg51a
23.5 scf303c
24.2 scf5k
29.6 NA1792
33.2 3ms2g09
60.9 scf36l
69.7 scf31h
77.2 scf137c
84.2 6ms4e4b
99.3 scf2s
100.1 scf1594
ACID
Vm4
0.0 lg6523b
0.8 2ms2g09
14.6 CA1413b
27.2 CA1413a
31.5 scf4860
38.3 NA800F
57.9 scf22m
68.3 scf19e
78.5 scf3514
80.6 scf2505a
86.8 lg729b
96.3 scf1p
Vm5
0.0 scf239d
10.4 2ms2a02
28.4 scf283b
32.0 scf207d
33.4 lg13662a
48.4 scf27L
55.8 vccj5
58.1 NA824
61.7 scf112c
87.1 ctg652
92.0 lg9279a
97.1 scf17d
102.1 ctg428
105.0 scf44a
MFW1
Vm6
0.0 scf37h
22.7 scf15b
27.4 scf79c
36.6 scf6213
44.1 scf248
48.8 CA39R
Vm7
0.0 NA172
5.1 NA800c
6.1 ctg480
22.7 scf10688
23.9 scf142e
28.9 scf5304
30.5 SCAR2122
55.1 scf9025
67.7 scf2253d
0.0 ctg130
1.2 scf511
2.8 scf20g
2007RR
Vm8
0.0 scf28l
12.0 scf41c
13.8 CA325
27.4 scf2000b
36.5 scf12916
48.4 scf300f
50.2 scf25m
55.5 scf14j
69.2 CA187F
70.7 ctg704
71.6 scf23c
76.4 vccb3
82.1 lg15420a
2010SFY
MFW2
Vm9
0.0 scf262a
6.7 scf11l
13.8 scf72c
21.8 scf8l
36.4 scf258d
Vm10
0.0 CA933
8.6 vccj3
18.7 scf9e
19.8 lg21768b
41.5 CA794F
42.4 CA421
Vm11
0.0 scf15903c
3.8 CA1413d
8.2 scf94a
11.8 SCAR5051
0.0 vccj1a
0.1 vccj1b
6.3 scf6341b
20.6 scf3072b
44.0 scf171f
50.5 scf46g
52.2 scf108b
57.0 scf30g
57.4 scf12i
MRR
Vm12
0.0 scf4b
3.5 scf2882
5.7 scf6355
7.6 lg28559a
9.4 vccj1d
10.0 scf39e

◀ **Fig. 1** Unified genetic map of cranberry. Loci in *blue* were identified using blueberry SSR primers; those in *red* are near putative flavonoid pathway genes; those in *green* are near putative defense genes; those in *purple* are near putative conserved orthologous set (COS) sequences. Loci in *bold* are distorted ($p < 0.01$) in CNJ98-153; loci in *italics* are distorted in CNJ98-154; loci distorted in CNJ98-164 are underlined. Quantitative trait loci (QTL) are shown next to the genetic map as 2-LOD support intervals, based on results of MQM (multiple QTL mapping) using the closest marker(s) to the QTL as a cofactor(s). QTL in *blue* are from combined analysis of CNJ98-153 and CNJ98-154 data (2009RR: 2009 rot rating; ACID: 2010 % titratable acidity); the QTL in *green* is from CNJ98-154 data (2007RR is 2007 rot rating). QTL in *red* are from CNJ98-153 data: 2010SFY is 2010 SFY. MFW is mean (September 2010 and September 2011) fruit weight; MFW2 became significant when 2ms2a02 (MFW1) was used as a cofactor in MQM. MRR is mean of August and September rot ratings from years without fungicide sprays (2005–2007 and 2009)

BLASTN and TBLASTX searches of the grape sequence with *A. thaliana* ESTs provided genomic locations for putative grape homologs of 69 total COS, defense, and flavonoid pathway genes (a grape homolog was not recovered for *A. thaliana* locus At4g04780). Synteny conservation between grape and cranberry was predictably more extensive than between *A. thaliana* and cranberry. In a region of Vm01 encompassing the conserved syntenic pair with At2, conservation of synteny with grape expanded to a third cranberry locus and a total of four grape genes (Table 9). None of the simulations contained more than pairs of genes shared between genomes. There were six additional conserved syntenic pairs between cranberry and grape. Based on simulations (Fig. 2), this is significantly ($p < 0.05$) higher than that can be expected by chance. It is also unlikely that the same gene pair would be associated by chance in more than two species. There are $69 \times 68/2$, or 2,346 possible pairs of 69 genes. Counting each of the six possible pairs between the four grape genes in the extended conserved syntenic region on Vm01, plus the six individual pairs, or 12 pairs total, the probability of a chance match between at least one of these and one of the three pairs conserved with *A. thaliana* is $\{1-[1-(12/2346)]^3\}$, or 0.015. The six syntenic pairs conserved with grape included cranberry scaffolds 13 and 144, two of the four scaffolds containing pairs of COS genes split in *A. thaliana* between At4 and At5. These grape syntenic pairs provide additional evidence of an ancestral gene organization, preserved in cranberry and grape, which was duplicated and underwent complementary gene loss during the evolution of *A. thaliana*.

QTL mapping

Seven QTL were positioned on the cranberry map (Table 10; Fig. 1). Both Kruskal–Wallis tests (KW) and interval mapping (IM) identified family specific loci associated with fruit-rot rating: on Vm11 in CNJ98-153 (mean rot rating from years with no fungicide applications) and on Vm7 in CNJ98-154 (mean rot rating in the KW test; August 2007 rot rating in IM). The component alleles of scf2253d were originally scored dominant to investigate their allelic relationships, and were fortuitously included in

Table 9 Regions of conserved synteny in cranberry, grape and *A. thaliana*

Grape chromosome	Grape sequence location	*A. thaliana* locus[a]	Cranberry locus	Cranberry linkage group	Cranberry map position
Vv7	2926435–2923509	**AT2G47760**	scf45	Vm01	0
Vv7	2859510–2860707	AT2G27490	scf45	Vm01	0
Vv7	1892240–1891525	**AT2G48070**	scf191	Vm01	0.9
Vv7	1061822–1066749	AT1G03110	scf55	Vm01	5.5
Vv1	956366–956424	AT4G20130	scf13	Vm01	61
Vv1	958667–959488	AT5G44710	scf13	Vm01	61
NC		**AT3G06490**	scf3298	Vm02	0
NC		**AT3G06350**	scf105	Vm02	4.4
NC		**AT4G18780**	scf6955	Vm02	49.5
Vv2	5653352–5655889	**AT4G17620**	scf144	Vm02	49.6
Vv2	5157517–5148347	AT5G47570	scf144	Vm02	49.6
Vv13	4413530–4413275	AT2G39940	scf2000	Vm08	27.4
Vv13	2164010–2162546	AT3G55120	scf12916	Vm08	36.5
Vv12	4563729–4474431	AT4G03560	scf6341	Vm11	6.3
Vv12	5456074–5463682	AT1G05180	scf3072	Vm11	20.6
Vv19	9630949–1398225	AT3G14910	scf171	Vm11	44
Vv19	6852626–6845729	AT4G26900	scf108	Vm11	52.2
Vv18	8077587–8077935	AT4G24210	scf2882	Vm12	3.5
Vv18	9165158–9165077	AT2G26560	scf6355	Vm12	5.7

NC synteny not conserved

[a] Boldface highlights *A. thaliana* loci showing conservation of synteny with cranberry

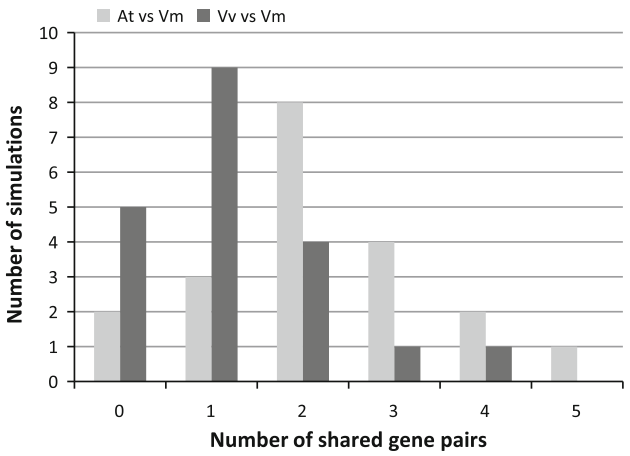


Fig. 2 Frequency of apparent conserved syntenic pairs in randomized data. A total of 20 randomizations were generated each for cranberry, *A. thaliana* and grape. At versus Vm (*A. thaliana* vs. *V. macrocarpon*): 70 genes were randomly assigned to the positions of comparative mapping genes/loci in the *A. thaliana* genome and on the cranberry genetic map. Vv versus Vm (*Vitis vinifera* vs. *V. macrocarpon*): 69 genes were randomly assigned to the positions of comparative mapping genes/loci in the grape genome and on the cranberry genetic map

the QTL analysis; the KW test statistic for the co-dominantly scored scf2253d was significant only at the 0.005 level. A fruit-weight QTL on Vm5 near 2ms2a02 was also identified in CNJ98-153 by both KW (2-year average data) and IM (2010 and 2-year average data). When 2ms2a02 was used as a cofactor in multiple QTL mapping (MQM), a second fruit-weight QTL was detected, on Vm8 near CA187F; including both loci as model cofactors improved the LOD score for the first QTL on Vm5. IM and MQM failed to confirm the QTL for proanthocyanidin (PAC) content identified at scf262a on Vm9 by the KW test. IM additionally identified a QTL for SFY (2010 data, CNJ98-153 family) on Vm8, which did not have a significant KW test statistic. The sound-fruit-yield QTL is adjacent to the second fruit-weight QTL. IM combined across populations detected two additional QTL: one associated with rot rating in 2009, on the top of Vm1, and one associated with titratable acidity on the bottom of Vm3. Determining the locations of QTL near the ends of linkage groups is problematical (Hyne et al. 1995), and the anomalously short QTL intervals seen here may reflect that difficulty. Neither QTL was detected in CNJ98-153 or CNJ98-154 when the data were analyzed separately, although the KW statistic for titratable acidity was significant at the 0.005 level for scf2s in CNJ98-153 (Supplemental Table 3). Several additional marker–trait associations approached significance in KW tests (Supplemental Table 3), but were not significant in IM or MQM, including (among others) associations with the following additional traits: first

Table 10 Significant marker–trait associations

Trait and mapping population	Locus[a]	KW statistic[b] (*df*)	LOD[c]	Variance explained (%)[d]
Field fruit rot				
20 Aug 2007 rot rating				
CNJ98-154	Vm7: scf9025		5.42	34.0
4 Sept 2009 rot rating				
CNJ98-153	Vm1: scf45d		5.51	19.8
CNJ98-154				18.0
Mean Aug–Sept 2005–2007 and 2009 rot rating				
CNJ98-153	Vm11: scf6341b	14.019 (1)		
CNJ98-153	Vm11: scf3072b		5.16	31.0
CNJ98-154	Vm7: scf2253d444[e]	12.970 (1)		
Fruit yield and quality				
13 Sept 2010 proanthocyanidin				
CNJ98-154	Vm9: scf262a	17.828 (3)		
13 Sept 2010 sound fruit yield (g/ft^2)				
CNJ98-153	Vm8: scf300f		4.16	25.9
13 Sept 2010 fruit weight (g/berry)				
CNJ98-153	Vm5: 2ms2a02		4.30	26.6
Mean Sept 2010–2011 fruit weight (g/berry)				
CNJ98-153	Vm5: 2ms2a02	20.263 (3)	6.36	30.2
CNJ98-153	Vm8: CA187F		4.08	17.8
13 Sept 2010 titratable acidity				
CNJ98-153	Vm3: scf2s		6.12	21.9
CNJ98-154				19.0

df degree(s) of freedom

[a] Map marker (KW) or nearest map marker (IM/MQM)

[b] Kruskal–Wallis statistic significant at the 0.0005 level, and showing a gradient in the test statistic (mapped markers)

[c] Significant at the 0.05 level based on permutation test (IM or MQM)

[d] IM or MQM

[e] Marker scf2253d444 is one allele of the tetra-allelic mapped marker scf2253d, which was significant at the 0.005 level

bloom, last bloom, total yield, TAcy, berry diameter, and percent rot of toothpick-inoculated fruit. Some of these associations may prove to be significant in future, larger studies with more statistical power.

The percentage of trait variance explained by the QTL was modest. 2007RR on Vm7 accounted for 34 % of the variance in the August 2007 rot ratings in CNJ98-154; MRR on Vm11 accounted for 31 % of the variance in mean rot ratings without fungicide in CNJ98-153. Also in CNJ98-153, MWF1 on Vm5 and MFW2 on Vm8 accounted for 30.2 and 17.8 % of variance in mean fruit-weight; 2010SFY on Vm8 accounted for 25.9 % of variance in 2010 SFY. 2009RR on Vm1 explained 19.8 % (CNJ98-153) and 18 % (CNJ98-154) of variance in 2009 rot ratings, and ACID on Vm3 explained 21.9 % (CNJ98-153) and 19.0 % (CNJ98-154) of variance in titratable acidity.

For at least one QTL, the favorable allele appears to have come from the phenotypically inferior parent. US88-70, rather than the large-fruited 'Stevens', appears to have provided the favorable allele for fruit weight at MFW1 on Vm5. At other QTL, there appear to be better or worse allelic combinations rather than better or worse alleles. CNJ98-153 individuals homozygous at locus scf45d (QTL 2009RR on Vm1) or scf3072b (MRR on Vm11) had higher rot ratings (more rot) than the various heterozygotes; individuals heterozygous at locus CA187 (MFW2 on Vm8) or scf300f (2010SFY on Vm8) had higher fruit weights or higher SFYs, respectively, than the various homozygotes. At QTL 2007RR and 2009RR in CNJ98-154, and at ACID in CNJ98-153, all individuals were heterozygous, with certain allelic pairings associated with better phenotypes.

Two of the fruit-rot QTL (2009RR on Vm1 and MRR on Vm11) span markers developed from sequence scaffolds containing defense-related genes (Table 4). These two regions are also among those showing conservation of synteny with grape (Table 9).

Discussion

The draft cranberry genome assembly derived from a single flowcell lane of Illumina GAIIx sequence data acquired in the course of this project is superior at the contig level to our previously reported draft cranberry genome assembly using SOLiD sequence data (Georgi et al. 2012). The newer assembly contains more sequence (almost 420 Mbp) in a smaller number of contigs (231,033) than does the earlier one (310 Mbp in 441,159 contigs). The size of the cranberry genome is estimated to be about 570 Mbp (Costich et al. 1993). The success rate for the ten SSR primers designed from the Illumina genomic assembly was 100 %. Although this is a relatively small sample size for meaningful comparison, it does reflect well on the quality of the assembly derived from the Illumina sequence data.

There is some suggestion from the cranberry genetic map (Fig. 1) that the COS markers (purple) and the defense markers (green) may be located in separate genomic regions, for example, on Vm9 and Vm12. Such an arrangement could prove selectively advantageous. In selecting conserved orthologs, that is, genes that are recognizably the same and map one-to-one among species of organisms, there is a bias toward genes that are under selection against duplication or other changes in sequence, such as genes encoding subunits of essential multiprotein complexes like housekeeping enzymes and structural components of cells (Koonin et al. 2004). On the other hand, for defense genes, there is a potential selective advantage to duplication with subsequent functional divergence of the resulting paralogs. One mechanism whereby genes may be duplicated in a genome is through failure of the cellular machinery responsible for blocking re-initiation of DNA synthesis at the many origins of replication in a eukaryotic genome (Green et al. 2010). Conceivably, some of these origins of replication could be less stringently regulated than others. This could be either selectively advantageous or disadvantageous depending on the nature of the genes whose replication they control. Consequently, selection could lead to segregation of highly conserved core essential housekeeping genes, subject to stringent purifying selection, in separate genomic compartments from defense-related genes, which are under diversifying selection.

Although field fruit-rot manifests itself as the fruit ripens late in the season, fruit is infected at or shortly after bloom (Tadych et al. 2012), and fungicide applications during flowering are most important for disease control (Oudemans et al. 1998). Apparently, the fungi are quiescent in or on the immature fruit (Latunde-Dada 2001; Tadych et al. 2012). Consequently, it is difficult to perform controlled inoculations that are both effective and biologically relevant. On the other hand, natural field infections have a plethora of uncontrolled and unknown variables that can be expected to interfere with genetic dissection of fruit-rot resistance. When breeding for fruit-rot resistance, it would be desirable to identify both broad-spectrum resistance, such as the resistance reflected in field fruit-rot ratings, and resistance against specific fungal pathogens. For this reason, it is disappointing that the toothpick inoculations attempted in the current study failed to produce a convincing resistance QTL. Further work will be required to develop an inoculation protocol that is useful for the identification of QTL associated with fruit-rot resistance and resistance to specific fungal pathogens.

Detecting QTL is easier for traits when numbers of contributing loci are low and heritability is high (Li et al.

2006). Studies done specifically to measure heritability in cranberry are limited, but estimates have been obtained (Vorsa and Johnson-Cicalese 2011). In one 3-year study measuring midparent–progeny mean regression of 16 crosses, heritability for berry weight ($r^2 = 0.73$–0.92) and TAcy (0.61–0.80) were found to be consistently high, while yield (0.29–0.47) was more moderate, and Brix (0.05–0.51) and titratable acidity (0–0.34) were variable and generally lower (Vorsa and Johnson-Cicalese 2011). Recent progeny screens including highly resistant parents obtained a moderately high heritability estimate for fruit-rot resistance ($r^2 = 0.52$, Johnson-Cicalese and Vorsa, unpublished). Detection of QTL for mean fruit weight and FFRR, but not for many of the other traits measured in this study, is generally consistent with their relative heritability. While the heritability of titratable acidity in cranberry was found to be low, acidity in other fruits has been found to be conditioned by alleles of large effect at single loci; for example, in peach, the low-acid trait (evaluated organoleptically) segregates as a single-locus dominant trait (D, on G8) (Etienne et al. 2002). Obviously, such QTL are also easier to detect.

Fruit-rot QTL have also been mapped in an advanced backcross population derived from an interspecific cross between *Lycopersicon esculentum* × *L. pennellii* (Frary et al. 2004), although there is no mention of whether the rot was due to fungal or bacterial infection. These authors also report QTL for a variety of fruit quality traits, including yield, fruit weight, Brix, and pH (which is negatively correlated with titratable acidity: Causse et al. 2004), with fruit-rot QTL on chromosomes 3, 5, 8, 9, and 12, and pH QTL on 3 and 12. Unfortunately, only two COS markers linked to cranberry QTL (scf171f, flanking MRR on Vm11; and scf1594, linked to ACID on Vm3) correspond to markers mapped in tomato (Wu et al. 2006). The corresponding tomato markers map to chromosome 7:61.0 and chromosome 10:25.9 on the high-density tomato genetic map (http://www.sgn.cornell.edu/markers/cosii_markers.pl). While Frary et al. (2004) did not discover fruit rot or pH QTL in these locations, a QTL for titratable acidity was found on chromosome 10 (and chromosomes 2, 3, 4, 5, 7, 8, 9, 11, and 12) in a study performed by Causse et al. (2004) on a population of introgression lines also derived from a cross between *L. esculentum* × *L. pennellii.* This study also found that the small-fruited, wild species *L. pennellii* provided alleles that increased fruit weight at seven of 13 fruit-weight QTL (Causse et al. 2004).

Conclusions

The first genetic map of cranberry has been constructed using primarily SSR markers, including a substantial fraction developed from blueberry sequences. Additional markers were designed using cranberry SSRs near various sequences of interest, including putative defense-related and flavonoid biosynthetic pathway genes and conserved orthologous set sequences. Mapped markers also included a number that have been used for fingerprinting cranberry cultivars and accessions. The utility of these highly polymorphic and transferrable markers is evidenced by the successful mapping of most of the markers in all four of the mapping populations used in the study. These populations, though small, were well established, with multiple years of associated phenotypic data, and either shared a parent or had a parent that bore a strong genetic similarity to a parent in other crosses. An exploration of the inheritance of quantitative traits in these populations was undertaken with the expectation that only a small number of QTL of large effect would be detectable. Such loci are also most readily exploitable in breeding programs. A handful of QTL were detected in these populations. Most of the QTL were population specific; however, in two instances, combined analysis across populations permitted the detection of QTL that did not reach significance in the populations when analyzed separately. Two QTL were located in regions of conserved synteny with grape. This offers the possibility of mutually beneficial transfers of knowledge between the cranberry and grape research communities with regard to economically important fruit traits. Larger cranberry mapping populations are under development that will provide greater statistical power for QTL detection and localization.

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