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ORIGINAL PAPER

A major QTL associated with *Fusarium oxysporum* race 1 resistance identified in genetic populations derived from closely related watermelon lines using selective genotyping and genotyping-by-sequencing for SNP discovery

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Abstract

Key message **A major quantitative trait locus (QTL) for *Fusarium oxysporum* Fr. f. sp. *niveum* race 1 resistance was identified by employing a "selective genotyping" approach together with genotyping-by-sequencing technology to identify QTLs and single nucleotide polymorphisms associated with the resistance among closely related watermelon genotypes.**

Abstract Fusarium wilt is a major disease of watermelon caused by the soil-borne fungus *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *niveum* (E.F. Sm.) W.C. Snyder & H.N. Hans (*Fon*). In this study, a genetic population of 168 F_3 families (24 plants in each family) exhibited continuous distribution for *Fon* race 1 response. Using a "selective genotyping" approach, DNA was isolated from 91 F_2 plants whose F_3 progeny exhibited the highest resistance (30 F_2 plants) versus highest susceptibility (32 F_2 plants), or moderate resistance to *Fon* race 1 (29 F_2 plants). Genotyping-by-sequencing (GBS) technology was used on these 91 selected F_2 samples to produce 266 single nucleotide polymorphism (SNP) markers, representing the 11 chromosomes of watermelon. A major quantitative trait locus (QTL) associated with resistance to *Fon* race 1 was identified with a peak logarithm of odds (LOD) of 33.31 and 1-LOD confidence interval from 2.3 to 8.4 cM on chromosome 1 of the watermelon genetic map. This QTL was designated "Fo-1.1" and is positioned in a genomic region where several putative pathogenesis-related or putative disease-resistant gene sequences were identified. Additional independent, but minor QTLs were identified on chromosome 1 (LOD 4.16), chromosome 3 (LOD 4.36), chromosome 4 (LOD 4.52), chromosome 9 (LOD 6.8), and chromosome 10 (LOD 5.03 and 4.26). Following the identification of a major QTL for resistance using the "selective genotyping" approach, all 168 plants of the F_2 population were genotyped using the SNP nearest the peak LOD, confirming the association of this SNP marker with *Fon* race 1 resistance. The results in this study should be useful for further elucidating the mechanism of resistance to Fusarium wilt and in the development of molecular markers for use in breeding programs of watermelon.

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Introduction

Watermelon production in the USA has doubled from 2.2 billion pounds (1.0 kg) in 1980 to over 4.1 billion pounds (1.86 kg) in 2010, with a farm-gate value of $492 million and an average annual consumption of 15.4 pounds (6.86 kg) per capita. As a result of many years of domestication and selective breeding for desirable fruit qualities, the resulting sweet-red watermelon possesses little genetic diversity (Levi et al. 2001, 2013). Consequently, resistance to diseases and pests is poor among watermelon cultivars. In recent years the watermelon crop has experienced numerous losses from soil-borne diseases, including Fusarium wilt (FW) (Bruton and Damicone, 1999; Bruton et al. 1988) and gummy stem blight (Gusmini et al. 2005).

FW [caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *niveum* (E.F. Sm.) W.C. Snyder & H.N. Hans (*Fon*)] is the major soil-borne disease of watermelon (Martyn 1985, 1987; Netzer 1976). In recent years, frequent occurrence of FW in the southeastern regions of the USA has caused significant damage to the watermelon crop and led to the loss of several susceptible varieties from the market. Currently, there are four described pathogenic *Fon* races: 0, 1, 2, and 3 (Martyn and Bruton 1989; Martyn and Netzer 1991; Netzer 1976; Netzer and Martyn 1989; Zhou et al. 2010). *Fon* race 2 is considered to be more virulent than race 0 or 1 and infects all watermelon cultivars (Martyn 1987). Resistance to *Fon* races 0, 1, or 2 but not to race 3 has been incorporated to some degree into various commercial watermelon cultivars. Still, the resistance to *Fon* is not sufficient in most cultivars and the pathogen can quickly reproduce in watermelon production areas. It has been reported that the frequent *Fon* occurrences have reduced yield by 40 % in watermelon fields in the southeastern regions of the United States (Keinath et al. 2010).

Effective control of *Fon* has been accomplished by preplant treatments with soil fumigants. Recently, the most economical and most effective soil fumigant, methyl bromide, has been phased out (Montreal Protocol and the Clean Air Act, 1998), leaving only a few effective alternatives for controlling soil-borne disease and pests. There is great concern among growers that soil-borne pathogens will increase dramatically in the near future, and there is a pressing need to enhance resistance to all races of FW in watermelon cultivars (Wechter et al. 2012).

Significant advances in genomic technologies and high-throughput genotyping techniques have led to the development of efficient marker-assisted selection (MAS) systems useful for plant breeders and geneticists. In addition to traditional phenotype-based selection systems, MAS can be a useful tool in selecting for quantitative trait loci (QTL) associated with disease-resistant traits that exhibit low to intermediate heritability. Although several sources of resistance to Fusarium have been identified in watermelon germplasm (Martyn and Nezter 1991; Wechter et al. 2012), to date there is no known DNA marker that is linked to FW resistance. Recently, the genomes of the elite Chinese watermelon line 97103 (Guo et al. 2013) and the American heirloom watermelon cultivar Charleston Gray (Levi et al. 2011; manuscript in preparation) have been sequenced and assembled. In addition, molecular genetics tools and genomic resources (www.icugi.org), valuable for breeding programs aiming to improve watermelon cultivars, have been developed. Our immediate objective was to develop molecular markers closely linked to genes conferring resistance to *Fon* race 1 for use in breeding of watermelon.

In this study, we employed a selective genotyping approach (Lebowitz et al. 1987; Darvasi and Soller 1992) using a large set of phenotypic data for segregating genetic populations and utilized GBS technology (Elshire et al. 2011) to identify single nucleotide polymorphisms (SNP) linked to *Fon* race 1 resistance QTLs.

Materials and methods

Genetic populations

Genetic populations (F_1, F_2, and F_3) were constructed using the homozygote-resistant (HMw017) and susceptible (HMw013) parental line. These populations were used in a genetic study (at the HM.CLAUSE greenhouse in Davis, California) to determine inheritance of *Fon* race 1 resistance. The F_2 plants were self-pollinated to generate 168 F_3 families that were tested (24 plants in each family) for *Fon* race 1 resistance as described below. DNA was isolated from leaf samples of each of the F_2 parental plants (each representing an F_3 family tested for *Fon* race 1 resistance) as described by Levi et al. (2013). In addition to the genetic populations, the watermelon cultivars Sugar Baby and Charleston Grey were included as highly susceptible and moderately susceptible controls, respectively; Calhoun Grey was used as the resistant control in all tests (Dane et al. 1998).

Fungal isolate and growth conditions

Fusarium oxysporum f. sp *niveum* (*Fon*) race 1 was originally isolated from a watermelon plot in California. The isolate was confirmed to be race 1 by use of differential watermelon varieties as described by Martyn and Netzter (1991). The *Fon* race 1 isolate was maintained on potato dextrose agar (PDA). Isolates were grown under a 12-h diurnal lighting regime using high-intensity fluorescent lighting at 25 ± 2 °C. Inoculum for tests was generated by placing ten 1-cm mycelium/agar plugs from a 10-day-old PDA plate into 1,000-mL Erlenmeyer flasks containing 500 mL of Fusarium medium as described by Esposito and Fletcher (1961). The inoculated flasks were placed on a shaker at 1,000 rpm and incubated at room temperature for 4–6 days. On the day of inoculation, the liquid culture was blended, and the inoculum slurry was adjusted to a spore concentration of 5×10^8 spores/ml distilled water.

Plant growth and fungal inoculations

Seeds were sown into sand in a 240-cell plug tray (Hummert International, Earth City, Missouri) and grown in a greenhouse set at 24–30 °C on top of a heat pad (30 °C). Once the cotyledons were fully expanded, the seedlings were uprooted from the sand, gently washed in water and

trimmed to create wounds for pathogen penetration into the plant. The seedlings were set in the inoculum for 1 min and transplanted in a mix of 1:1 sand and soil in 806-cell plug trays (Hummert International, Earth City Missouri) and placed in a greenhouse at 24–30 °C. Cotyledons of susceptible plants began to wilt about 1 week after inoculation; final disease rating was taken 4 weeks after inoculation according to the following scale: 1 = healthy; 2 = stunted 50 % or less compared to resistant control; 3 = yellow leaf lesions, wilt, poor root system; 4 = dead. A rating of 1 was considered resistant, 2 moderately susceptible, and 3–4 were considered susceptible. As shown in Table 1 and Fig. 1, F_3 families that had a survival rate of 21–24 of 24 plants at the 28th day after inoculation were considered resistant, while F_3 families that had a survival rate of 0–4 of 24 plants were considered highly susceptible. F_3 families that had a survival rate of 5–11 of 24 plants were considered susceptible, while F_3 families that had a survival rate of 12–20 of 24 plants were considered to have moderate resistance.

Selective genotyping

Following the phenotyping of the 168 F_3 families, we used a "selective genotyping" procedure (Lander and Botstein 1989; Darvasi and Soller 1992). We primarily selected the F_2 plants that produced F_3 families with the highest resistance (F_2 plants where their F_3 families showed a survival rate of 87.5–100 %; 21–24 plants survived at the 28th day after *Fon* race 1 inoculation) or susceptibility (F_2 plants where their F_3 families showed a survival rate of 0–16 %; 0–4 plants survived at the 28th day after *Fon* race 1 inoculation). F_2 plants that produced F_3 families with moderate susceptibility (F_3 family showed a survival rate of 21.0–46.0 % at the 28th day after *Fon* race 1 inoculation) also were included for GBS–SNP analysis (Table 1).

Genotyping by sequencing

In collaboration with the team at the Institute for Genomic Diversity, Cornell University, we have optimized the "genotyping by sequencing" (GBS) procedure (Elshire et al. 2011) for watermelon. Of the several restriction enzymes tested in the GBS experiment, *ApeK*I produced a large number of fragments in the low-copy fraction of DNA samples of the cultivars Charleston Gray and Black Diamond. Also, the in silico digest of the Chinese elite watermelon line 97103 genome (Guo et al. 2012) shows that *ApeKI* produced a large number of fragments on all chromosomes (Table 2) and therefore, was considered the most suitable for GBS and SNP analysis of the watermelon genome. Following phenotyping of F_3 families for resistance to *Fon* race 1 (Table 1), high-quality DNA [1 *u*g/10 *u*L] of the parental F_2 plants and their *Fon* Race 1 resistant and susceptible parental lines were used in GBS analysis, as described by Elshire et al. (2011). The TASSEL software Version: 3.0.160 was used for GBS analysis pipeline. The SNP calls were provided in both HapMap and VCF format and were filtered using TASSEL Version 4.0 program, as described at the following website http://www.maizegenetics.net/Table/Genotyping-By-Sequencing/ (Elshire et al. 2011).

SNP analysis and genetic mapping

Ninety-one F_2 plants were selected for GBS analysis based on the phenotypes of their derived F_3 families (Table 1). SNPs with high-quality genotyping data were chosen for QTL analysis, defined as those with less than 20 % missing data for the 91 F_2 plants, polymorphic between the resistant and susceptible control lines, and showing a heterozygous genotype for the F_1 of the cross between the resistant and susceptible lines.

For QTL analysis, each SNP was assigned to a position on the integrated map constructed by Ren et al. (2014). Each SNP in this study was assigned to a position on the integrated map according to the integrated map marker closest to its physical map position. MapQTL 6 (Van Ooijen, 2009) was then used to analyze the association of SNPs with the phenotype. Logarithm of the odds (LOD) significance thresholds ($p < 0.05$) was determined per chromosome and genome wide using a permutation test in MapQTL. Interval mapping using MapQTL 6 was done to determine QTL associated with *Fon* race 1 resistance. Since the analysis was on a selectively genotyped population, single QTL interval mapping was done. Parameters for MapQTL6 were 10,000 iterations, step 0.1 cM, maximum 5 neighboring markers, algorithm: regression, test statistic: LOD, and dominance fitted for F_2. The genetic linkage map and QTLs were visualized using MapChart 2.2 (Voorrips 2002).

Genotyping of entire F_2 population

Genotyping of the 168 plants of the F_2 population with the SNP "S1_67050" closest to the peak LOD of the major QTL was performed using a KASP (LGC Genomics) SNP assay according to the manufacturer's protocol for 384-well plate, wet DNA method. Primer sequences for this assay were as follows: susceptible parent allele-specific primer, 5′-GAA GGT GAC CAA GTT CAT GCT CCT GAA TTG GGT AAG CAG ATC CTT A-3′; resistant parent allele-specific primer, 5′-GAA GGT CGG AGT CAA CGG ATT CTG AAT TGG GTA AGC AGA TCC TTG-3′; common primer, 5′-GAG AAC TAT GGG GAT CCA TCA GAG AA-3′. PCR was done on a Veriti 384-well thermal

Table 1 The resistant (R), susceptible (S), and moderately susceptible (MS) F_3 Families representing their F_2 parental plants selected for genotyping-by-sequencing (GBS) and SNP discovery

Line	Plants germinated	Plants survived	Classification	Line	Plants germinated	Plants survived	Classification
7,174	24	21	[a]R	7,244	24	2	S
7,212	24	21	R	7,263	24	2	S
7,226	24	21	R	7,267	24	2	S
7,252	24	21	R	7,292	24	2	S
7,224	24	22	R	7,302	24	2	S
7,268	24	22	R	7,328	24	2	S
7,275	24	22	R	7,259	24	3	S
7,318	24	22	R	7,261	24	3	S
7,326	24	22	R	7,339	24	3	S
7,329	24	22	R	7,176	24	3	S
7,198	18	18	R	7,214	24	3	S
7,186	24	23	R	7,240	24	3	S
7,187	24	23	R	7,251	24	3	S
7,192	24	23	R	7,221	24	4	S
7,193	24	23	R	7,202	24	4	S
7,201	24	23	R	7,314	24	4	S
7,231	18	17	R	7,323	24	5	[b]MS
7,280	24	23	R	7,305	24	5	MS
7,288	24	23	R	7,270	22	5	MS
7,324	24	23	R	7,230	24	5	MS
7,171	24	24	R	7,196	24	5	MS
7,205	24	24	R	7,213	24	6	MS
7,219	24	24	R	7,223	24	6	MS
7,220	24	24	R	7,271	24	6	MS
7,242	24	24	R	7,181	24	7	MS
7,264	24	24	R	7,331	24	7	MS
7,269	24	24	R	7,183	24	8	MS
7,274	17	17	R	7,209	24	8	MS
7,289	24	24	R	7,238	24	8	MS
7,343	24	24	R	7,265	24	8	MS
7,283	24	0	[c]S	7,266	24	8	MS
7,296	24	0	S	7,301	24	8	MS
7,236	24	0	S	7,272	24	9	MS
7,256	24	0	S	7,313	24	9	MS
7,257	24	0	S	7,177	24	10	MS
7,178	24	1	S	7,211	24	10	MS
7,185	24	1	S	7,225	24	10	MS
7,195	24	1	S	7,241	24	10	MS
7,199	24	1	S	7,248	24	10	MS
7,262	24	1	S	7,188	24	11	MS
7,278	24	1	S	7,203	24	11	MS
7,284	24	1	S	7,239	24	11	MS
7,310	24	1	S	7,243	24	11	MS
7,330	24	1	S	7,286	24	11	MS
7,222	24	2	S	7,333	24	11	MS
7,228	24	2	S				

[a] F_3 families classified as resistant (*R*), having survival rate of 87.5–100 % (21–24 plants) at the 28th day following inoculation with *Fon* race 1

[b] F_3 families classified as moderately susceptible (*MS*), having survival rate of 21–46.7 % (0–4 plants) at the 28th day following inoculation with *Fon* race 1

[c] F_3 families classified as susceptible (*S*), having survival rate of 0–16.7 % (0–4 plants) at the 28th day following inoculation with *Fon* race 1

Table 2 Summary of in silico digest of the Chinese elite watermelon line 97103 genome with the *ApeK*I restriction enzyme used for preparing the genotyping-by-sequencing (GBS) libraries in this study (kindly provided by Dr. Zhangjun Fei, Boyce Thompson Institute, Ithaca NY)

Chromosome	No. fragments
Chr0[a]	24,670
Chr1	36,519
Chr2	36,033
Chr3	29,578
Chr4	26,906
Chr5	37,938
Chr6	29,071
Chr7	32,377
Chr8	28,442
Chr9	37,311
Chr10	29,241
Chr11	29,339

[a] *Chr0* refers to all sequences that could not be assembled on the 11 watermelon *chromosomes* in the sequencing project of the Chinese elite watermelon line 97103 genome (Guo et al. 2012)

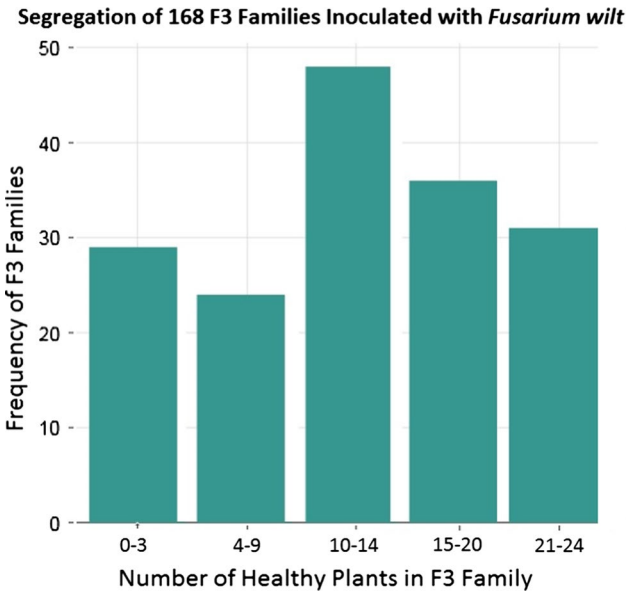

Fig. 1 Histogram showing the segregation pattern of 168 F_3 *families* (24 plants in each family) based on number of plants in each F_3 *family* that survived at the 21 days after inoculation with *Fon* race 1

cycler (Applied Biosystems). Fluorescence was measured on a FLUOstar Omega microplate reader (BMG Labtech) and SNP calling was done using KlusterCaller software (LGC Genomics). One-way analysis of variance (ANOVA) and a post hoc Tukey–Kramer honestly significant difference (HSD) test of mean survival rate after *Fon* race 1 inoculation of each of the genotype categories were done using JMP 9.0 software (SAS Institute Inc., Cary, NC, 1989–2007).

Results

Segregation for resistance to *Fon* race 1

All 18 F_1 plants tested were resistant to *Fon* race 1, while the 168 F_3 families, each representing their F_2 parental plant, exhibited a continuous segregation pattern for resistance to *Fon* race 1 (Fig. 1). The large number of F_3 plants that survived the *Fon* race 1 inoculation (2128 of the 4,019 plants inoculated with *Fon* remained healthy at the 28th day post-inoculation) indicates that the resistance might be controlled by a major effect QTL and several independent QTLs of smaller effect.

Genotyping-by-sequencing

The GBS analysis in this study produced 527,844 tags (64 bp each). Of these tags, 379,125 (71.8 %) were aligned to unique positions, 49,349 (9.3 %) were aligned to multiple positions, while 99,370 (18.8 %) could not be aligned to the watermelon genome. The 379,125 tags that aligned to unique positions were used for SNP data analysis. These unique position tags represent about 5.7 % of the watermelon genome (425 Mb; Arumuganathan and Earle, 1991) [(379,125 tags × 64 bp each)/425 Mb = 5.7 %]. However, these 379,125 unique position tags produced a limited number of useful SNPs (266 SNPs; Table 3).

QTL analysis

The analysis using MapQTL 6 (Van Ooijen, 2009) identified a major QTL significantly associated with resistance to *Fon* race 1 (Fig. 2). This QTL was designated "Fo-1.1" and showed a peak LOD of 33.31 and 1-LOD confidence interval from 2.3 to 8.4 cM on chromosome 1 of the integrated genetic map. The closest SNP marker to the resistance is S1_67050, with LOD of 31.84. Also, the SNP markers S1_169305 and S1_181862 showed significant association (LOD 30.88). Additional independent, but minor QTLs were identified on chromosome 3 (at position 29.2 cM; LOD 4.36), chromosome 4 (at position 13.8 cM; LOD 4.52), chromosome 9 (at position 47.1 cM; LOD 6.8), and on chromosome 10 (at positions 41.8 cM and 76.9 cM; LOD 5.03 and 4.26, respectively) (Fig. 2). The QTL designations, estimated percentage of variance explained, name of the SNP closest to the peak LOD for each QTL and the flanking sequence for the SNP are given in Table 4.

Table 3 The number of single nucleotide polymorphisms (SNPs) produced by the GBS for each of the 11 watermelon chromosomes

Chromosome	Number of SNPs	Genetic distance covered (cM)	Genetic distance in reference map (cM)
1	38	0.0–110.9	115.0
2	9	7.2–90.6	97.0
3	33	0.0–65.8	68.0
4	9	2.2–60.5	62.5
5	18	39.6–96.4	98.0
6	21	0.0–63.3	64.0
7	29	0.6–64.6	66.5
8	23	2.3–29.2	31.0
9	48	6.8–73.9	80.0
10	25	3.9–76.9	96.0
11	13	2.6–30.7	36.5

The genetic distance the *SNP* markers covered in each chromosome versus the genetic distance discovered in the *SNP*-based reference genetic map constructed for watermelon by Ren et al. (2014)

Genotyping of entire F_2 population

SNP S1_67050, closest to the peak LOD of the major QTL for resistance to *Fon* race 1, was genotyped on the entire F_2 population to confirm the QTL which was identified by selective genotyping. There was a statistically significant difference in mean fraction surviving at the 28th day following inoculation with *Fon* race 1 between the genotypic classes (homozygous for resistant parent allele, heterozygous, and homozygous for susceptible parent allele) as determined by one-way ANOVA [$F(2, 159) = 81.13$, $p < 0.01$]. Post hoc comparisons of the mean fraction surviving for each genotypic class using the Tukey–Kramer HSD showed that the mean of each genotypic group was significantly different from each other at $p < 0.01$ (Table 5).

Genomic region of QTL

Exploring the "Fo-1.1" genomic region in the 1-LOD interval around the peak LOD using the 97,103 watermelon genome (Guo et al. 2013) and the Charleston Gray genome data (manuscript in preparation) revealed 212 genes within this 2.03-Mb region, including Cla004884 and Cla004990 that encode glucan endo-1,3-β-glucosidase precursors, Cla004914, Cla004920 and Cla004921 that encode acidic class III chitinase (www.icugi.org), and three other genes which had been identified as putative disease resistance-related genes: Cla004959 that encodes a lipoxygenase, and two receptor-like kinases, Cla004916 and Cla011391 (Guo et al. 2013).

Discussion

The pattern of continuous segregation in response to *Fon* race 1 is in accordance with our finding here that the resistance is associated with a major QTL and with several independent QTLs of small effect. Utilizing the selective genotyping approach proved effective in this study. When a genetic population with a large number of individuals is phenotyped and shows continuous segregation (Fig. 1), selective genotyping of individuals from both ends can be highly effective in the identification of QTL (Lebowitz et al. 1987; Darvasi and Soler 1992). Here, of the 168 F_3 families (Fig. 1), we selected for GBS–SNP analysis 30 F_2 plants that produced F_3 families with the highest survival rate (87.5–100 % at the 28th day after Fon race 1 inoculation) and 32 F_2 plants that produced F_3 families with lowest survival rate (0–16 %; at the 28 day after inoculation). Also, 29 F_2 plants that produced F_3 families with moderate survival rate (21.0–46.0 % at the 28th day after inoculation) were selected for GBS–SNP analysis (Table 1; Fig. 1).

Lander and Botstein (1989) demonstrated that for traits with continuous segregation, the power of QTL analysis increases when the test is based on the quantitative values of individuals at the high and low ends of the population. They used a maximum likelihood approach for the data analysis and named their approach "selective genotyping." In "selective genotyping", individuals from the high and low phenotypic extremes of the entire sample population are genotyped to identify quantitative trait loci (QTL) associated with the trait of interest. Selective genotyping markedly decreases the number of individuals genotyped for a given trait at the expense of an increase in the number of individuals phenotyped (Lander and Botstein, 1989; Darvasi and Soller 1992). Here, the large number of F_3 plants (24 plants in 168 families) phenotyped provided a robust platform for the identification of the most resistant versus most susceptible F_2 parent lines used for GBS–SNP analysis. The further genotyping of entire F_2 population using the SNP S1_67050 (Table 5) validated that the major QTL "Fo-1.1" identified first using the selective genotyping approach is indeed associated with resistance to *Fon* race 1.

The low number of SNPs identified in this study may reflect the overall narrow genetic diversity among watermelon cultivars (Levi et al. 2001). Limited polymorphism was also detected in our earlier PCR experiment with the DNA samples of the resistant versus susceptible parent and of F_2 plants representing the most resistant versus most susceptible F_3 families (data not shown). In these early experiments we tested 2,151 PCR primers, including target region amplification polymorphism, expressed sequenced tag, simple sequence repeat, inter-simple sequence repeat, randomly amplified polymorphic DNA or sequence-related amplified polymorphism primers. These primers

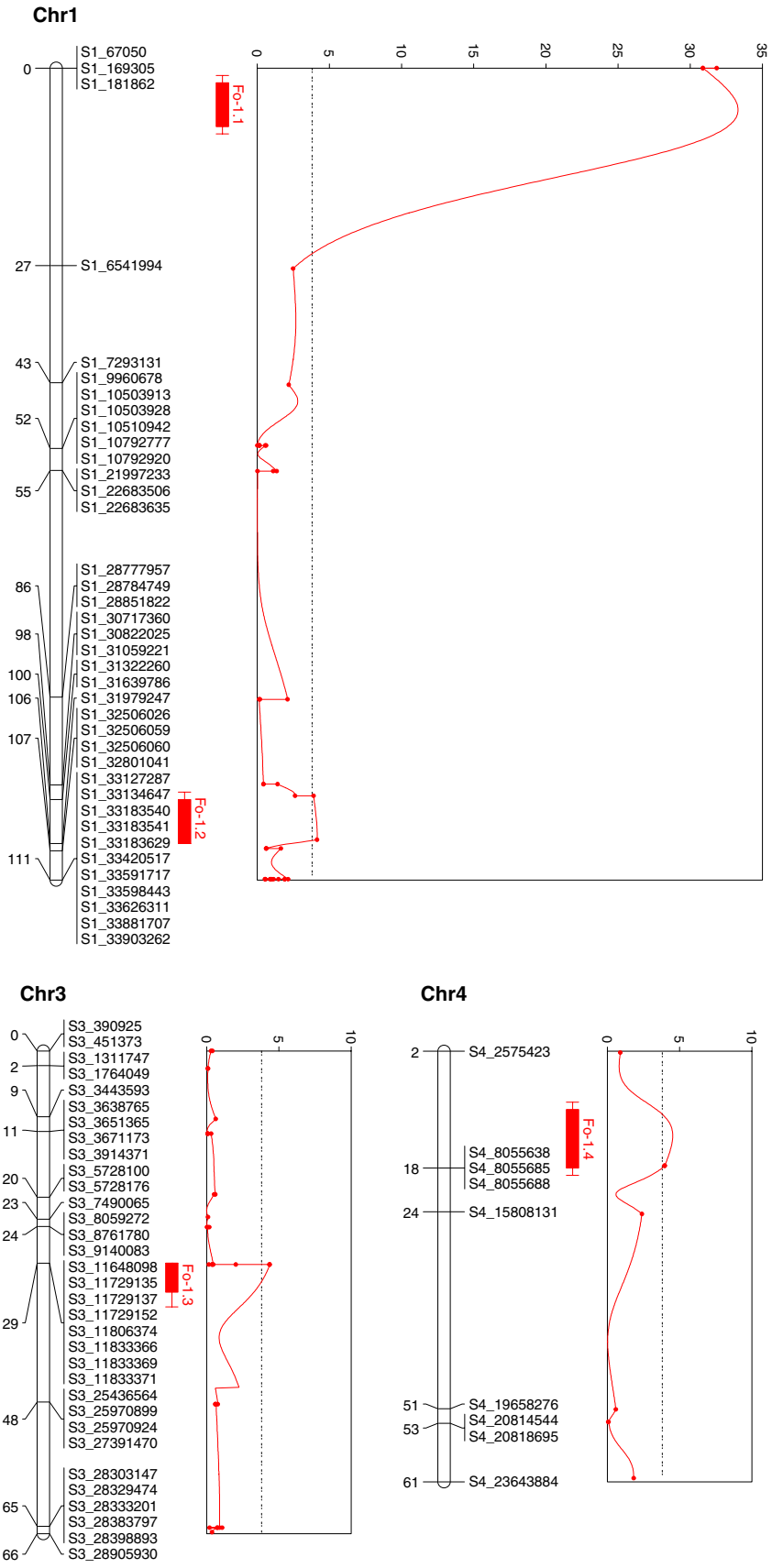

Fig. 2 The genetic linkage map showing the position of SNP markers and QTLs associated with *Fon* race 1 resistance on chromosomes 1 (0.00 cM), 3 (29.2 cM), 4 (13.8 cM), 9 (47.1 cM), and 10 (41.8 and 76.9 cm). The likelihood of QTL associated with Fon race 1 resistance with respect to recombination units (cM) for chromosomes 1, 3, 4, 9 and 10 is graphed to the right of each linkage group. The genome-wide logarithm of odds threshold for declaring significance ($p < 0.05$) (LOD = 3.8) is presented with a broken line. The SNP names indicate the chromosome and physical position of each SNP on the 97103 watermelon genome (Guo et al. 2013)

Fig. 2 continued

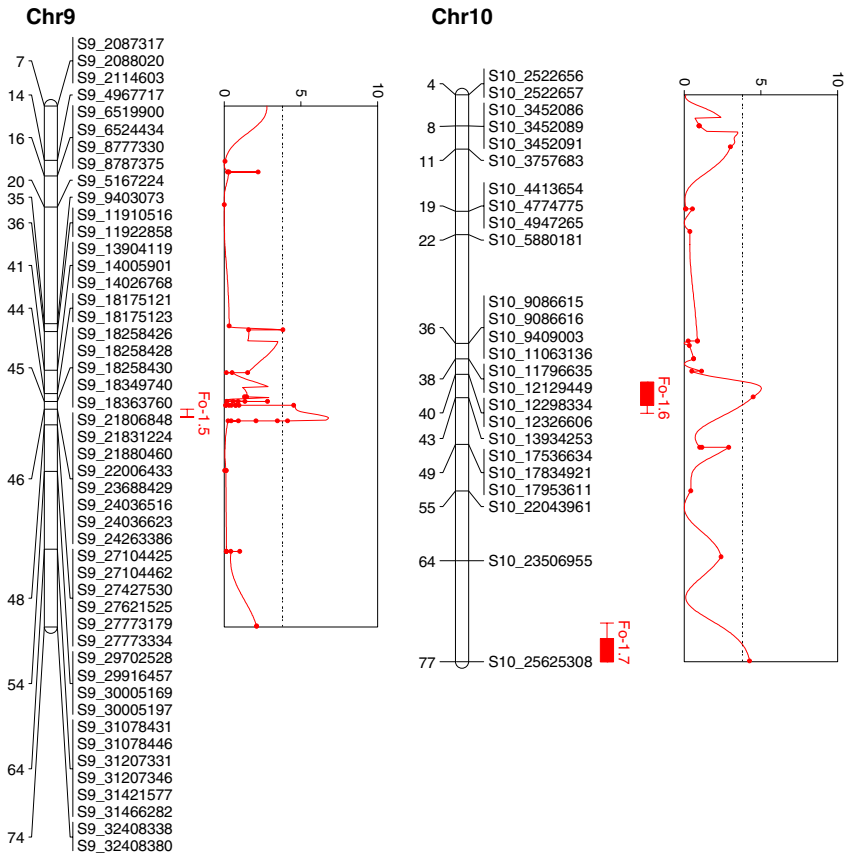

Table 4 The percentage of variance explained by each QTL discovered in the study, the name of the mapped SNP closest to the QTL peak LOD, and the flanking sequence of each SNP

QTL designation	% Variance explained by QTL[a]	Name of closest SNP/variant	SNP or variant (susceptible/resistant parent genotype) and flanking sequence
Fo-1.1	59.9	S1_67050	CAATTGGTATGGCCATTATTATGTCATCTTGGACTGAGA-ACTATGGGGATCCATCAGAGAA[T/C]AAGGATCTGCT-TACCCAATTCAGGGGGGCTGCTGTTGCCATTGCGTCTG-GTATTCTCTCA
Fo-1.2	10.8	S1_31979247	CAGCAACAAATTTCCTCAGCTAGGAGTTCCCTTTCTTCTGT-GCAGATAGATCAGGATCTGAA[G/A]G
Fo-1.3	11.3	S3_11833366, S3_11833369, S3_11833371	CAGCCGCCACCGCTCCAGCTTTCTATCAACGCATATAAATAT AAATTATATAATATAT[GTGGGA/ATGTGG]
Fo-1.4	11.7	S4_8055638	CTGCCCA[C/T]ATGCCTTCCTTTCATCCTTGAGCATTGGGTC CCATTTCTATTTTTC
Fo-1.5	17	S9_27104425	CAGCACCGTCA[T/C]GAACTCTTTGGCTCTCACGTCGGACTT CGAAGGGCACCAA
Fo-1.6	12.9	S10_13934253	CAGCATCAGAGTTT[A/G]CAGCTTCAGCTCCACCGGCTG
Fo-1.7	11	S10_25625308	CAGCTTTATGCTTATCCAATTAAAAAAATTATTAAAAAAAAA AAAAA[A/G]ACAAAAAAAAAAAA

[a] *QTL* analysis was done by interval mapping; the estimated percent variance explained by each *QTL* is not additive

produced only 155 polymorphic markers, and in bulked segregant analysis (Michelmore et al. 1991) none of these polymorphic DNA markers could be associated with *Fon* Race 1 resistance (Harris et al. 2009, 2010). The narrow genetic base among watermelon cultivars is also reflected by the low SNP density (an average of one SNP for 1,431 bp) that exist between the genome of the American heirloom watermelon cultivar Charleston Gray (elongated

Table 5 The mean fraction surviving at the 28th day following inoculation with *Fon* race 1 for each genotype class of SNP S1_67050 on the whole F_2 population

Genotype class	Number	Mean fraction surviving	Standard deviation
R/R[a]	55	0.850	0.136696
R/S[b]	68	0.607	0.210337
S/S[c]	39	0.283	0.259358

Genotype "*R/R*" indicates plants homozygous for the resistant parent allele, "*R/S*" plants heterozygous at this locus, and "*S/S*" plants homozygous for the susceptible parent allele. Genotype classes which do not share the same superscript letter have significantly different *mean fractions surviving* (Tukey–Kramer HSD, $p < 0.01$). Six samples which could not be scored for the SNP marker were excluded from the analysis

watermelon with pink flesh and light grey-green speckled rind) and the elite Chinese line 97103 (small globular watermelon with red flesh and light and dark green stripes) (Levi et al. 2012).

Although GBS analysis produced only a limited number of SNPs, identification of a QTL associated with resistance was still possible. This suggests that SNP data produced by GBS can be efficient in identifying QTL among closely related genotypes when using the "selective genotyping" approach based on a large phenotypic population, as previously indicated (Lebowitz et al. 1987; Darvasi and Soller 1992). As shown here, different studies indicated that a quantitative trait may consist of one or a few (3–4) major QTL and of several QTL with smaller effect (Mackay 2001; Roff 2007).

The 1-LOD interval of the major QTL for resistance to *Fon* race 1 contained 212 genes, several of which could be noteworthy in future investigations of resistance to Fon in watermelon. The presence of pathogenic-related (PR) gene sequences that encode glucan endo-1,3-β-glucosidase precursors (Cla004884 and Cla004990) or gene sequences that encode acidic class III chitinase (Cla004914, Cla004920 and Cla004921) in the Fo-1.1 genomic region may require further testing to determine if any of these gene loci are associated with resistance to *Fon* in watermelon. A recent study (Mazzeo et al. 2014) showed that infecting tomato roots with *Fusarium oxysporum* f. sp. *radicis-lycopersici* induced accumulation of pathogen-related proteins (PR proteins) displaying glucanase and endochitinase activity. The PR proteins glucan endo-1, 3-β-glucosidase A and an acidic 26-endochitinase were present in higher levels in inoculated versus non-inoculated tomato roots. PR proteins are manufactured in plants in response to pathogen attack. Their production and accumulation are an integral part of the plant defense mechanism against pathogen infection (Hou et al. 2012). Several of the PR proteins play a role in signal transduction in response to pathogen attack, while other PR proteins interfere with the phytopathogen by stimulating cross-linking of molecules in the cell wall and in the deposition of lignin, slowing the pathogen's ingress or progression in the plant. Among the PR proteins, the glucan endo-1, 3-β-glucosidases function as endochitinases that inhibit pathogenic fungal expansion by degrading chitin and b-glucan that constitute fungal cell walls (van Loon et al. 2006). In addition, the role of the lipoxygenase gene sequence (Cla004959) and the receptor-like kinases (Cla004916 and Cla011391) should also be investigated. Lipoxygenase proteins are known to be involved in biotic and abiotic defense mechanisms of plants (Akram et al. 2008; Yan et al. 2013), while the receptor kinases belong to a large family of proteins that play important roles in pathogen recognition and in growth and defense of plants (Goff and Ramonell 2007).

Most of the watermelon heirloom cultivars have been developed utilizing germplasm with a narrow genetic base and using traditional breeding procedures that involved recurrent testing and selection for plants with desired fruit qualities, with less emphasis on multi-disease resistance (Levi et al. 2001). For this reason there is a need for enhancing disease resistance in watermelon cultivars using the *Citrullus* spp. germplasm collected throughout the world (Levi et al. 2013). The narrow genetic base among watermelon cultivars is reflected by the low number of SNPs, as shown in this study. However, genetic studies that employ sizeable genetic populations for phenotyping in conjunction with robust SNP analyses for QTL discovery can be useful to identify genomic regions and/or putative gene loci associated with disease resistance. This strategy should allow researchers to identify additional candidate sequences that might be associated with disease resistance or any other horticultural quality in this important specialty crop. Here, the identification of putative PR and putative disease resistance-related gene sequences near the major QTL "Fo-1.1" may contribute to elucidating the mechanisms of resistance to *Fon* race 1 in future studies of watermelon. The results in this study should enhance breeding schemes using MAS for incorporation of disease resistance in watermelon cultivars.

Author contributions Shaunese Lambel Constructed the genetic maps, analyzed the GBS data and used MapQTL to identify the QTL for Fon race 1. Also, she had an active part in writing the manuscript and illustrating the major results. Brenda Lanini and Julie Fauve had an active role in conceiving and planning this study and in constructing and testing the genetic populations. Elisabetta Vivoda conducted the Fusarium wilt experiments and phenotyped the genetic populations for resistance to Fusarium wilt.

W. Patrick Wechter had an active role in planning this study and in optimizing Fusarium wilt inoculation tests and in testing different watermelon cultivars and plant introductions (PIs) used as differentials. Also, he contributed to writing and editing this manuscript. Karen Harris-Shultz extracted DNA from the F2 population, tested a large number of PCR primers and performed BSA with polymorphic markers. She also contributed to data analysis and critical reviewed and edited the manuscript. Laura Massey isolated DNA and prepared DNA samples for GBS. Also, she tested different PCR primers and conducted BSA. Amnon Levi had an active role in conceiving and planning this study, coordinated all details of the project and wrote the manuscript.

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