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Assay development and marker validation for marker assisted selection of *Fusarium oxysporum* f. sp. *niveum* race 1 in watermelon

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Abstract Fusarium wilt, *Fusarium oxysporum* f. sp. *niveum* (FON), of watermelon (*Citrullus lanatus*) is a fungal pathogen that causes significant yield losses in the US watermelon industry. FON damages watermelon through invasion of the root system and remains a difficult pathogen to manage due to its long-lasting survival spores which persist in the soil. Chemical control options for this pathogen are lacking, making development of genetic resistance the best option. There are four known races of FON (0, 1, 2, and 3) which are distinguished based on their pathogenicity of differential cultivars. Most modern cultivar releases have FON race 1 (FON-1) resistance, which has been mapped on the end of chromosome 1. Application of marker assisted selection (MAS) would improve the efficiency of FON-1 resistance breeding. In order to identify markers for selection in the FON-1 region, the QTL-seq method was utilized on an F_2 population segregating for FON-1 resistance. Single nucleotide polymorphism (SNP) markers in the region were developed into Kompetitive allele-specific PCR (KASP™) assays and tested for trait association on the segregating $F_{2:3}$ population. Marker validation was done using an F_2 population from a cross between FON-1 susceptible "New Hampshire Midget" and FON-1-resistant "Calhoun Gray." Further validation on a panel of susceptible and resistant cultivars and Plant Introductions identified SNP marker UGA1_502161 as a useful marker for selection of FON-1 resistance from Calhoun Gray.

Keywords *Citrullus lanatus* · MAS · SNP · QTL-seq · Fusarium wilt · QTL

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Introduction

Fusarium (*Fusarium oxysporum*) is a major yield-reducing disease that causes vascular wilts in over 150 crops, including watermelon. Fusarium wilt of watermelon, caused by *F. oxysporum* f. sp. *niveum* (E.F. Sm.) W.C. Snyder & H.N. Han. (FON), is a soil-borne pathogen that was described in the early 1890s and was first studied in detail by Erwin F. Smith (Smith 1894). The fungus enters the plant through the roots, where it inhabits the xylem resulting in plant wilt and eventual plant death (Martyn 2014). The characteristic wilting symptoms caused by the pathogen are thought to be a result of xylem plugging by the plant to reduce spread of the pathogen; however, recent research on the fusarium species affecting cucumber (*Fusarium oxysporum* f. sp. *cucumerinum*) suggests that toxin release may also be involved (Sun et al. 2017). Spread of the pathogen can result from planting of infected material, contaminated

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soil transfer, or use of infected seed. Once in the soil, fusarium wilt chlamydospores can survive for as long as 8 years without a host, making replanting difficult. Furthermore, the phasing out of the fumigant methyl bromide, which has been used to control fusarium wilt in the past, makes development of genetic resistance to this pathogen critical (Martyn 2014).

There are four known races of FON, race 0, 1, 2, and 3 which are distinguished based on pathogenicity (Armstrong and Armstrong 1978; Correll 1991; Martyn and Netzer 1991; Zhou et al. 2010). Distinction between FON races is determined through virulence tests using differential cultivars, with higher race numbers indicating greater pathogenicity. The cultivars "Black Diamond" and "Sugar Baby" are susceptible to all races of FON, and "Charleston Gray" is resistant to race 0, but susceptible to races 1, 2, and 3. "Calhoun Gray" is resistant to races 0 and 1 and susceptible to races 2 and 3. PI 296341-FR, a selection from a South African accession, is resistant to races 0, 1, and 2, and susceptible to race 3 (Zhou et al. 2010). Further distinction between races 1 and 2 may soon be possible due to recent research focusing on an effector gene, SIX6, which has been shown to play a key role in activation of R-protein-mediated immunity (Houterman et al. 2007; Niu et al. 2016; Schmidt et al. 2013). SIX6 was functional in FON-1 isolates (fonSIX6) and may be responsible for initiating a resistance response in FON-1-resistant genotypes, while FON-2 isolates lacked the fonSIX6 effector gene resulting in escape from the plant's immune system and higher disease severity (Niu et al. 2016).

Soon after the pathogen was first identified in watermelon fields, development of resistant varieties was undertaken by Orton (1907). Due to the lack of resistance in the cultivated germplasm, Orton used a novel approach at the time and crossed a non-edible citron melon (*Citrullus amarus*) showing FON resistance with the edible cultivar "Eden." Subsequent selection and selfing of the progeny produced the first FON-resistant cultivar, "Conqueror" (Martyn 2014; Orton 1907). Although the use of Conqueror was not adopted in all states, Orton made an important contribution to the watermelon breeding community by demonstrating the value of introgression of disease resistance from wild germplasm.

Breeders have developed a wide variety of cultivars with FON-1 resistance (Martyn 2014). Development of the cultivar Calhoun Gray was focused specifically on introgressing FON-1 resistance into the Charleston Gray cultivar type. Resistance in Calhoun Gray (North Louisiana Experiment Station, 1965) was derived from "Florida Seedling 124," a selection developed at the Leesburg research station in Florida in 1936 (Crall 1981a). The wild *C. amarus* accession PI 296341-FR was used to develop "SP-4" in the Super Pollenizer™ series of pollinizers (Brusca and Zhang 2012; Zhang 2008), which have resistance to races 0, 1, and 2. Resistance to races 0 and 1 is common in modern seeded watermelon cultivars such as the cultivars "Sangria" and "Top Gun" (Syngenta) as well as triploid seedless varieties, e.g., "Sweet Dawn," "Fascination" (Syngenta), "Joy Ride," "Road Trip," (Seminis), and "Millionaire" (HM Clause).

When selecting for FON-1 resistance, current watermelon breeding programs typically use disease screening methods which are slow and inefficient compared to molecular breeding methods (Tester and Langridge 2010; Wehner 2008). Application of MAS in FON-1 breeding programs would increase breeding efficiency by eliminating the need to screen for resistance at each selection cycle. A major QTL for FON-1 resistance was mapped on the end of chromosome 1, providing a region for development of markers for selection (Lambel et al. 2014). The same FON-1 QTL was found in both PI 296341-FR (0.08–0.66 Mb) and Calhoun Gray (0–1.1 Mb), further underscoring the usefulness of markers in this region (Meru and McGregor 2016; Ren et al. 2015). Markers in the FON-1 QTL region have been identified (Lambel et al. 2014; Meru and McGregor 2016; Ren et al. 2015), including two SNP markers for potential selection of FON-1 resistance in watermelon [S1_67050 (Lambel et al. 2014) and Chr1_502124 (Ren et al. 2015)]. However, these markers have either not been validated in diverse genetic backgrounds or have not been developed for high-throughput genotyping, which is essential for practical application in breeding programs.

Limited marker development for FON-1 resistance is likely due to the low density of markers in the FON-1 region of chromosome 1. The QTL-seq method utilizes a bulk segregant analysis approach (Michelmore 2000) paired with whole-genome sequencing to identify SNP markers highly associated with a trait of interest and may be useful to identify more markers in the FON-1 region associated with resistance (Michelmore 2000; Takagi et al. 2013). Following SNP identification, high-throughput genotyping assays need to be

developed for routine MAS in breeding programs. Kompetitive allele-specific PCR (KASP™; LGC Genomics LLC, Teddington, UK) has become increasingly popular for SNP genotyping as it is highly specific, cost-effective, and high-throughput (Semagn et al. 2013; Shi et al. 2015).

The purpose of this study was to utilize the QTL-seq method to saturate the FON-1 resistance locus on chromosome 1 with SNPs and develop high-throughput KASP™ assays validated in different genetic backgrounds for MAS of FON-1 resistance in watermelon breeding programs.

Materials and methods

Plant material

For QTL-seq, the same $F_{2:3}$ population ($n = 87$) previously used to map FON-1 (Meru and McGregor 2016) was used in the current study. The population is a cross between the susceptible cultivar Sugar Baby ("SB") and the highly FON-1 resistant cultivar Calhoun Gray ("CALG"). An additional F_2 population of 110 individuals was developed from a cross between the FON-1 susceptible cultivar "New Hampshire Midget" ("NHM") and CALG for marker validation. Additionally, a validation panel was constructed that included cultivars of various different pedigrees and Plant Introductions (PI) of different species to provide a wide variety of genetic backgrounds (Table 1). The panel consisted of both susceptible (11) and resistant (6) cultivars as well as susceptible (5) and resistant (1) PI accessions, and FON-1 resistance of all cultivars and PIs was verified through disease screening.

FON-1 inoculation, disease rating, and DNA extraction of validation germplasm

Seedlings of the NHM × CALG F_2 population and the validation panel were inoculated with FON-1 [(B05-7) provided by Anthony Keinath, Clemson University] in the greenhouse, and disease severity ratings were performed using the protocol in Meru and McGregor (2016) with the following adjustments. Seedling trays were filled with Fafard3B (Sun Gro Horticulture, Agawam, MA) soil amended with osmocote 14N-4.2P-11.6K, and severity ratings were taken 27 days after inoculation rather than 26 days for cultivars screened in 2017. Sugar Baby and Black Diamond were included as susceptible controls, and CALG as the resistant control. For the F_2 population screen, NHM (susceptible parent) and F_1 seedlings were also included. Three replicates of 4 plants for each cultivar or PI were screened for the validation panel with 14 cultivars and PIs in the summer of 2016 and 9 additional cultivars and PIs in the summer of 2017. DNA was extracted for both the NHM × CALG F_2 population and the validation panel using the King et al. (2014) extraction method, with the following modifications. For both the F_2 population and the validation panel, samples were frozen on liquid nitrogen prior to adding the first buffer, tissue was macerated using a TissueLyser II (QIAGEN, Hilden, Germany), 500 µl of the combined NaCl and extraction buffer [40% (*v*/*v*) 5 M NaCl and 60% (*v*/*v*) extraction buffer (200 mM Tris/HCl pH 7.5, 250 mM NaCl,

Table 1 Watermelon cultivars and PI accessions used for marker validation

Accession names	Subspecies	FON-1 severity rating ± SE[b]
Calhoun Gray	*C. lanatus*	0.26 ± 0.09
Allsweet	*C. lanatus*	0.75 ± 0.41
Sunsugar	*C. lanatus*	0.75 ± 0.43
Sugarlee[a]	*C. lanatus*	0.00 ± 0.00
PI 296341-FR	*C. amarus*	0.20 ± 0.13
Crimson Sweet	*C. lanatus*	1.58 ± 0.57
AU-Producer	*C. lanatus*	0.67 ± 0.31
Sugar Baby	*C. lanatus*	5.00 ± 0.00
Black Diamond	*C. lanatus*	5.00 ± 0.00
Charleston Gray	*C. lanatus*	2.17 ± 0.53
Orangeglo	*C. lanatus*	4.33 ± 0.29
Congo	*C. lanatus*	4.70 ± 0.31
New Hampshire Midget	*C. lanatus*	4.83 ± 0.11
Florida Giant	*C. lanatus*	5.00 ± 0.29
Golden Midget	*C. lanatus*	5.00 ± 0.35
Klondike Black Seeded	*C. lanatus*	5.00 ± 0.00
Desert King	*C. lanatus*	5.00 ± 0.31
Blacktail Mountain	*C. lanatus*	3.00 ± 0.30
PI 279461[a]	*C. lanatus*	5.00 ± 0.00
PI 560023[a]	*C. mucosospermus*	5.00 ± 0.00
PI 248178[a]	*C. mucosospermus*	4.89 ± 0.11
PI 249010[a]	*C. mucosospermus*	5.00 ± 0.00
PI 595203[a]	*C. mucosospermus*	4.50 ± 0.40

[a] USDA-ARS Plant Genetic Resources Unit (Griffin, GA)

[b] 26 or 27 DAI FON-1 severity ratings and standard error

25 mM EDTA, 0.5% SDS)] was used, and all centrifuging was done for 10 min at 3600 rpm.

For the NHM × CALG F_2 population, leaf tissue was collected from individual seedlings by taking a single hole punch (approximately 5 mm) from the cotyledon just prior to inoculation, while leaf samples for the validation panel were collected from non-inoculated controls after the completion of the screen.

DNA extraction and pooled sample sequencing for QTL-seq

The resistant and susceptible bulks were each comprised of 10 F_2 individuals from the SB × CALG $F_{2:3}$ population previously described (Meru and McGregor 2016; Meru 2014). First, the 15 most resistant and most susceptible $F_{2:3}$ lines were identified based on the average FON-1 disease severity scores at 26 days after inoculation. For each bulk, the 10 lines with the smallest standard error across the three disease screens were then selected. For each selected F_2 individual, DNA was extracted using the E.Z.N.A. Plant DNA kit (Omega Bio-Tek Inc., Norcross, GA) from approximately 50 mg of young leaf tissue frozen in liquid nitrogen. The DNA concentration was measured using an Infinite M200Pro plate reader (Tecan, Group Ltd., Switzerland). Equal amounts of DNA from the 10 individuals comprising a bulk were pooled and shipped on dry ice for sequencing. Library construction and whole genome Illumina sequencing on an Illumina HiSeq X (Illumina, Inc., San Diego, CA) were performed at HudsonAlpha Genomic services laboratory (Huntsville, AL). Constructed libraries were then subjected to 151-bp paired-end whole genome sequencing in the same lane.

Read analysis and SNP identification

The short reads were aligned against the "97103" watermelon reference genome sequence (icugi.org) (Guo et al. 2013). The genome was indexed using BWA, and reads were aligned using BWA MEM (Li and Durbin 2009). SAMtools was then used to convert from SAM to BAM format, to sort and index the converted files, and to calculate genotype likelihoods. SNP calling was performed using BCFtools, and SNPs were then filtered with a depth of 10 using a Python script (Python Software Foundation, Beaverton, OR). For each SNP locus, the number of reads matching the reference was divided by the total number of reads for that locus. This was done for each bulk, and this "SNP-index" value was then used to calculate the ΔSNP-index by subtracting the SNP-indices (FON-1 resistant and FON-1 susceptible) of each bulk (Takagi et al. 2013). A 1-Mb sliding window with a 10-Kb increment increase was used to plot the ΔSNP-index to identify regions of the genome with high association to FON-1 resistance.

KASP™ assay development, genotyping, and genetic mapping

KASP™ assays (LGC Genomics LLC) were designed and optimized using Primer3Plus (Untergasser et al. 2007) for SNPs spanning the FON-1 QTL region on chromosome 1 (Online Resource 1). A total of 26 assays were designed, with 19 showing expected segregation allowing them to be used in analysis. Assays were named based on marker location according to the 97103 genome (Guo et al. 2013) using the following convention: UGA[chromosome_number]_[marker_loci_(bp)]. KASP™ assays were performed in a 4-μl reaction volume with 1.94 μl 2× low rox KASP™ master mix (LGC Genomics LLC), 0.06-μl primer mix with a final primer concentration of 0.81 μM, and 2 μl of 50–100 ng/μl genomic DNA. The PCR conditions used for the KASP™ assays were as follows: 15 min at 95 °C, followed by 10 cycles of touch down PCR with 20 s of 95 °C, 25 s of primer annealing temperature + 9 °C with 1 °C decrease each cycle, and 15 s of 72 °C, then 35 cycles of 10 s at 95 °C, 1 min at primer annealing temp, and 15 s at 72 °C. KASP™ florescent end-point readings were measured using an Infinite M200Pro plate reader (Tecan, Group Ltd.), and genotype calls were made using KlusterCaller™ (LGC Genomics LLC). Marker performance was compared using JMP software (JMP, Version 13.0, SAS Institute Inc., Cary, NC, 2016). A one-way ANOVA followed by a Tukey-Kramer HSD test was used to determine marker/trait association in the SB × CALG and the NHM × CALG F_2 populations. The Kruskal-Wallace test ($P = 0.05$) was used to test the association of genotypes with FON-1 severity ratings in the cultivar panel. Flapjack version 1.16.10.31 (Milne et al. 2010) was used for haplotype visualization.

Results

FON-1 severity of F_2 populations and validation panel

FON-1 severity scores for the SB × CALG $F_{2:3}$ population showed clear segregation of FON-1 resistance (Meru and McGregor 2016). The majority of the NHM × CALG F_2 individuals had an intermediate disease severity rating (2–3), and the parents and controls had the expected disease severity with NHM being completely dead and CALG showing little to no disease symptoms (Online Resource 2). Disease symptom development in susceptible plants in this population seems to be accelerated, leading to a larger percentage of individuals with severity ratings of 5 (dead) than observed in the SB × CALG population. FON-1 severity ratings for the validation panel agreed with expected resistance level based on previous reports (Table 1). Susceptible control cultivars SB and Black Diamond had high FON-1 severity ratings, while the resistant control CALG had very low FON-1 severity.

QTL-Seq

The QTL-seq method used in this study yielded a total of 206,959,301 (99.41%) and 260,854,970 (98.65%) mapped reads for the "resistant" and "susceptible" bulks, respectively. A total of 354,688 SNPs were identified between bulks for the whole genome of 11 chromosomes. There were 34,352 SNPs on chromosome 1, providing the marker density needed for a more refined map of the QTL. The location of the FON-1 QTL was confirmed on the end of chromosome 1 (Fig. 1) in the region previously reported to control resistance (Lambel et al. 2014; Meru and McGregor 2016; Ren et al. 2015). No other significant regions were identified in the genome. The QTL region obtained from the smoothed ΔSNP-index spanned from 0.01 to 1.41 Mb ($P = 0.001$) with maximum significance at approximately 0.38 Mb. Numerous markers in this region were available; however, there was a gap, with no significant markers from 0.75–1.07 Mb, and it was not possible to design primers for this region. The low number of SNPs identified in the 0.75–1.07 Mb region prompted further examination of QTL-seq data obtained for each bulk. Both the susceptible and resistant bulks maintained sufficient read depth through the entire region, but no additional SNPs were observed.

Marker performance on segregating populations

KASP™ assays were designed for SNPs across the 0.01–1.41-Mb region (Online Resources 1 and 3) and were then tested on the SB × CALG F_2 population to determine which markers showed the highest association with the FON-1 trait (Fig. 2). One-way ANOVA and Tukey-Kramer HSD test results showed significant association with FON-1 resistance for all markers in the QTL region ($P < 0.0001$). Many individuals in the population had fixed genotypes in the FON-1 region, with either the CALG haplotype, or the SB haplotype, and displayed either the resistant or susceptible phenotype, respectively. The majority of the population was heterozygous across the whole FON-1 region and displayed intermediate disease severity;

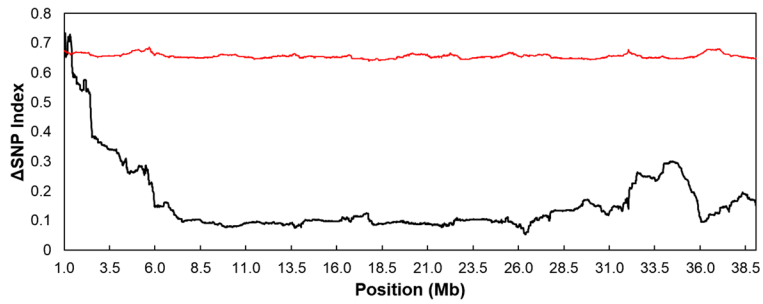

Fig. 1 ΔSNP-index graph from QTL-seq analysis. *X*-axis indicates the marker position on chromosome 1 and *Y*-axis indicates SNP-index. ΔSNP-index values (black) were calculated using a 1 Mb sliding window with a 10-Kb increment increase and plotted with statistical confidence intervals (red) under the null hypothesis assumption of no QTL ($P < 0.001$). The FON-1 QTL was confirmed on watermelon chromosome 1 (0.01–1.41 Mb) with the criteria that the ΔSNP-index was higher than the confidence value ($P < 0.001$)

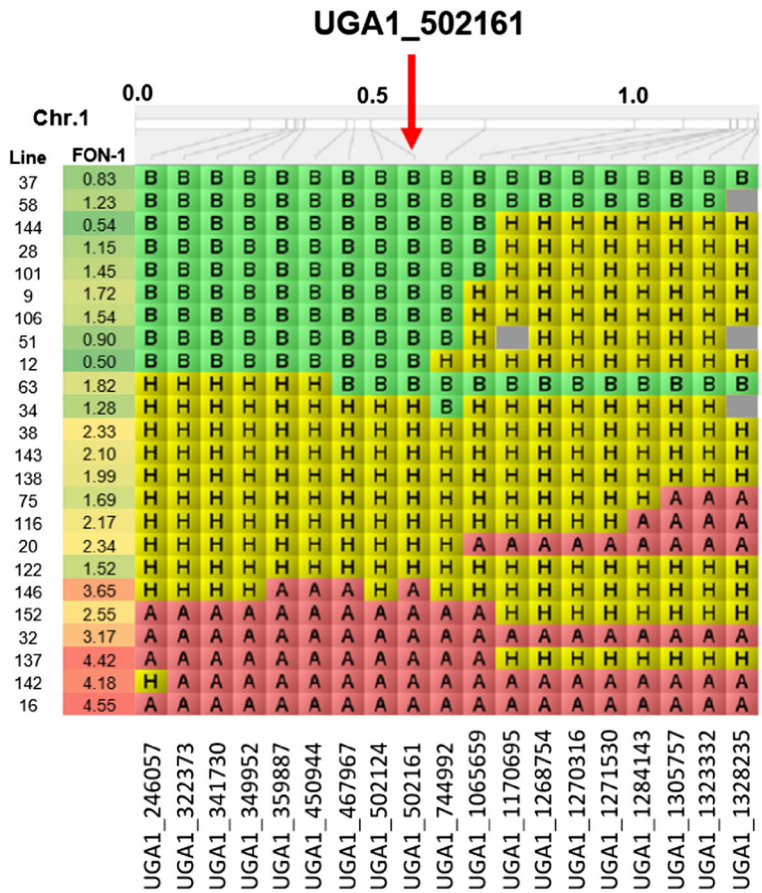

Fig. 2 Haplotype analysis in the FON-1 region of recombinant individuals in the SB × CALG F_2 watermelon population. FON-1 severity rating and marker genotype for recombinant lines at each marker location (Mb). Genotype indicated in relation to the susceptible (SB, red, (A)) and resistant (CALG, green, (B)) parental genotypes, and yellow blocks represent heterozygotes (H). Marker locations on chromosome 1 are indicated above the figure (Mb), and marker names are below the figure with UGA1_502161 indicated with an arrow

however, there were some individuals that showed recombination within the FON-1 region (Fig. 2). Closer analysis of these recombinant individuals identified a pattern of better performance of markers from UGA1_246057 to UGA1_744992. Several resistant recombinant individuals displayed the CALG haplotype and then were heterozygous for the remainder of the markers. Additionally, susceptible recombinant lines 152 and 137 displayed the SB haplotype from marker UGA1_246057 to marker UGA1_1065659 and were heterozygous for the rest of the markers. Further analysis showed that markers in the 0.45–0.51 Mb region (UGA1_450944, UGA1_467967, UGA1_502124, and UGA1_502161) had the greatest difference between average FON-1 severity of the SB and CALG genotypes in the one-way ANOVA and Tukey-Kramer HSD test (data not shown).

The NHM × CALG F_2 population was used to further validate these highly significant markers in a different genetic background. Markers located in the 0.45 to 0.47 Mb region (UGA1_450944 and UGA1_467967) were both monomorphic for the parents and thus could not be validated on this population. UGA1_502124 (SNP first identified by Ren et al. (2015)) did not produce distinct genotyping clusters and therefore could also not be validated in this population. However, UGA1_502161 gave clear genotyping results and a high association was found between this marker and FON-1 resistance in the NHM × CALG F_2 population ($P < 0.0001$; $R^2 = 0.59$) (Fig. 3). Marker UGA1_744992 was also highly significantly associated with resistance ($R^2 = 0.46$), but less so than UGA1_502161 (data not shown).

Marker performance on validation panel

To test the utility of UGA1_502161 further, marker performance was then tested on a validation panel (Fig. 4). UGA1_502161 had good performance in the susceptible germplasm, having the SB genotype for all susceptible cultivars and PIs. However, when tested on

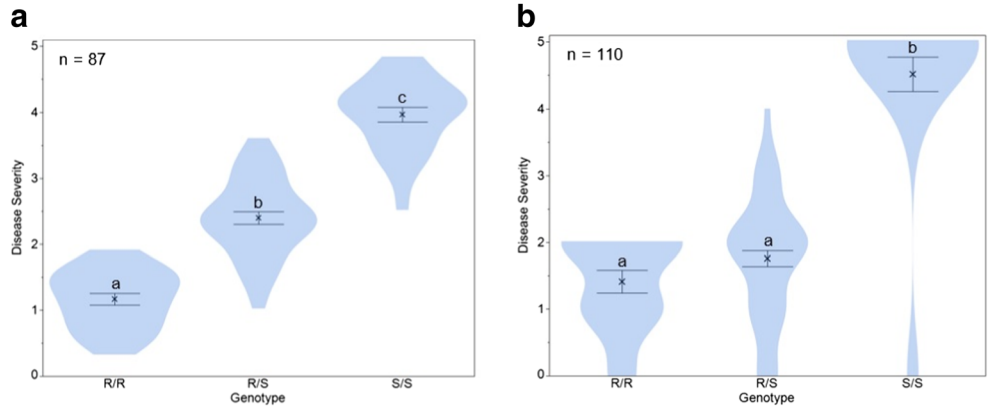

Fig. 3 **A** Marker performance of UGA1_502161 on the SB × CALG $F_{2:3}$ watermelon population ($n = 87$) ($R^2 = 0.80$) and further validation on **B** NHM × CALG F_2 watermelon population ($n = 110$) ($R^2 = 0.59$). R/R homozygous resistant type allele, S/S homozygous susceptible type allele, R/S heterozygous. ANOVA results indicated high marker/trait association ($P < 0.0001$) for UGA1_502161 in both populations

resistant cultivars and PIs, UGA1_502161 had the SB genotype for the two resistant cultivars, "Crimson Sweet" and "AU-Producer" and the resistant selection PI 296341-FR.

To determine if another marker might have better performance, all markers were tested on the validation panel (Fig. 4). Markers from UGA1_246057 to UGA1_1065659 all had the SB genotype for Crimson Sweet and AU-Producer. PI 296341-FR had the CALG genotype for marker UGA1_322373 to marker UGA1_450994, and the SB genotype from marker UGA1_467967 to UGA1_1170695.

Discussion

FON-1 resistance breeding is a time-consuming and laborious process that would be improved with application of MAS. In this study, we increased the marker density of the FON-1 region using the QTL-seq method. Furthermore, we identified markers in this region to develop KASP™ assays for high-throughput selection of FON-1 resistance. Previous studies mapping the FON-1 QTL were conducted with few markers in this region (Lambel et al. 2014; Meru and McGregor 2016; Ren et al. 2015). High marker density in the FON-1

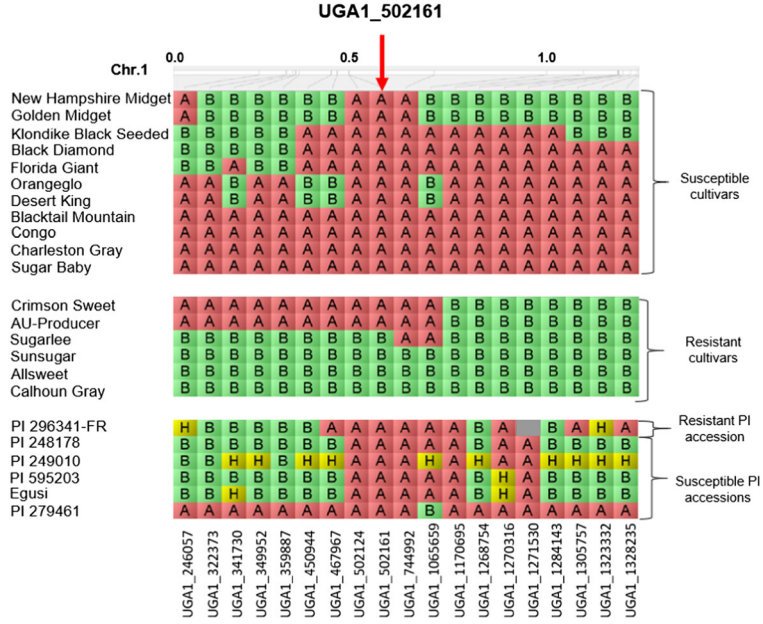

Fig. 4 Haplotype analysis for markers in the watermelon FON-1 region with a diverse panel of cultivars and PI accessions. Red blocks represent "SB" genotype (A), green blocks represent the "CALG" genotype (B), and yellow blocks represent heterozygotes (H). Marker locations on chromosome 1 are indicated above the figure (Mb), and marker names are below the figure with UGA1_502161 indicated with an arrow

region (19 markers in a 1.3 Mb region) allowed us to detect recombination patterns in the SB × CALG F_2 population that narrowed the region of interest from 0.01 to 1.41 Mb to the region between markers UGA1_246057 and UGA1_744992. Testing of markers in this region identified marker UGA1_502161 as a candidate for MAS of FON-1 resistance, which was then further validated in an additional F_2 population (NHM × CALG) and showed high association with the trait (Fig. 3). Two individuals in the NHM × CALG F_2 population had the NHM genotype but displayed the resistant phenotype. This may be due to recombination, or to the fact that phenotyping was carried out on individual F_2 plants, where replication is not possible, thus increasing the chance of escapes. There were more individuals that had a severity rating of "5" than expected in the population distribution (data not shown), which may be due to faster disease symptom development resulting from the hole punch tissue collection method used. However, this did not affect marker performance since these individuals all had the susceptible genotype.

Previous lack of markers in the FON-1 region has also limited the genotypic characterization of watermelon germplasm in this region. Cultivars provide resistant and susceptible germplasm that has gone through many rounds of recombination, providing an excellent background for marker testing (Holdsworth et al. 2016; Menda et al. 2014; van der Beek et al. 1992). Although many cultivars had fixed genotypes in the FON-1 region, with either the CALG haplotype, or the SB haplotype, recombination was observed in several cultivars. Black Diamond, which is often used as a susceptible control due to its high susceptibility to FON-1, had the CALG haplotype from marker UGA1_246057 to UGA1_359887. Several other highly susceptible cultivars also had the CALG genotype in this region (Fig. 4), including NHM, indicating that control of FON-1 resistance is not found in this region on chromosome 1. Susceptible cultivar Charleston Gray, which is fixed in the FON-1 region for the SB haplotype, is a parent of many different cultivars, including the resistant cultivars Crimson Sweet, "Allsweet," and CALG (Crall 1981a). The FON-1 region appears to have been completely replaced in both Allsweet and CALG, while Crimson Sweet still has the SB genotype up to marker UGA1_1065659. Crimson Sweet is a parent of AU-Producer (Crall 1981b; Hall 1963; Norton et al. 1983), which shares an identical haplotype, as well as "Sunsugar" which has the CALG haplotype, and "Sugarlee" which shows double recombination in the region between markers UGA1_502161 and UGA1_117695. Additional efforts to develop more markers in the FON-1 region were not possible in this study due to lack of SNPs detected in the 0.75–1.07-Mb region (Online Resource 3). Several markers in the FON-1 region had the SB genotype for PI 296341-FR, which has been used previously to map the FON-1 resistance QTL in this region of chromosome 1 (Ren et al. 2015). It should be noted that although FON-1 resistance in both PI 296341-FR and CALG has been mapped to the same location, CALG shows a higher level of resistance than PI 296341-FR (Ren et al. 2015). Currently, it is not known whether this is due to a different allele at the chromosome 1 resistance locus, additional resistance loci in CALG, loci interaction, or some other mechanism. FON-1 resistance in Crimson Sweet (and AU-Producer) has not been mapped, but it is assumed to be the same locus on chromosome 1, based on the shared resistant African stock citron in their pedigrees. Once again, the level of FON-1 resistance in Crimson Sweet is lower than that observed in CALG (Elmstrom and Hopkins 1981). Further research will be needed to dissect the cause(s) of the different levels of resistance observed in FON-1-resistant germplasm.

Conclusion

FON-1 screening in the greenhouse for selection of resistant lines is a time-consuming and tedious process. Current disease resistance breeding in watermelon requires seedlings to be screened for resistance in each selection cycle. Additionally, ensuring accurate phenotyping of FON resistance in these screens is difficult and depends upon many critical factors, including greenhouse temperatures, soil moisture and composition, pathogen strain, and physiological stage of seedlings (Martyn 2014; Meru and McGregor 2016). Balancing all of these factors is difficult and makes disease resistance breeding in watermelon a time-consuming and costly practice.

Using MAS, breeders can reduce costs and labor by performing FON-1 screens on final selections, rather than each round of selection. The high association found between marker UGA1_502161 and FON-1 resistance in both the SB × CALG and NHM × CALG F_2 populations indicates its usefulness as a selection tool in

watermelon breeding programs. The validation panel indicated that UGA1_502161 will be useful for selection when using CALG as a source of resistance, and its utilization in watermelon breeding programs will aid in the development of FON-1-resistant cultivars by allowing selections to be made using efficient, high-throughput genotyping.

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