Mapping Resistance to *Alternaria cucumerina* in *Cucumis melo*

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ABSTRACT

Infection with *Alternaria cucumerina* causes Alternaria leaf blight (ALB), a disease characterized by lesion formation on leaves, leading to substantial yield and quality losses in *Cucumis melo* (melon). Although fungicides are effective against ALB, reduction in the frequency of application would be economically and environmentally beneficial. Resistant melon lines have been identified but the genetic basis of this resistance has not been determined. A saturated melon genetic map was constructed with markers developed through genotyping by sequencing of a recombinant inbred line population (F_6 to F_{10} ; $n = 82$) derived from single-seed descent of a F_2

population from a cross between the ALB-resistant parent MR-1 and the ALB-susceptible parent Ananas Yokneum. The population was evaluated for *A. cucumerina* resistance with an augmented block greenhouse study using inoculation with the wounded-leaf method. Multiple quantitative trait loci (QTL) mapping identified two QTL that explained 33.9% of variation in lesion area. Several candidate genes within range of these QTL were identified using the *C. melo* v3.5 genome. Markers linked to these QTL will be used to accelerate efforts to breed melon cultivars resistant to ALB.

Melon (*Cucumis melo* L.), also called cantaloupe or muskmelon, is an economically important crop worldwide and is severely affected by fungal pathogens. *Alternaria cucumerina* (Ellis & Everh.) J. A. Elliott, the causal agent of Alternaria leaf blight (ALB), is one such pathogen that can cause extensive damage (Latin and Egel 2001). In the United States, ALB is a significant problem in the melon-producing Midwestern and eastern states and has been responsible for yield losses approaching 50% (Evans et al. 1992; Suheri and Latin 1991). In addition to quantity losses, fruit quality is also significantly affected by serious ALB infection (Latin et al. 1994). In order for cantaloupe to be considered of “good internal quality,” the edible portion of the melon must have minimum soluble solid content (SSC) of 9% (USDA Agricultural Marketing Service 2008). Latin et al. (1994) found a significant decrease in SSC as *Alternaria* infection increased and that SSC less than 9% frequently occurred in fields with high infection rates.

Under severe infection, rapidly expanding lesions coalesce and cause defoliation and sun-scalding of the fruit and become a source of conidia that are spread via wind and water (Jackson and Weber 1959; Latin et al. 1994). Infection severity varies according to several factors: presence of overwintered mycelia in the field (Jackson and Weber 1959), leaf wetness and temperature (Chandler and Thomas 1991; Evans et al. 1992; Thomas 1983), foliar damage by insects (Chandler and Thomas 1991), mechanical foliar damage (Thomas et al. 1990), fungicide treatments (Egel and Harmon 2001; Keinath et al. 2007; Latin et al. 1994; Suheri and Latin 1991), and degree of cultivar resistance (Boyhan and Norton 1992; Carmody et al. 1985; Egel 1999a,b; Thomas 1984; Thomas and Caniglia 1997; Thomas et al. 1990). Fungicide application is the primary control method and has been enhanced by the introduction of MelCast (Latin and Egel 2001), a proven tool which allows for strategic fungicide use based on duration of temperature and wetness. However, ALB severity could be further mitigated through the use of

resistant varieties (Thomas and Caniglia 1997), which would have immediate financial benefits for growers who would potentially require significantly fewer fungicide applications to control ALB.

Resistance to ALB has been identified in several melon lines (Boyhan and Norton 1992; Egel 1999a,b; Sitterly 1972; Thomas and Caniglia 1997; Thomas et al. 1990). Decreased lesion formation is the hallmark trait of resistance to ALB. For example, resistant melons, after 10 days of infection, were shown to have markedly decreased lesion growth (2 to 6% of susceptible plant lesion growth) and sporulation of lesion area (9 to 47% of susceptible plant sporulation) (Thomas 1984). The inheritance of ALB resistance has been evaluated in two studies (Boyhan and Norton 1992; Thomas et al. 1990). In one case, Boyhan and Norton (1992) demonstrated the inheritance of ALB resistance in muskmelon breeding line AC-82-37-2 to be additive, epistatic, and highly heritable. Thomas et al. (1990) reported the inheritance of a single, dominant resistance gene, *Ac*, in the United States Department of Agriculture MR-1 breeding line that confers a high level of resistance. Currently, molecular markers linked to ALB resistance in melon have yet to be identified.

In this study, genotyping by sequencing (GBS) (Elshire et al. 2011) was used to create a high-density genetic map of a recombinant inbred line (RIL) population of ‘MR-1’ crossed with the susceptible Israeli ‘Ananas Yokneum’ (‘AY’), and quantitative trait loci (QTL) analysis was conducted to identify QTL affecting *Alternaria* lesion area. The *C. melo* genome v3.5 (Garcia-Mas et al. 2012) was used to identify potential candidate genes collocating with ALB resistance QTL.

MATERIALS AND METHODS

Plant materials. In order to compare contrasting ALB resistance levels, 89 MR-1 × AY RIL (F_6 to F_{10}) were used for genotyping and ALB resistance phenotyping. MR-1 was originally selected and inbred from plant introduction (PI) 124111, an Indian melon line, for its high levels of powdery mildew (*Podosphaera xanthii*) and downy mildew (*Pseudoperonospora cubensis*) resistance (Thomas 1986). However, MR-1 also has been shown to possess resistance to races of Fusarium wilt (*Fusarium oxysporum* f. sp. *melonis*) (Wechter et al. 1995; Zink and Thomas 1990) and ALB (Thomas et al. 1990), making it an excellent source of resistance genes. AY is susceptible to powdery mildew, downy mildew, and

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*The e-Xtra logo stands for “electronic extra” and indicates that two supplementary figures are published online.

Fusarium wilt (Wang et al. 1997). Although AY is reported as resistant to *A. cucumerina* (Thomas and Caniglia 1997), our results indicate susceptibility relative to MR-1, as well as segregation for ALB resistance in the MR-1 × AY RIL. 'Hales Best Jumbo' and 'Perleta' melon were included as susceptible checks in addition to AY.

Plant growth conditions. Plants were grown in Metro-mix 360 (Sun Gro Horticulture, Agawam, MA) in 50-cell trays (PRO050R5; Myers Industries Lawn & Garden Group, Middlefield, OH) until the one to two fully expanded leaf stage, at which time the plants were transferred to plastic pots (10 by 10 by 10 cm, SVT-400 4 × 5; T.O. Plastics, Inc., Clearwater, MN) and fertilized weekly (Peters Professional Water Soluble Fertilizer 20-20-20; Everris International B.V., Geldermalsen, The Netherlands) at 10 g/liter weekly. Plants were maintained year-round in a greenhouse where temperatures averaged 25°C and ranged from 18 to 38°C (nighttime low and daytime high, respectively) throughout the experiment.

Inoculum preparation. The *A. cucumerina* isolate AC-4B used in this study was collected in South Carolina in 2001 from *C. melo* (A. P. Keinath, personal communication). *A. cucumerina* cultures were grown for 30 to 40 days on quarter-strength potato dextrose agar (Becton, Dickinson and Company, Franklin Lakes, NJ) on a 12-h diurnal cycle at room temperature (approximately 25°C) under fluorescent lights. In order to avoid carbon dioxide inhibition of sporulation, the petri dishes were not sealed (Cotty 1987). Conidia were harvested for immediate use by flooding the 100-by-15-mm petri dish with sterile distilled water (sdH₂O) to approximately half the volume and then, using a 1-ml pipetter, repeatedly aspirating and pipetting directly into the dish for 30 s to dislodge conidia. The liquid was then poured into a 250 ml media bottle containing approximately 50 ml of sdH₂O and vigorously shaken for 30 s to separate clumped conidia. Conidia were counted using a hemacytometer (Fisher Scientific, Waltham, MA) and sdH₂O was used to adjust the liquid to 2.5×10^4 conidia/ml.

***A. cucumerina* inoculation and resistance evaluations.** Each third expanded leaf of plants at the fourth expanding leaf stage was inoculated using a modified wounded-leaf method (Batta 2003). Leaves were lightly scratched with a 22-gauge needle, creating three to eight wounds (approximately 4 mm in length) approximately equidistant between the midrib and margin. The number of wounds depended on leaf area, which varied among RIL. A buffer zone of approximately 15 mm between wound locations allowed for lesion expansion in even the most susceptible lines without coalescing. A 10- μ l droplet of 2.5×10^4 conidia/ml of inoculum (approximately 250 conidia) was pipetted directly onto each wound and allowed to air dry prior to being placed in a humidity chamber at room temperature and maintained as described below. Controls were wounded, then treated as above but replacing the fungal inoculant with sdH₂O. All plants were randomized within the chamber.

Plants were maintained on a 10-day regimen used by Thomas et al. (1990): 16 h in an indoors humidity chamber followed by 8 h of natural daylight in the greenhouse. This was done each day of the 10-day regime. The humidity chamber (4.6 by 2.4 by 1.2 m) consisted of a wooden frame, covered in 5-mil polyethylene sheeting. It had a capacity of approximately 500 plants arranged in two tiers using four expanded metal platforms. A large, commercial humidifier (PA-600; Pharos Inc., Springdale, AR) was centrally located on one end of the chamber and was set to cycle as follows: initial 60 min to produce dew that was subsequently maintained by four evenly distributed 30-min intervals, for a total of 3 h of active humidification during the 16 h in the chamber. The humidity chamber was located in a temperature-controlled building that averaged 21°C.

Disease severity was evaluated on day 10 by measuring lesion area. Lesion areas of plants were recorded by measuring the length and width and using the equation for the area of an ellipse [$(\text{Diameter} - A/2) \times (\text{Diameter} - B/2) \times \pi$], because lesions were occasionally elliptical. All measurements were recorded in millimeters using digital calipers (Digimatic Caliper CD-6'B; Mitutoyo Corporation, Aurora, IL).

Statistical analyses. Statistical analysis of RIL lesion area was conducted using augmented incomplete blocks in JMP Pro 10 (SAS Institute Inc., Cary, NC) software. The leaf lesions of each plant were averaged and used for the analysis. The RIL were divided into nine incomplete blocks in which lines were completely randomized, with at least eight replications per line. Lines were tested one or two additional times in separate blocks, and MR-1, AY, and (AY × MR-1) F₁ checks were included in every test. The use of checks in every block is a fundamental facet of augmented block design and provides an estimate of the blocking effect and experimental error variance, significantly reducing error (Federer and Raghavarao 1975). Planting date and planting date by line effects were designated as random. The means of individual plants from each planting date were compared using Fisher's Protected least significant difference ($P=0.05$).

A restricted maximum-likelihood mixed model was used to calculate variance components for estimation of broad-sense heritability (H^2) and best linear unbiased predictors (BLUP) (Henderson 1950) of leaf lesion area. The lmer function in the lme4 package (Bates et al. 2015) of R v3.2.3 (R Core Team 2015) was used for model estimation. RIL, test, the interaction between RIL and test, and leaf within test were all included as random variables in the model. The RIL variance divided by the phenotypic variance was reported as H^2 . The random effects of each RIL and BLUP were estimated from the model with the ranef function of the lme4 package.

DNA extractions for GBS. DNA aliquots of 89 MR-1 × AY RIL, 3 MR-1 plants, and 3 AY plants were submitted to Cornell University Institute for Genomic Diversity (IGD) for GBS. DNA was extracted using the ChargeSwitch gDNA Plant Kit (Life Technologies, Carlsbad, CA) from 100 mg of leaf tissue. The tissues were first homogenized in 1.5-ml microcentrifuge tubes in a solution containing 100 μ l of Reagent A (300 mM CaCl₂, 15% polyvinylpyrrolidone [10,000 average molecular weight], and ChargeSwitch Lysis Buffer), 100 μ l 10% sodium dodecyl sulfate, 2 μ l of RNase A, and 900 μ l of ChargeSwitch Lysis Buffer using a FastPrep FP120 homogenizer on setting 4 m/s for 20 s (Thermo Fisher Scientific, Waltham, MA) with a quarter-inch Ceramic Sphere (MP Biomedicals, Santa Ana, CA) in each microcentrifuge tube. The manufacturer's recommended protocol was then followed to completion. For DNA quantification, 1 μ l of DNA was prepared using the Quant-iT dsDNA Assay Kit, broad range (Thermo Fisher Scientific), and quantified on a Qubit Fluorometer (Thermo Fisher Scientific). Concentrations exceeding 100 ng/ μ l were diluted to 50 to 100 ng/ μ l by adding more ChargeSwitch Elution Buffer. Aliquots (50 μ l) of the extracted DNA were pipetted into a 96-well plate (VWR 83007-374; VWR International, Radnor, PA) and sealed using polymerase chain reaction (PCR) tube strip caps (VWR 20170-000; VWR International). The samples were immediately placed on dry ice and shipped overnight to IGD for GBS.

Marker development. Biallelic single-nucleotide polymorphisms (SNP) with indels removed and less than 90% missing data were identified with the GenoSummary plugin of TASSEL v5.2.16 (Bradbury et al. 2007). The cluster algorithm of the FSFhap Imputation plugin of TASSEL (Swarts et al. 2014) was run twice on the dataset. After the first imputation, lines with more than 7.5% heterozygous sites were removed, because they were likely the result of unintended outcrossing. The original data were then imputed with the remaining lines. The ABH plugin of TASSEL removed SNP where the parental genotype was missing, ambiguous, or heterozygous and coded the remaining SNP into parental genotypes. All subsequent analyses were done in Rqtl (Broman et al. 2003). SNP with less than 20% missing data, less than 15% heterozygosity, and a minor allele frequency greater than 5% were chosen to generate a high-quality genetic map. Markers with identical segregation patterns were binned. SNP with significant segregation distortion were identified by a χ^2 test and a false-discovery rate (FDR) correction (Benjamini and Hochberg 1995). Significant segregation distortion of single markers in areas with expected Mendelian segregation patterns were regarded as genotyping errors and removed from further analysis.

Linkage map construction. A genetic linkage map for the *C. melo* RIL population was constructed in Rqtl (Broman et al. 2003). Twelve linkage groups were formed with a logarithm of the odds (LOD) score of nine and marker order was determined with a window size of seven in the ripple function. Genetic distances were calculated from recombination fractions with the Kosambi mapping function (Kosambi 1943).

QTL mapping. QTL associated with *A. cucumerina* resistance BLUP were identified in Rqtl (Broman et al. 2003) with a two-step approach. First, the maximum number of QTL was determined through forward selection using the scanone function set to standard interval mapping with the multiple imputation method (Sen and Churchill 2001). Genotypes were imputed with the sim.genofunc function and settings of 100 draws and a step size of 0.5 centimorgans (cM). Second, multiple QTL mapping (MQM) was done with stepwiseqtl (Broman and Sen 2009), which chooses the model of highest penalized LOD score (Manichaikul et al. 2009) through automated forward and backward selection up to a maximum of five QTL. LOD score penalties and the genome-wide significance threshold (LOD = 3.15) were determined through 1,000 permutations in scantwo. *C. melo* genome v3.5 was used to identify potential candidate genes within the 1.5-LOD intervals of the QTL (Garcia-Mas et al. 2012).

Data archiving. All phenotypic and genotypic data associated with this research are available from the Dryad Digital Repository (Dryad Tables D1 through D5; <http://datadryad.org/resource/doi:10.5061/dryad.5404r>).

RESULTS

Screening and rating for resistance in MR-1 × AY RIL. Lesion areas of 89 MR-1 × AY RIL were continuous and ranged from 20 to 111 mm²; MR-1, (AY × MR-1) F₁, and AY had means of 25, 37, and 72 mm², respectively (Dryad Table D1). The *H*² of mean leaf lesion area was relatively low at 0.20. Generally, lesion formation at a wound site was visually detectable on day three postinoculation. Initially, the wound site exhibited slight chlorosis, progressing to minor lesion formation, similar to that obtained with spray inoculation (Thomas 1984). As disease progressed over the 10-day infection regime, lesions expanded and dark-brown rings, characteristic of ALB infection, would often become evident within the lesion. In resistant lines, lesion formation was markedly slower than susceptible-line lesion formation and often was surrounded by a chlorotic ring that was noticeably wider than in susceptible lines. The

Fig. 1. Disease reactions of *Cucumis melo* MR-1 and Ananas Yokneum (AY) to *Alternaria cucumerina* using the leaf-droplet method. A, MR-1 water control; B, MR-1 (two leaves) inoculated with *A. cucumerina*; C, AY water control; and D, AY inoculated with *A. cucumerina*. Reactions are shown 10 days postinoculation with 10- μ l droplets of *A. cucumerina* at 2.5×10^4 conidia/ml.

perforations from the wounding expanded as lesions expanded, leading to ragged, gaping holes in highly susceptible plants (Fig. 1). Water-inoculated control plants exhibited no symptoms of infection.

GBS. GBS produced 176 million good-quality barcoded reads at an average depth of 12 and resulted in 24,055 SNP, which were filtered to 9,024 high-quality biallelic SNP. FSFhap imputation reduced the percentage of missing data from 39.9 to 1.5%. Redundant SNP with identical segregation patterns were binned to 2,200 SNP (Dryad Table D2) and used for QTL mapping. No markers had significant segregation distortion (FDR < 0.05). Seven

Fig. 2. Genetic linkage map of chromosomes 10 and 12 from *Cucumis melo* MR-1 × Ananas Yokneum recombinant inbred line population depicting single-nucleotide polymorphisms associated with quantitative trait loci qALB-10 and qALB-12.

RIL showed evidence of outcrossing (i.e., excessive heterozygosity as compared with the rest of the population) and were removed before map construction.

Map construction and QTL mapping. The 2,200 SNP formed 12 linkage groups for a total map length of 1,410.4 cM (Supplementary Fig. S1). The length of linkage groups ranged from 81 to 170 cM. Markers were well dispersed across the genome, with 140 to 243 SNP per linkage group, an average distance of 0.6 cM between SNP, and a maximum distance of 17.9 cM (Dryad Table D3). Linkage groups were named by the corresponding chromosome and there was a 99.4% match between them. The 13 misplaced SNP were all grouped to the beginning of linkage group 8 and were physically located on chromosome 5 (Supplementary Figs. S1 and S2).

Two significant QTL identified by MQM (exceeded the genome-wide significance threshold of 3.15 LOD score) were associated with *A. cucumerina* resistance in the *C. melo* RIL population and explained 33.9% of the variation (Fig. 2). The QTL on chromosome 10 (qALB-10) and chromosome 12 (qALB-12) had LOD scores of 3.4 and 4.1 and explained 14.2 and 17.4% of the variation in lesion area, respectively (Table 1). Alleles for the decrease in lesion area for both QTL came from the resistant parent (MR-1). The 1.5-LOD interval for qALB-10 extended 34.1 cM (9.2 Mb) and collocated with 479 genes, whereas qALB-12 was 17.1 cM (1.98 Mb) with 288 genes (Fig. 3; Dryad Table D4). Candidate genes prioritized by function based on gene ontology (GO) terms included 42 kinases, 13 transcription factors, and 5 genes potentially involved in systemic acquired resistance (SAR) (Dryad Table D5).

DISCUSSION

The majority of ALB resistance screenings in melon are greenhouse based. In melon assays, leaf selection ranges from cotyledons and first leaves at the respective growth stages (Carmody et al. 1985), to first and second leaves of plants at the third expanded leaf stage (Chandler and Thomas 1991; Thomas and Caniglia 1997; Thomas et al. 1990), to second and third leaves of plants at the expanding fourth leaf stage (Evans et al. 1992). During preliminary testing, we found that first and second leaves of plants at the fourth expanding leaf stage were often small and irregular compared with third leaves, thus decreasing testable leaf area. Furthermore, lesion expansion on the first leaves was often accompanied by early senescence, particularly in MR-1 plants, which exacerbated abnormal lesion expansion and produced inconsistent results, similar to observations by Thomas et al. (1990). Hence, we selected third leaves of plants at the fourth expanding leaf stage for testing, which ultimately provided consistent results on plants small enough to allow large-scale testing.

Although melon resistance screenings involve foliar inoculations on whole plants, Batta (2003) demonstrated in cucumber that ALB resistance could be assayed by applying inoculum directly to artificial wounds on leaf discs, providing greater control over lesion placement and saving space. Our initial attempts to adapt the wounded-leaf method to melon leaf discs and detached leaves were irreconcilably fraught with inconsistent lesion expansion; however, wounded-leaf assays on whole plants produced consistent results that were in accord with expected relative resistance ratings for previously studied lines. Although whole plants were successfully used in this study, it required the use of extensive

space and resources; adaptation of the leaf disc assay to melon would allow higher throughput resistance testing and fewer resources.

Carmody et al. (1985) reported that lesion size and mean number of lesions on cotyledons were predictive of lesion size, number of lesions, and resistance rating in field conditions. In addition, they rated Hales Best Jumbo and Perlita as susceptible in both greenhouse and field conditions, comparable with our ratings for these two cultivars using the wounded-leaf assay. The similarities suggest that the wounded-leaf method may also be predictive of resistance and susceptibility under field conditions; nevertheless, future field comparison studies need to be conducted.

Wounded-leaf inoculated resistant and susceptible lines differed significantly in lesion area, analogous to observations by Batta (2003) in cucumber leaf-disk assays. Lesion size differences among the RIL became increasingly noticeable over the 10-day assay; thus, clear differences between resistance, intermediate resistance, and susceptibility could be statistically determined. Although rare, some AY controls were excluded from analysis because of high levels of powdery mildew on third leaves, which severely restricted lesion area relative to the other AY controls.

A high-density consensus map (1,592 markers) was developed by the International Cucurbit Genome Initiative using eight previously published genetic maps ranging from 877 to 1,654 cM in length (Diaz et al. 2011). The saturated genetic map presented here is comparable in both size (1,410 cM) and marker number (2,200 SNP).

We were able to identify two QTL significantly associated with *C. melo* resistance to *A. cucumerina* but the 1.5-LOD intervals for both QTL were large and collocated with hundreds of genes, likely

Fig. 3. Logarithm of the odds (LOD) scores across linkage groups A, 10 and B, 12. Shaded regions mark the 1.5-LOD intervals of qALB-10 and qALB-12, respectively. The genome-wide significance threshold of 3.15 is shown by the dashed horizontal line.

TABLE 1. Quantitative trait loci (QTL) associated with *Alternaria cucumerina* lesion area best linear unbiased predictors (BLUP) in a *Cucumis melo* MR-1 × Ananas Yokneum recombinant inbred line (RIL) population

QTL	LG ^a	Peak (cM) ^b	Range (cM) ^b	LOD	V _P (%) ^c	P value	N ^d	Candidate ^e
qALB-10	10	64.4	46.0–79.5	3.4	14.2	1.0 × 10 ^{−4}	479	28
qALB-12	12	80.5	65.5–82.9	4.1	17.4	2.1 × 10 ^{−5}	288	32

^a Linkage group.

^b Peak and range in centimorgans (cM) of the 1.5-logarithm of the odds (LOD) intervals of the QTL.

^c Percentage of the phenotypic variation explained by the QTL.

^d Number of genes located within the boundaries of the 1.5-LOD interval of the QTL.

^e Number of prioritized candidate genes by function collocated with QTL.

due to our small population size. In a previous study, Thomas et al. (1990) reported a single dominant gene in MR-1 conferring resistance to ALB. Our identification of at least two putative genetic regions, located on different chromosomes, expands our current knowledge of the genetics of inheritance to ALB resistance in MR-1 and may help breeders in the incorporation of this resistance into new cultivars. Although the QTL we have identified in this study comprise large numbers of gene loci (Table 1), they should still serve as an important starting point useful in further genetic mapping studies of gene loci associated with resistance to ALB. Despite the low resolution of QTL location, a few genes are strong candidates for molecular validation based upon functional relevance of their encoded products. Previous work in *Arabidopsis thaliana* and *Glycine max* has shown that glycerol-3-phosphate (G3P) is required for induction of SAR (Chanda et al. 2008, 2011). MR-1 resistance to ALB may be due to an enhanced SAR response because five of the genes in this pathway collocated with the resistance QTL. G3P can be produced through the action of either a glycerol kinase or a G3P-dehydrogenase (Mandal et al. 2011). Four genes encoding products involved in the metabolic pathway of G3P collocated with qALB-10. Two encoded transferases from the pathway may transport G3P from the wound site to the rest of the plant to initiate SAR (*MELO3C011715* and *MELO3C023876*). *MELO3C20157* and *MELO3C20158* both encode enzymes with G3P dehydrogenase activity, which produces G3P. The allele from the resistant parent may increase levels of G3P relative to that of the sensitive parent in response to infection, thereby inducing an enhanced SAR response. There was an additional gene, *MELO3C025184*, annotated with the GO term for SAR but its role in this process is unknown.

None of the genes that collocate with qALB-12 encode products previously implicated in disease resistance. Resistance may also be conferred through the action of transacting regulatory proteins causing increased expression of resistance genes. The 1.5-LOD interval for qALB-12 contains genes encoding 24 kinases and 8 transcription factors.

Contrary to expectations, MR-1 *Alternaria* resistance did not exert complete dominance in MR-1 × AY RIL, as was reported by Thomas et al. (1990) in a Perlita × MR-1 F₂ population investigating inheritance of *Alternaria* resistance. Because of the continuous distribution of lesion area among the RIL and intermediate resistance of the F₁ (AY × MR-1), we expected to identify several contributing QTL; however, only two QTL were identified. It is likely that, with increased population size, additional QTL affecting lesion area would be detected. Because of this variability, we suggest that breeders interested in introgressing MR-1 ALB resistance should conduct preliminary tests to determine the level of resistance that can be expected.

Based on the observed overall additive nature of *Alternaria* resistance, the ability to generate RIL with high levels of resistance to ALB in the absence of selection, and the identification of qALB-10 and qALB-12 in the MR-1 × AY RIL, this study is in agreement with Boyhan and Norton (1992) that a pedigree or backcross method would lead to improved resistance to *Alternaria cucumerina* in melon. Marker-assisted selection may accelerate the introgression of ALB resistance into commercial melon lines, given the low heritability available for traditional phenotypic selection.

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LITERATURE CITED

Bates, D., Mächler, M., Bolker, B., and Walker, S. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67:1-48.

- Batta, Y. 2003. *Alternaria* leaf spot disease on cucumber: Susceptibility and control using leaf disk assay. *An-Najah Univ. J. Res.* 17:269-279.
- Benjamini, Y., and Hochberg, Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B.* 57:289-300.
- Boyhan, G. E., and Norton, J. D. 1992. Inheritance of resistance to *Alternaria* leaf blight in muskmelons. *HortScience* 27:1114-1115.
- Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., and Buckler, E. S. 2007. TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics* 23:2633-2635.
- Broman, K. W., and Sen, S. 2009. *A Guide to QTL Mapping with R/qtl*, Vol. 46. Springer, New York.
- Broman, K. W., Wu, H., Sen, S., and Churchill, G. A. 2003. R/qtl: QTL mapping in experimental crosses. *Bioinformatics* 19:889-890.
- Carmody, B. E., Miller, M. E., and Grisham, M. P. 1985. A technique to screen muskmelons for resistance to *Alternaria* leaf blight. *Plant Dis.* 69:426-428.
- Chanda, B., Venugopal, S. C., Kulshrestha, S., Navarre, D. A., Downie, B., Vaillancourt, L., Kachroo, A., and Kachroo, P. 2008. Glycerol-3-phosphate levels are associated with basal resistance to the hemibiotrophic fungus *Colletotrichum higginsianum* in *Arabidopsis*. *Plant Physiol.* 147:2017-2029.
- Chanda, B., Xia, Y., Mandal, M. K., Yu, K., Sekine, K.-T., Gao, Q.-M., Selote, D., Hu, Y., Stromberg, A., Navarre, D., Kachroo, A., and Kachroo, P. 2011. Glycerol-3-phosphate is a critical mobile inducer of systemic immunity in plants. *Nat. Genet.* 43:421-427.
- Chandler, L. D., and Thomas, C. E. 1991. Effect of leaf miner feeding-activity on the incidence of *Alternaria* leaf-blight lesions on muskmelon leaves. *Plant Dis.* 75:938-940.
- Cotty, P. J. 1987. Modulation of sporulation of *Alternaria tagetica* by carbon dioxide. *Mycologia* 79:508-513.
- Diaz, A., Fergany, M., Formisano, G., Ziarsolo, P., Blanca, J., Fei, Z., Staub, J. E., Zalapa, J. E., Cuevas, H. E., Dace, G., Oliver, M., Boissot, N., Dogimont, C., Pitrat, M., Hofstede, R., van Koert, P., Harel-Beja, R., Tzuri, G., Portnoy, V., Cohen, S., Schaffer, A., Katzir, N., Xu, Y., Zhang, H., Fukino, N., Matsumoto, S., Garcia-Mas, J., and Monforte, A. J. 2011. A consensus linkage map for molecular markers and Quantitative Trait Loci associated with economically important traits in melon (*Cucumis melo* L.). *BMC Plant Biol.* 11:111.
- Egel, D. S. 1999a. Severity of *Alternaria* leaf blight on muskmelon varieties, 1996. *Biol. Cult. Tests* 14:157.
- Egel, D. S. 1999b. Severity of *Alternaria* leaf blight on muskmelon varieties, 1997. *Biol. Cult. Tests* 14:158.
- Egel, D. S., and Harmon, P. 2001. Effects of nozzle type and spray pressure on control of *Alternaria* leaf blight of muskmelon with chlorothalonil. *Plant Dis.* 85:1081-1084.
- Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., and Mitchell, S. E. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One* 6:e19379.
- Evans, K. J., Nyquist, W. E., and Latin, R. X. 1992. A model based on temperature and leaf wetness duration for establishment of *Alternaria* leaf blight of muskmelon. *Phytopathology* 82:890-895.
- Federer, W. T., and Raghavarao, D. 1975. On augmented designs. *Biometrics* 31:29-35.
- Garcia-Mas, J., Benjak, A., Sanseverino, W., Bourgeois, M., Mir, G., González, V. M., Hénaff, E., Cámara, F., Cozzuto, L., Lowy, E., Alioto, T., Capella-Gutiérrez, S., Blanca, J., Cañizares, J., Ziarsolo, P., Gonzalez-Ibeas, D., Rodríguez-Moreno, L., Droege, M., Du, L., Alvarez-Tejado, M., Lorente-Galdos, B., Melé, M., Yang, L., Wang, Y., Navarro, A., Marques-Bonet, T., Aranda, M. A., Nuez, F., Picó, B., Gabaldón, T., Roma, G., Guigó, R., Casacuberta, J. M., Arús, P., and Puigdomènech, P. 2012. The genome of melon (*Cucumis melo* L.). *Proc. Natl. Acad. Sci. USA* 109:11872-11877.
- Henderson, C. R. 1950. Estimation of genetic parameters. *Biometrics* 6:186-187.
- Jackson, C. R., and Weber, G. F. 1959. Morphology and taxonomy of *Alternaria cucumerina*. *Mycologia* 51:401-408.
- Keinath, A. P., Everts, K. L., Langston, D. B., Egel, D. S., and Holmes, G. J. 2007. Multi-state evaluation of reduced-risk fungicides and Melcast against *Alternaria* leaf blight and gummy stem blight on muskmelon. *Crop Prot.* 26:1251-1258.
- Kosambi, D. D. 1943. The estimation of map distances from recombination values. *Ann. Eugen.* 12:172-175.
- Latin, R. X., and Egel, D. S. 2001. Melcast: Melon Disease Forecaster. *Purdue Univ. Coop. Ext. Bull.* Lafayette, IN BP-64-W.
- Latin, R. X., Rane, K. K., and Evans, K. J. 1994. Effect of *Alternaria* leaf blight on soluble solids content of muskmelon. *Plant Dis.* 78:979-982.
- Mandal, M. K., Chanda, B., Xia, Y., Yu, K., Sekine, K., Gao, Q.-M., Selote, D., Kachroo, A., and Kachroo, P. 2011. Glycerol-3-phosphate and systemic immunity. *Plant Signal. Behav.* 6:1871-1874.

- Manichaikul, A., Moon, J. Y., Sen, S., Yandell, B. S., and Broman, K. W. 2009. A model selection approach for the identification of quantitative trait loci in experimental crosses, allowing epistasis. *Genetics* 181:1077-1086.
- R Core Team. 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. Online publication. <http://www.R-project.org/>
- Sen, S., and Churchill, G. A. 2001. A statistical framework for quantitative trait mapping. *Genetics* 159:371-387.
- Sitterly, W. R. 1972. Breeding for disease resistance in cucurbits. *Annu. Rev. Phytopathol.* 10:471-490.
- Suheri, H., and Latin, R. X. 1991. Retention of fungicides for control of *Alternaria* leaf blight of muskmelon under greenhouse conditions. *Plant Dis.* 75:1013-1015.
- Swarts, K., Li, H., Romero Navarro, J. A., An, D., Romy, M. C., Hearne, S., Acharya, C., Glaubitz, J. C., Mitchell, S., Elshire, R. J., Buckler, E. S., and Bradbury, P. J. 2014. Novel Methods to optimize genotypic imputation for low-coverage, next-generation sequence data in crop plants. Online publication. *Plant Genome* 7. doi:10.3835/plantgenome2014.05.0023
- Thomas, C. E. 1983. Fungicide applications based on duration of leaf wetness periods to control *Alternaria* leaf blight of cantaloupe in south Texas. *Plant Dis.* 67:145-147.
- Thomas, C. E. 1984. Resistant reaction type against *Alternaria* leaf-blight in *Cucumis melo*. (Abstr.) *Phytopathology* 74:865.
- Thomas, C. E. 1986. Downy and powdery mildew resistant muskmelon breeding line MR-1. *HortScience* 21:329-336.
- Thomas, C. E., and Caniglia, E. J. 1997. Evaluation of US honeydew-type melons for resistance against downy mildew and *Alternaria* leaf blight. *HortScience* 32:1114-1115.
- Thomas, C. E., McCreight, J. D., and Jourdan, E. L. 1990. Inheritance of resistance to *Alternaria cucumerina* in *Cucumis melo* line MR-1. *Plant Dis.* 74:868-887.
- USDA Agricultural Marketing Service. 2008. United States Standards for Grades of Cantaloups. Online publication. United States Department of Agriculture. https://www.ams.usda.gov/sites/default/files/media/Cantaloup_Standard%5B1%5D.pdf
- Wang, Y.-H., Thomas, C. E., and Dean, R. A. 1997. A genetic map of melon (*Cucumis melo* L.) based on amplified fragment length polymorphism (AFLP) markers. *Theor. Appl. Genet.* 95:791-798.
- Wechter, W. P., Whitehead, M. P., Thomas, C. E., and Dean, R. A. 1995. Identification of a randomly amplified polymorphic DNA marker linked to the *Fom 2* *Fusarium* wilt resistance gene in muskmelon MR-1. *Phytopathology* 85:1245-1249.
- Zink, F. W., and Thomas, C. E. 1990. Genetics of resistance to *Fusarium oxysporum* f. sp. *melonis* races 0, 1, and 2 in muskmelon line MR-1. *Phytopathology* 80:1230-1232.