Discrimination of Mint Strains of *Verticillium dahliae* Using High Resolution Melting Analysis

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Introduction

Verticillium wilt is the major disease impacting mint production in the Pacific Northwest. Black Mitcham peppermint and Scotch spearmint are very susceptible to Verticillium wilt while other mints, including native spearmint and some peppermint varieties exhibit varying degrees of resistance or tolerance to the disease (1, 4, 6). Verticillium wilt is caused by *Verticillium dahliae*, a soilborne fungus which has a wide host range and can survive for ten years or more as microsclerotia in field soils. Although the fungus can colonize and infect a wide range of dicot crops, a mint strain exists which belongs to vegetative compatibility group (VCG) 2B, exhibits a reduced host range, and is highly aggressive on mint (1, 3, 5). In addition, co-infection by *V. dahliae* and the root-lesion nematode *Pratylenchus penetrans* can result in increased disease incidence and severity and this interaction on mint is most synergistic when the mint strain of *V. dahliae* is involved.

Detection and quantification of microsclerotia in soils can be an important component of Verticillium wilt management. However, management options for Verticillium wilt should generally be implemented prior to planting in order to reduce disease levels. This is especially true for a perennial crop such as mint where fumigation and other control practices are difficult or impossible to perform once the field is established. Most methods to detect and quantify microsclerotia of *V. dahliae* rely on plating soils onto semi-selective medium, but these methods are time-consuming and labor-intensive and can take several weeks to obtain results. However, these tests usually do not include the identification or quantification of the mint VCG 2B strain in the assay, which can take weeks or months to complete using traditional methods.

Double-stranded DNA disassociates, or melts, into single-stranded DNA at different temperatures depending upon its length and sequence composition. High-resolution melting (HRM) analysis incorporates a fluorescent dye that disassociates from double-stranded DNA when it becomes single-stranded DNA, and this reduction in fluorescence can be used to determine the melting temperature of a particular region of DNA. The objective of this project was to determine if HRM analysis can be used to differentiate the VCG 2B mint strain of *V. dahliae* using differences in melting temperature of DNA after PCR amplification.

Materials and Methods

HRM analysis was conducted on a total of 112 *V. dahliae* isolates (49 isolates from mint, 39 isolates from potato, and 24 isolates from other hosts) using previously developed PCR primers that target the intergenic spacer (IGS) of DNA (9). The IGS region has previously been shown to exhibit sequence variation among *V. dahliae* isolates from different hosts, including mint and potato (7). A total of 86 HRM products were sequenced to identify differences in DNA sequences and IGS regions of DNA from 62 of the *V. dahliae* strains were sequenced to validate the results of HRM analysis. HRM analysis was repeated on 82 *V. dahliae* isolates (40 mint, 23 potato, and 19 other hosts) using a set of 3 representative mint strain as controls. HRM analysis was conducted using High Resolution Melt Software ver.3.0.1 (Applied Biosystems).

Results and Discussion

HRM analysis originally grouped mint isolates into three different groups based on melting temperatures of Q-PCR products. Some isolates from potato and other hosts were also grouped among some mint isolates based on melting temperatures, initially suggesting that HRM analysis would not reliably differentiate mint strains of *V. dahliae*. However, post-HRM sequencing revealed that one of the reference isolates from mint that was used as a control (isolate 111) contained a sequence that was atypical of the rest of the mint isolates. HRM analysis was repeated using control isolates that were representative of peppermint and spearmint isolate sequences. As a result, HRM analysis grouped all mint strains into a single HRM group (red), with the exception of the aforementioned mint strain with the atypical sequence (blue) (Figure 1). In addition, HRM analysis discriminated mint strains (red) from potato isolates (blue) (Figure 1) and separated mint strains from 16 of the 19 isolates from other hosts (Figure 1). The 3 isolates that grouped with mint strains contained the same sequence composition as typical mint strains (data not shown). One of these isolates was obtained from skullcap (*Scutellaria lateriflora*, Lamiaceae) and corresponded to the VCG 2B mint strain; this isolate likely originated from a mint crop that was planted in the field prior to the skullcap crop (2). Additional experiments are necessary to determine if HRM analysis can be used in environmental samples containing multiple strains.

Acknowledgements

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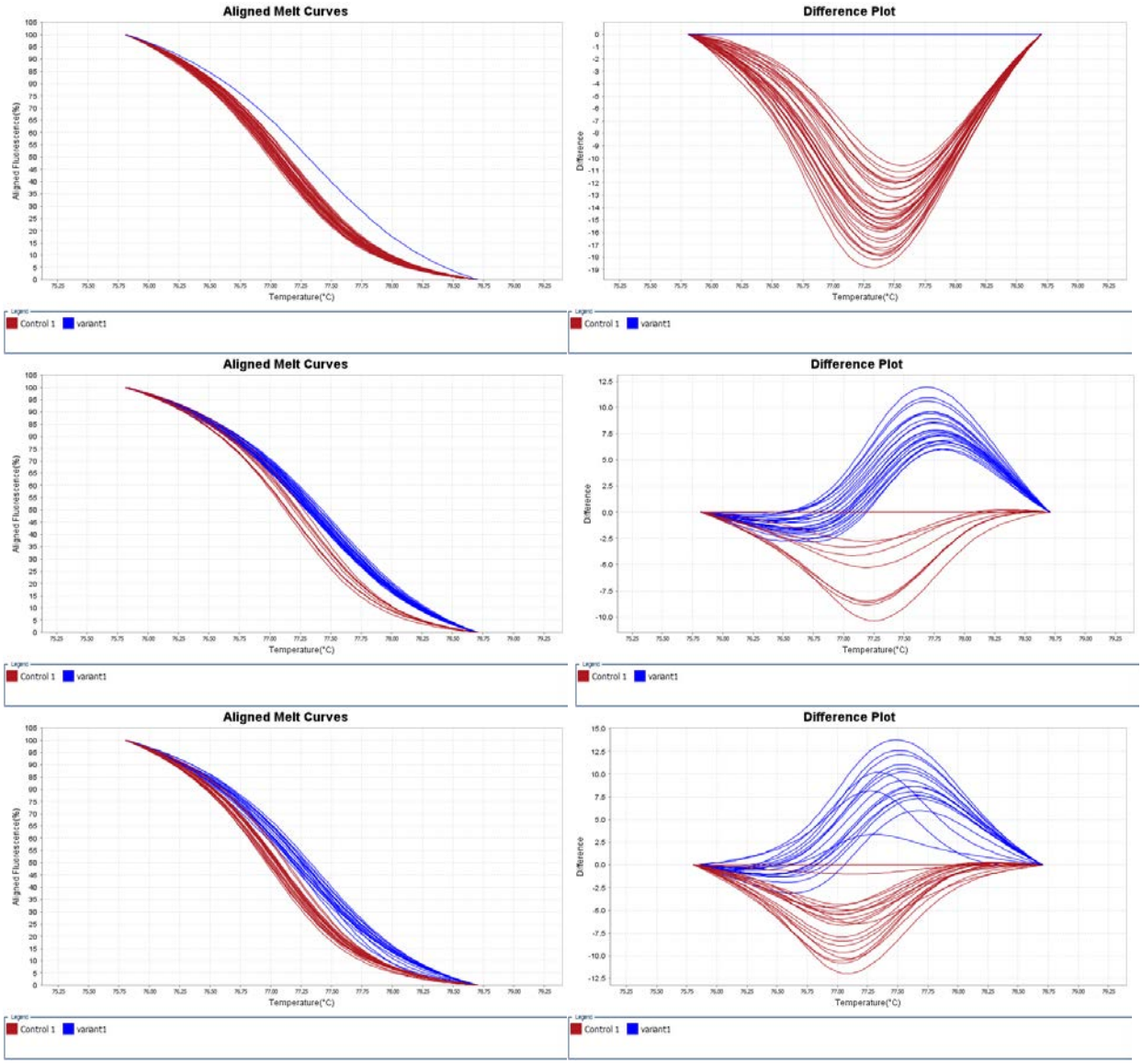

Figure 1. HRM analysis of *V. dahliae* strains from mint (top), potato (middle), and other hosts (bottom). Aligned melt curves (left) and difference plots (right) showing the differentiation of typical mint strains (red), potato strains and atypical mint strains (blue) and strains from other hosts (red or blue depending on sequence similarity).