Mint Varietal Improvement Project

Kelly Vining, Oregon State University
Mark Lange, Washington State University
Jeremiah Dung, Oregon State University

Objective: Cross ancestral peppermint species *Mentha longifolia* and *Mentha suaveolens* to regenerate interspecific hybrids (*Mentha spicata*)

Our long-term breeding goal is to generate Verticillium wilt resistant peppermint cultivars by hybridization between ancestral mint species. Nature originally took this route, giving rise to Black Mitcham peppermint. The two-step hybridization process involves first crossing *M. longifolia* with *M. suaveolens*, then crossing the resulting *M. spicata* type with *M. aquatica*. We initiated work on this objective by testing USDA accessions of each species for relative wilt susceptibility, and then chose four resistant accessions from each species for crossing as previously reported. After encountering pest problems in the greenhouse, we moved into a growth chamber in fall 2016 to keep plants under a long-day lighting regime in order to force continued flowering. However, while the *M. longifolia* accessions continued flowering as expected, all of the *M. suaveolens* accessions reverted to vegetative growth. In January 2017, the plants were moved into a greenhouse section with stronger lighting than that of the growth chamber. With no apparent disease or pest problems, the *M. suaveolens* accessions are now flowering, and crosses are proceeding (Fig. 1). Some of the pollinated flowers' ovules are already expanding, which is an early indication of successful fertilization. After onset of ovule expansion, mature seeds can be expected in approximately three weeks.

Figure 1. *Mentha suaveolens* accession CMEN 28 flowering under lights at the OSU greenhouse. Crossing tags mark flowers that have been pollinated with pollen from *M. longifolia* accessions.

Objective: Compare gene expression in resistant and susceptible mints in order to identify genes conferring wilt resistance.

This work focused initially on two *M. longifolia* accessions: wilt-resistant CMEN585 and wilt-susceptible CMEN584. These are the two grandparents of the *M. longifolia* SAF2 population, which is segregating for wilt resistance, oil constituents, and other traits. A genome assembled for CMEN585 provides the

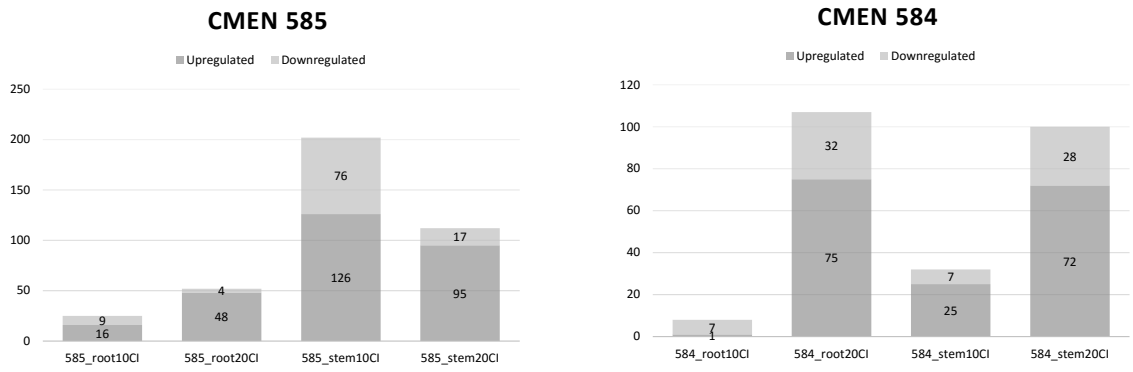

Figure 2. Plant defense genes switched on earlier in stems of resistant mint. Numbers of genes upregulated or downregulated in response to *Verticillium dahliae* inoculation in resistant *M. longifolia* CMEN 585 (left) and susceptible *M. longifolia* CMEN 584 (right). Results are shown for 10 and 20 days post-inoculation (DPI).

reference point from which we can compare sets of genes that are switched on or off, or turned up or down, in response to fungal invasion. These profiles of gene expression changes have enabled identification of candidate genes for wilt resistance. We started with *M. longifolia* because, with just two genome copies, it is genetically simpler than Black Mitcham peppermint (six genome copies) and Native spearmint (four genome copies), for which gene expression data was also collected. We previously reported that, when plants were inoculated with *Verticillium dahliae*, a range of 50-150 genes changed expression level in roots of the different mints, and a range of 200-1,100 genes changed expression level in stems. We have now categorized these genes into different functional categories, prioritizing those with relevance to plant defense against pathogens. We have determined that there is early, increased expression in resistant CMEN 585 stems of genes in two key categories: plant defense signaling (Fig. 2), and secondary metabolite biosynthesis. We are now comparing the variants of these genes in CMEN 585 and CMEN 584. Each *M. longifolia* accession has two copies of every gene; the two copies may be the same, or may have small differences. We will also look at these genes in Black Mitcham (six copies of each gene), in Native spearmint (four copies of each gene), and in the USDA accessions we are using in crosses. We can exploit these small differences to develop molecular markers for these genes that can be employed in the breeding program.

Objective: Compare *Verticillium dahliae* isolates to assess genetic diversity and potential for pathogenicity.

This work focuses on a collection of mint *V. dahliae* isolates that were mainly collected from mint-growing sites in Washington and Oregon. We previously showed that, although nearly all of the mint isolates belong to the same vegetative compatibility group (2B), they have underlying genetic differences that define four distinct groups. We have chosen representative isolates from each of the four groups for further testing of two types: 1) DNA tests of specific mating type and pathogenicity-related genes; and 2) Inoculation tests to determine relative pathogenicity on known resistant and susceptible mints. For the DNA tests, the Vining lab obtained cultures of the representative isolates from the Dung lab, and isolated DNA. Whole-genome DNA sequence information that we had previously generated was then searched to

determine whether there were any single-point DNA differences in any of the genes listed in Table 1. Tests are currently underway to determine the mating type of these isolates.

Table 1. Examples of *Verticillium dahliae* pathogenicity-related genes showing evidence of DNA sequence differences among surveyed isolates. Gene name, expected function, chromosome site and number of DNA sequence differences are listed.

Gene Name	Function	Chromosome	Start Position	Stop Position	Number SNPs
MAT1-2	Mating type associated gene - pathogenicity related	3	1,655,533	1,647,040	1
VdRasGTPase	Ras GTPase Activator Protein - pathogenicity related	3	3,707,621	3,698,265	2
VMK1	MAP Kinase - pathogenicity related	1	5,080,214	5,082,141	1