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Mentha longifolia (L.) L.: A Model Species for Mint Genetic Research

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Abstract. ***Mentha longifolia*****, a wild relative of the polyploid, cultivated *Mentha* (mint) species, was evaluated as a potential model system for genetic research relevant to the cultivated mints. Fourteen *Mentha longifolia* accessions maintained by the US Department of Agriculture (USDA), Agricultural Research Service, National Clonal Germplasm Repository (NCGR), were highly diverse with respect to geographic origin, oil composition, verticillium wilt resistance, aspects of morphology, and molecular marker polymorphism. Accession CMEN 584 was the only carvone chemotype, while CMEN 682 was the only accession with high menthol content. Trans-piperitone oxide was the primary oil component of accessions CMEN 17 and CMEN 18, while pulegone was most abundant in CMEN 20, CMEN 500, CMEN 501, and CMEN 585. Four accessions—CMEN 585, CMEN 17, CMEN 501, and CMEN 81—were consistently resistant to verticillium wilt, while CMEN 584 and CMEN 516 were highly susceptible. Pairwise similarity coefficients were calculated and a UPGMA (unweighted pair-group analysis) tree was constructed on the basis of 63 informative randomly amplified polymorphic DNA (RAPD) marker bands. CMEN 585 and CMEN 584 shared the greatest number of bands (16), and formed a distinct cluster in the UPGMA tree. Seven pairs of accessions had no bands in common, emphasizing the high degree of molecular diversity represented by these accessions. The favorable features of diploid (2n = 2x = 24) genome constitution, comparatively small genome size (400 to 500 Mb), self-fertility, fecundity, and diversity with respect to economically relevant traits, contribute to *M. longifolia*'s potential usefulness as a model system for the cultivated mints. As a perennial species amenable to vegetative propagation, *M. longifolia*'s spectrum of susceptibility/resistance to an important vascular wilt disease encourages its further evaluation as a system for broader studies of plant–microbe interactions and disease resistance mechanisms.**

The principal *Mentha* (Lamiaceae = mint family) species of commerce in the United States are vegetatively propagated polyploids, making them difficult or intractable subjects for transmission genetic analysis and conventional breeding. Native spearmint (*Mentha* ×*villosonervata* Opiz) is triploid ($2n = 3x = 36$), although morphologically similar to fertile, tetraploid spearmint (*M. spicata* L., $2n = 4x = 48$). Scotch spearmint (*M.* ×*gracilis* Sole) is heptaploid ($2n = 7x = 84$). 'Mitcham' peppermint (*M.* ×*piperita* L.) is hexaploid ($2n = 6x = 72$) (Tucker and Naczi, 2005; Tucker and Fairbrothers, 1990; Udo et al., 1962). Polyploidy increases composite genome size and allelic complexity, hampering structural and functional genomics studies, and may be accompanied by poor fertility. Not surprisingly, no genetic linkage maps have been constructed for *Mentha*. Other than an extensive literature on the genetics of oil quality, both classical (Hefendehl and Murray, 1976; Hendriks et al., 1976) and molecular (Croteau and Gershenzon, 1994), few traits have been characterized genetically, and few genomic resources have been developed. Gene identification in *Mentha* has been limited to genes encoding enzymes involved in essential oil biosynthesis. These genes have been extensively characterized, and genetic manipulation of peppermint oil biosynthesis has been initiated (Mahmoud et al., 2004; Burke et al., 2004; Mahmoud and Croteau, 2001).

A diverse and widely distributed *Mentha* germplasm base has been documented (Tucker and Naczi, 2005). As of December 2004, the NCGR in Corvallis, Ore., maintained 441 *Mentha* accessions as vegetative clones and 52 as seed, representing 20 species and a diversity of interspecific hybrids (GRIN). Twenty-one accessions are *M. longifolia*, and six are listed as *M. longifolia* × *M. longifolia* hybrids. In addition, of the 67 accessions listed as simply *Mentha* hybrid, 30 include *M. longifolia* in the known or inferred pedigree. The USDA collection of *M. longifolia* accessions represents a wide range of geographic, phenotypic, and genetic diversity.

Mentha longifolia has the widest natural geographic distribution of any *Mentha* species, from western Europe to central Asia and in southern Africa. It may encompass 22 subspecies (Tucker and Naczi, 2005). Almost all are diploid ($2n = 2x = 24$), but some tetraploid ($2n = 4x = 48$) forms have also been described (Chambers and Hummer, 1994). The sexual fertility of the diploid, and even the tetraploid, forms has been documented (Fagbemi and Morton, 1982; Murray, 1960). The size of the *M. longifolia* genome was reported as 1C = 385 Mbp (Bennett and Leitch, 2005), and in the range of 2C = 0.84 to 0.99 pg (Gobert et al., 2002), or 1C = 405 to 477 Mbp. The *M. longifolia* C value is relatively small among those of cultivated plants, being comparable to that of rice (C = 400 to 466 Mbp) and about half that of tomato (C = 980 Mbp) (Bennett and Leitch, 2005). Phylogenetic analysis of *Mentha* indicates that *M. longifolia* is an ancestor of *M. spicata*, and may be the latter's organelle genome source (Bunsawat et al., 2004). In turn, *M. spicata* is a parent of *M.* ×*gracilis* and of *M.* ×*piperita* (Tucker and Naczi, 2005; Tucker et al., 1991; Tucker and Fairbrothers, 1990). *Mentha canadensis* is believed to have arisen as a hybrid of *M. longifolia* and *M. arvensis* (Tucker and Chambers, 2002).

We have examined a set of *M. longifolia* accessions maintained by the NCGR, with particular attention to two traits of economic relevance: oil composition and resistance to verticillium wilt, an important disease of peppermint. This paper documents the phenotypic and genetic diversity among these *M. longifolia* accessions and reviews the features that make *M. longifolia* a potentially useful model species for *Mentha* genetic and genomic research.

Materials and Methods

Germplasm. Fourteen accessions initially identified as *M. longifolia*, including 4 subspecies, were obtained as rooted plants or rhizomes from the NCGR. The USDA National Plant Germplasm System Plant Information (PI) numbers for each of these accessions, as well as their chromosome numbers (if known) and geographic origins, are listed in Table 1. Plants were maintained in a greenhouse at the University of New Hampshire in 22-cm pots, and were propagated vegetatively. Observations of morphology were made by direct visual examination and by light microscopy.

Oil composition. Oils from whole flowering plants were distilled with a neo-Clevenger of Moritz after Kaiser and Lang with the modification of Hefendehl (Kaiser and Lang, 1951; von Rudloff, 1969). Mass spectra were recorded with a 5970 Hewlett-Packard Mass Selective detector coupled to a HP 5890 GC using a HP 50 m × 0.2 mm fused silica column coated with 0.33 mm FFAP (crosslinked). The GC was operated under the following conditions: injector temperature 250 °C; oven temperature programmed to 60 °C held for 1 min to 115 °C at 2.5 °C per min, then to 210 °C at 1.0 °C per min and held for 30 min; injection size 1mL (about 50% solution in spectroscopy grade n-pentane) split 1:10. The MSD EI was operated at electron impact source 70 eV, 250 °C. Identifications were made by Kovats Indices and library searches of our volatile oil library supplemented with those of NBS, NIST, and Wiley.

Verticillium resistance screening. Qualitative assessment of verticillium resistance in all 14 accessions was conducted with a wild-type *Verticillium dahliae* strain provided by Dennis Johnson at Washington State University. Based on the outcome of these initial trials, a subset of resistant and susceptible accessions was chosen

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Table 1. Features of *Mentha longifolia* accessions used in this study. Accession numbers, status and geographic origin as listed on http://www.ars-grin.gov/cor/mentha/meninfo.html.

Accession	Status	$2n^z$	Collected from
CMEN 17 (PI 557755)	Breeding material	24	Unknown European country
CMEN 18 (PI 557756)	Wild material	24	Netherlands
CMEN 19 (PI 557757)	Wild material	24	France
CMEN 20 (PI 557770)	Wild material	24	Syria
CMEN 34 (PI 557758)	Wild material	---[y]	India
CMEN 500 (PI 212313)	Wild material	48	Afghanistan
CMEN 501 (PI 212314)	Cultivated material	48	Afghanistan
CMEN 516 (PI 557760)	Cultivated material	---	Italy
CMEN 584 (PI 557769)	Uncertain improvement status	24	South Africa
CMEN 585 (PI 557767)	Uncertain improvement status	24	South Africa
CMEN 592 (PI 557766)	Wild material	24	Uzbekistan
CMEN 635 (PI 557768)	Wild material	24	Nepal
CMEN 682 (PI617491)	Cultivar: 'Velvet'	---	Russia
CMEN 81 (PI 557759)	Probable hybrid of *M. longifolia* × *M. spicata*	---	United States

[z]Chambers and Hummer (1994).
[y]Undetermined chromosome number.

for closer examination and for use as crossing parents for future genetic studies. The latter trials differed from the initial assessments in that a quantitative rating scale was used, and a *V. dahliae* strain which was transformed with green fluorescent protein (GFP) (Lorang et al., 2001), provided by Linda Ciuffetti at Oregon State University, was used instead of the wild-type strain. Both *V. dahliae* cultures were maintained in petri plates on Czapek-Dox medium, which was supplemented with 45 $\mu g \cdot mL^{-1}$ hygromycin for the GFP strain.

Mentha longifolia cuttings of uniform size were rooted in 1206 cell packs with soilless Metro Mix 360 (The Scotts Co., Maryville, Ohio). They were maintained for 2 weeks in a growth chamber with fluorescent lighting (15 ± 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$), cool temperatures and a short-day cycle (22 °C, 10 h light/20 °C, 14 h dark) to minimize growth and prevent flowering.

Screening was performed using a modified root-dip inoculation technique based on that of Green and Simon (1996). An appropriate volume of *Verticillium* in liquid Czapek-dox medium was incubated at room temperature on a shaker for 1 to 2 weeks. The volume of liquid culture used depended on the number of cuttings to be inoculated. Liquid cultures were strained through a single layer of Miracloth to separate conidia from hyphae. The filtrate was centrifuged at 10,000 g_n for 5 min to pellet conidia. Pellets were resuspended in 100 mL distilled H_2O. This step was performed to eliminate any residual medium as well as any substances secreted by the fungus. A hemacytometer was used to count conidia with a compound microscope (20× objective). The conidial suspension was diluted with distilled H_2O to about 10^7 conidia/mL.

Screening trials had 12 replicate cuttings randomized within each treatment (control, inoculated). Control plants and inoculated plants were processed simultaneously. Cuttings were uprooted, soil was shaken from roots, and plants were placed in 50-mL beakers containing about 20 mL of either distilled H_2O (uninoculated control) or conidial suspension for 5 min. Plants were then replanted in new flats in Metro Mix 360. After inoculation, plants were kept in the growth chamber with minimal watering, continuing the aforementioned light and temperature conditions. After 2 weeks, plants were moved to the greenhouse under natural light and ambient temperature.

Observations were taken 8 weeks postinoculation. For plants with dead primary stems, stem sections were surface-sterilized and plated on water agar to confirm the presence of *Verticillium dahliae*. Degree of plant stunting, horizontal leaf curling (crescent leaf), and tissue chlorosis and necrosis were all observed in order to assign an overall symptom severity rating from 0 to 4 (Dennis Johnson, personal communication). A 0 rating indicated lack of visible symptoms; a 4 rating meant the plant was dead. Intermediate ratings described plants with mild to severe symptoms. Ratings data for inoculated plants were processed in Systat v.10.0 by ANOVA as a completely randomized design. Pairwise comparisons were made with Tukey's tests.

DNA extraction. DNA was extracted from fresh, unexpanded leaf tissue using the CTAB miniprep method of Torres et al. (1993), with the following modifications: sodium bisulphate was not used in the grinding buffer, chloroform–octanol was not added to the grinding slurry before 65 °C incubation, and the ethanol wash utilized 70% ethanol without sodium acetate. Concentration of DNA dissolved in 50 µL TE (Tris-EDTA, pH 8.0) was measured fluorometrically, then DNA was diluted with sterile H_2O to a standard stock concentration of 40 $ng \cdot \mu L^{-1}$.

Polymerase chain reaction. Fourteen oligonucleotide primers (Operon technologies, Alameda, Calif.) were used individually in PCR to detect randomly amplified polymorphic DNA (RAPD) polymorphisms. DNA was amplified in 25 µL reactions using 100 ng template DNA, 0.1 U Taq DNA polymerase (Eppendorf), 2.5 mM each of dNTP (Promega) and 0.8 µM primer. The reactions were performed in a thermal cycler programmed for one cycle of 2 min at 94 °C followed by 39 cycles of 1 min at 94 °C, 2 min 30 s at 35 °C, 30 s at 45°C, and a final elongation step of 10 min at 72°C.

Separation and visualization of amplification products. PCR products were separated on gels containing 1% NuSieve GTG agarose (FMC Bioproducts, Rockland, Maine) and 1% agarose (Shelton Scientific, Shelton, Conn.) run in 1× TBE, pH 8.0 at 90V for at least 3.5 h at 4 °C. Gels were stained with ethidium bromide and photographed under UV light.

RAPD marker diversity analysis. Only informative markers (bands that were present in at least two accessions and absent in at least two) were included in the analysis. A total of 63 bands were treated in a binary format and scored as 1 (band present) or 0 (band absent). Pairwise genetic similarities were calculated using the Jaccard similarity coefficient (a/(a + b + c)) (Jaccard, 1908). Additionally, a phenetic analysis was conducted using Paup 4.0b10. A dendrogram was generated using the unweighted pair-group method with arithmetic averages (UPGMA) method with 1000 bootstrap replications.

Genome size determination. Root tips were fixed in Farmer's solution (3 ethanol:1 glacial acetic acid) and sent to I.J. Leitch, Jodrell Laboratory, Royal Botanic Gardens, Kew, where the C values were measured by Feulgen microdensitometry (Bennett and Leitch, 2005).

Results

Among the morphological characters showing variation were leaf shape, flower color (Table 2) and growth habit. CMEN 584 and CMEN 585 had lanceolate leaves; the others' leaves were ovate (Table 2). Three accessions—CMEN 584, CMEN 585 and CMEN 34—had white flowers, while flowers of the other accessions were various shades of purple. Under the growth conditions in the UNH greenhouse, CMEN 584 and CMEN 585 had a tall upright growth habit, reaching a height of about 100 cm at flowering. CMEN 682 and CMEN 34 had a moderately upright growth habit, but only reached 50% to 75% of the height of CMEN 584 and CMEN 585. The other accessions had a shorter upright growth habit.

Oil composition was highly variable among the accessions (Table 2). Pulegone was the principal oil component of CMEN 20, CMEN 500, CMEN 501, and CMEN 585. These accessions, along with CMEN 682 and CMEN81, contained moderate levels of menthone. CMEN 17, CMEN 18, and CMEN 635 had high levels of *cis*- or *trans*-piperitone oxide. CMEN 584 was the only accession for which the principal oil component was carvone.

The *M. longifolia* germplasm showed diversity in response to inoculation with *V. dahliae*. Symptoms first became apparent 2 to 4 weeks

Table 2. Phenotypes of *Mentha longifolia* accessions. Only principal oil compounds (>5%) are listed. Verticillium resistance qualitative ratings are from initial screenings conducted with wild type *Verticillium dahliae* before a numerical rating system was implemented. Qualitative ratings are R = resistant, I = intermediate, S = susceptible. Quantitative ratings are from subsequent screenings of a subset of accessions chosen as crossing parents for future genetic studies. The latter trials were conducted with a GFP-transformed *V. dahliae* strain. Ratings are average scores for total numbers of plants screened for each genotype. The rating system is 0 = no visible symptoms; 0.5 to 2.5 = mild to moderate symptoms; 3 to 3.5 = severe symptoms; 4 = dead. Ratings followed by the same letter are not significantly different from one another ($p = 0.05$). Ratings with different letters are highly significantly different ($p < 0.01$) according to a Tukey's test.

	Leaf	Flower	Verticillium response		
Accession	shape	color	Qualitative	Quantitative	Oil composition
CMEN 585	Lanceolate	W	R	0[z]	32.8% Pulegone 24.3% menthone 11.3% 1,8-cineole
CMEN 501	Ovate	P	R	0[z]	30.4% Pulegone 25.3% menthone 11.0%menthol 5.0% limonene
CMEN 81	Ovate	P	R	0[z]	39.2% Menthone 22.5% iso-menthone 8.1% 1,8-cineole
CMEN 17	Ovate	P	R	0.3[a]	43.4% Trans-piperitone oxide 19.7% cis-piperitone oxide 7,0% 1,8-cineole
CMEN 635	Ovate	P	R	1[y]	45.6% Cis-piperitone oxide 26.6% piperitenone oxide 5.0% trans-piperitone oxide
CMEN 34	Ovate	W	S	2.0[x]	14.9% Piperitenone oxide 6.97% limonene
CMEN 682	Ovate	P	S	2.6[w]	56.5% Menthol 14.8% menthone
CMEN 516	Ovate	P	S	3.5[v]	21.9% Germacrene D 18.6% trans-piperitone oxide 11.7% limonene 8.0% (Z)-B-ocimene
CMEN 584	Lanceolate	W	S	3.8[v]	59.6% Carvone 12.3% limonene
CMEN 18	Ovate	P	I		56.4% Trans-piperitone oxide 7.2% cis-piperitone oxide 5.8% 1,8-cineole
CMEN 19	Ovate	P	S		
CMEN 20	Ovate	P	R		13.5% Pulegone 11.7% nonanal 7.8% menthone 7.0% trans-piperitone oxide 6.6% limonene
CMEN 500	Ovate	P	R		34.6% Pulegone 17.0% menthone 14.2% sabinene 6.1% limonene
CMEN 592	Ovate	P	S		22.4% (E)-β-farnesene 16.0% limonene 12.7% nonanal 11.0% B-caryphyllene 7.4% Gamma-muurolene

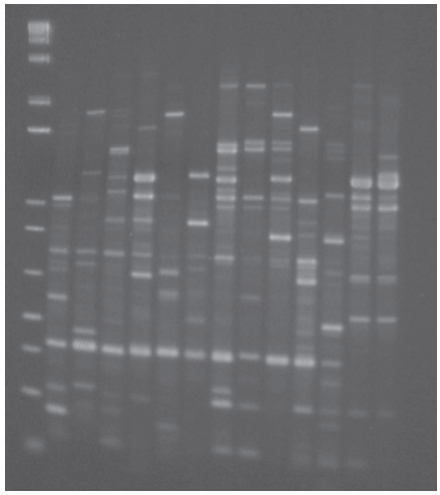

Fig. 1. Example of electrophoretic banding pattern of PCR-amplified DNA fragments produced from RAPD primer OPO20. Lane 1 = a molecular size marker (1Kb Plus DNA Ladder, Invitrogen). Lanes 2 to 14 = CMEN 17, CMEN 18, CMEN 19, CMEN 20, CMEN 34, CMEN 516, CMEN 592, CMEN 500, CMEN 501, CMEN 635, CMEN 682, CMEN 585, and CMEN 584, respectively.

postinoculation. Symptoms ranged from mild horizontal curling of apical leaves to complete necrosis. Nine accessions, selected as representing extremes of inoculation response and other traits of interest, were then screened more rigorously, using the quantitative rating scale of 0 to 4 (Table 2). Results of screenings conducted with the GFP strain were highly consistent with those obtained using the wild type *Verticillium* strain: the four accessions given 0 to 1.0 ratings in the second trial had all received R ratings in the first trial, while accessions given ratings 2.0 and above in the second trial all had S ratings in the first trial (Table 2). Overall, CMEN 585, CMEN 17, CMEN 501, and CMEN 81 were the most resistant, and CMEN 584 and CMEN 516 were the most consistently susceptible accessions.

Symptom development varied considerably among susceptible accessions. For example, by 4 to 6 weeks postinoculation, CMEN 516 exhibited overall chlorosis of leaf tissue, mild to moderate crescent leafing and little or no stunting, while CMEN 584 was consistently stunted >50% compared to controls and had substantial crescent leaf symptoms. Both CMEN 516 and CMEN 584 primary stems had died by the time final observations were recorded. However, asymptomatic shoot growth was sometimes seen emerging at the soil surface after complete death of primary stems above the soil line, indicating that at least some portion of these plants survived and escaped or recovered from fungal infection.

Similarly, *Verticillium*-resistant accessions showed differences in response to fungal inoculation. CMEN 585 occasionally had mild to moderate horizontal curling of apical leaves about 4 weeks postinoculation, followed by production of asymptomatic leaves. CMEN 17 commonly displayed shortened internodes and mild horizontal leaf curl about 4 weeks postinoculation, followed by apparent recovery. CMEN 501 and CMEN 81 rarely displayed any disease symptoms.

For RAPD analysis (Fig. 1), 14 oligonucleotide primers produced a total of 63 informative bands. The number of bands shared by any pair of accessions ranged from 16 to 0: for example, CMEN 584 had 16 bands in common with CMEN 585 and none in common with five of the accessions (Table 3). The Jaccard similarity indices ranged from a high of 0.7619 (CMEN 584 vs. CMEN 585) to a low of 0 (e.g., CMEN 584 vs. CMEN 682) (Table 3). A UPGMA tree had 5 nodes with bootstrap support of 50% or better (Fig. 2). CMEN 585 and CMEN 584 formed a group that was highly distinct from, and sister to, the other accessions.

The genome sizes of *M. longifolia* accessions CMEN 584 and CMEN 585 were determined to be 4C = 1.75 pg (1C = 440 Mbp) and 4C=1.64pg (1C = 410 Mbp), respectively (Lynda Hanson, pers. comm.). The genome size of CMEN 17 was estimated to be 4C = 1.57pg (1C = 385Mb) (Bennett and Leitch, 2005).

Discussion

Our examination of 14 NCGR accessions of *M. longifolia* detected considerable phenotypic

Table 3. Jaccard similarity coefficient matrix. Values above the diagonal are the number of bands shared by each pair of accessions. Values below the diagonal are Jaccard similarity coefficients.

	CMEN 17	CMEN 18	CMEN 19	CMEN 20	CMEN 34	CMEN 516	CMEN 592	CMEN 500	CMEN 501	CMEN 635	CMEN 682	CMEN 585	CMEN 584
CMEN 17		9	12	7	7	9	8	3	4	7	3	2	0
CMEN 18	0.4286		8	7	5	9	8	2	2	7	1	1	0
CMEN 19	0.5217	0.3478		11	6	9	10	4	3	8	3	1	0
CMEN 20	0.2414	0.2800	0.4231		7	7	9	3	1	6	0	4	2
CMEN 34	0.3043	0.2381	0.2400	0.2800		5	7	2	4	3	1	1	1
CMEN 516	0.3750	0.4500	0.3600	0.2500	0.2083		8	4	3	5	1	2	1
CMEN 592	0.3200	0.2857	0.3226	0.2727	0.2414	0.2581		9	10	5	2	2	1
CMEN 500	0.0968	0.0714	0.1290	0.0909	0.0714	0.1379	0.2903		7	2	1	1	2
CMEN 501	0.1290	0.0690	0.0909	0.0278	0.1481	0.0968	0.3226	0.2500		4	3	1	2
CMEN 635	0.3043	0.3684	0.3478	0.2308	0.1304	0.2083	0.1613	0.0714	0.1481		2	1	0
CMEN 682	0.1500	0.0556	0.1429	0.0000	0.0556	0.0476	0.0741	0.0455	0.1429	0.1176		0	0
CMEN 585	0.0588	0.0323	0.0278	0.1176	0.0323	0.0606	0.0500	0.0286	0.0278	0.0667	0.0000		16
CMEN 584	0.0000	0.0000	0.0000	0.0571	0.0333	0.0303	0.0250	0.0606	0.0588	0.0333	0.0000	0.7619	

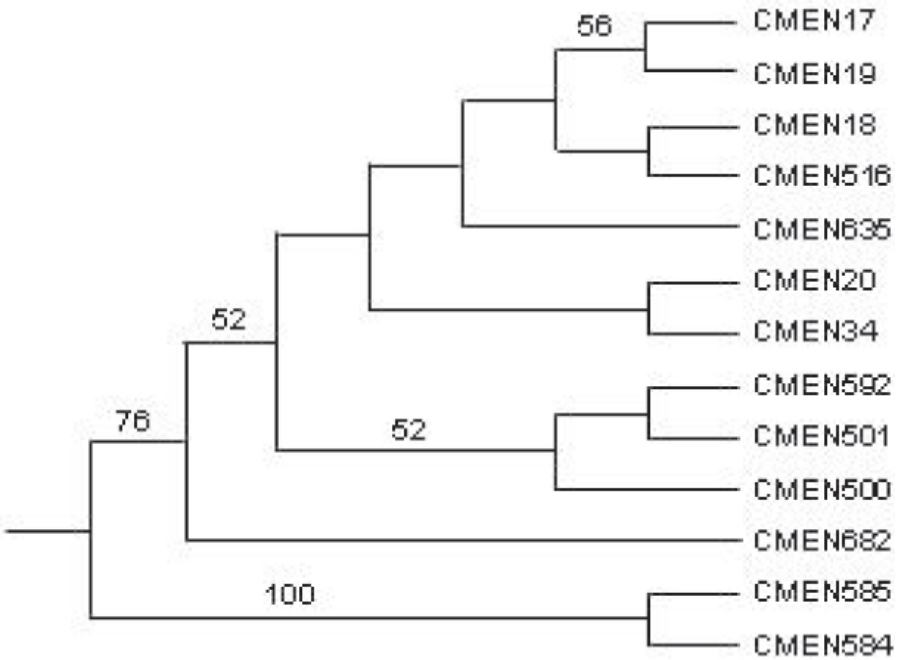

Fig. 2. RAPD marker-based, midpoint-rooted UPGMA dendrogram showing relationships of *Mentha longifolia* accessions**.** Numbers above branches are bootstrap values.

and genetic variation. Plant height, flower color, leaf shape and leaf trichome density were obviously variable among the accessions. Variation was noted but not systematically examined in other morphological features such as leaf color, leaf margin type and stem thickness. Although *Mentha* species are distinguished primarily by their essential oil contents, the range of morphological variation in *M. longifolia* points to its potential for development as an ornamental species as well as a genetic model system.

Mentha longifolia has been a subject of numerous oil composition studies (Ghoulami et al., 2001; Hefendehl, 1977; Kokkini et al., 1995; Kokkini and Papageorgiou, 1988; Shaiq et al., 2002; Venskutonis, 1996). *M. longifolia* oil composition has attracted recent attention due to its potential for antimicrobial and antifungal activity (Mimica-Dukic et al., 2003; Abou-jawdah et al., 2002). The present paper adds data on 14 NCGR accessions to the substantial body of knowledge about *M. longifolia* oil composition. CMEN 584 is the only carvone chemotype in the NCGR plant collection; however, other *M. longifolia* carvone chemotypes have been reported (Hefendehl, 1977; Kokkini et al., 1995).

One major focus of our research with *M. longifolia* is the identification of plants with differential responses to the fungal pathogen *V. dahliae.* Toward that end, all *M. longifolia* accessions were initially screened with a wild type strain of the fungus. When a GFP-transformed strain became available (Lorang et al., 2001), it was used for subsequent screenings of selected accessions, and of F1 and F2 populations developed from resistant × susceptible crosses (results to be presented elsewhere). Trials conducted with the GFP strain of *V. dahliae* produced results consistent with those performed with the wild type strain. The GFP strain is of interest as a potentially useful tool for the study of the early events of fungal penetration of a plant host (Lorang et al., 2001).

The disease resistance screening showed that some accessions are highly resistant to verticillium wilt and others are highly susceptible. Of the two most resistant accessions, CMEN 501 is a tetraploid and CMEN 81, as a probable hybrid between *M. longifolia* and *M. spicata* (tetraploid), is not likely to be diploid. The two most resistant diploids, CMEN 585 and CMEN 17, sometimes displayed mild disease symptoms followed by asymptomatic growth. The most susceptible *M. longifolia* accessions, CMEN 584 (diploid) and CMEN 516 (chromosome number undetermined), showed differences in symptom development, although the eventual outcome for both was primary stem death. Both genotypes occasionally exhibited secondary growth after death of primary stems, indicating that even when primary stems were completely dead above the soil line, some stem tissue survived and was capable of regeneration. It is possible that part of the disease resistance response in these plants involves blockage of part of the root vascular system in order to sequester the invading fungus. In the field, such a response could allow the plants to escape verticillium disease by growing via secondarily produced shoots and stolons to a noninfested area. A strategy for outgrowing soilborne pathogens is especially important for a perennial species with a primarily asexual mode of reproduction.

Mentha longifolia is recognized as the most phenotypically diverse species of the taxonomically complex *Mentha* genus (Gobert et al., 2002). These investigators used AFLP markers to analyze 62 *Mentha* accessions, 6 of which are represented in the present study. They found that *M. longifolia* grouped as a distinct taxon from other *Mentha* species, and is most closely related to *M. spicata* and *M. suaveolens*. The present study, which was aimed only at assessing genetic diversity in *M. longifolia*, demonstrated substantial molecular diversity as detected using RAPD markers. In pairwise comparisons of RAPD markers, only two pairs of accessions (CMEN 17 vs. CMEN 19, and CMEN 585 vs. CMEN 584) had >50% of informative markers in common.

The two South African accessions, CMEN 584 and CMEN 585, are remarkably different in appearance from the others. Both have a tall upright growth habit and lanceolate leaves. In addition, the RAPD marker data set these two accessions apart (Fig. 2, Table 3). However, despite their morphological similarity and the high number of shared RAPD markers, these two accessions were very different from each other in oil composition and verticillium wilt resistance. Our initial results indicate a need to expand the available germplasm collection to include a broader sampling of the South African representatives of *M. longifolia*.

Mentha longifolia is a suitable and valuable species to serve as a model species for mint genetics for several reasons. Of the 14 NCGR accession we examined, 8 are known to be diploid, a favorable feature for genetics and linkage mapping. The *M. longifolia* genome size in the 400 to 500 Mbp range is relatively small, making it a favorable subject for structural and functional genomics studies. The C values we obtained for CMEN 585 and CMEN 584 are the first reported for South African genotypes of *M. longifolia*. They are comparable to previously published C value measurements of other NCGR *M. longifolia* accessions (Gobert et al., 2002). Because of the abundant genetic/phenotypic diversity apparent in the species, crosses between appropriately chosen representatives could be used to study the genetic basis for variation in numerous characters of economic relevance. Examples of trait diversity documented here include plant morphology, disease resistance and oil composition. Given the broad geographic range of *M. longifolia*, the species is likely to contain considerable variation for responses to environmental stress factors as well.

Mentha longifolia is also an intriguing subject for the study of host–pathogen interactions because of its perennial habit, vegetative propagation, and stem morphology. Replication of screening experiments is facilitated because large numbers of cuttings (clones) can be quickly generated from a single plant. Plants can be maintained in a perpetual vegetative growth state under short-day light regimes, minimizing variation due to hormonal differences between flowering and vegetative growth stages. *Mentha longifolia* is particularly useful for the study of vascular wilt pathogens because of stem morphology: stems are square, and each stem possesses exactly four vascular bundles—one at each corner—making it possible to observe localized disease symptoms and correlate them to pathogen invasion of particular vascular bundles. Thus, the many favorable features of *M. longifolia* make this species a useful diploid system for studies of

Mentha genetics and genomics, and a vegetatively propagated model organism of potential interest for the study of plant–pathogen interactions in general.

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