Research Article

Flavour and Fragrance Journal

Received: 22 March 2009; Revised: 21 September 2009; Accepted: 6 October 2009; Published online in Wiley InterScience: 27 October 2009

(www.interscience.wiley.com) DOI 10.1002/ffj.1957

Assessment of menthol mint collection for genetic variability and monoterpene biosynthetic potential

Ajit K. Shasany,* Soni Gupta, Mahesh K. Gupta, Ali A. Naqvi, Janak R. Bahl and Suman P. S. Khanuja

ABSTRACT: Eighteen accessions of *Mentha arvensis* var. *piperascens* Holmes from the CIMAP collection (CIMAP/C01 to CIMAP/C18) consisting of wild collections and released varieties, their mutants, seed progeny and hybrids from the CIMAP gene bank were assessed for diversity through a combined morphochemical and molecular approach. Morphological characters, oil yield and essential oil components were taken into account to generate a cluster that outgrouped accession CIMAP/C05 from others. A total of 60 primers were used for RAPD and the tree generated after cluster analysis revealed accession CIMAP/C05 as the most diverse genotype. All the accessions exhibited differences in narrow range except CIMAP/C05. A wide range of compositional differences were observed in the essential oil profile of the genotypes indicating varying efficiencies of conversion of one component into other and/or existing metabolic blocks in the essential oil biosynthetic pathway in them. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: biosynthetic pathway; essential oil; *Mentha arvensis*; morphochemical; RAPD

Introduction

Mints (species of genus *Mentha* L.) belonging to the family Lamiaceae are commercially important, due to the essential oil produced by them. One of the *p*-menthane monoterpene constituting the major part of the essential oil is menthol, which along with other components such as limonene, carvone, linalool, etc. find various uses in the pharmaceutical, food and confectionery industries. The main component of mint oil menthol is being used as analgesic and this property is mediated through a selective activation of κ-opioid receptors.[1] Menthol also enhances the efficacy of ibuprofen in topical applications *via* vasodilation, which reduces skin barrier function.[2] In nature, *Mentha* species exhibit variability, not only morphologically but also in essential oil content and composition, resulting in differing aromas.[3] This variability can be accounted for by the fact that natural interspecific hybridization has led to the formation of hybrids differing widely from their parents and often designated as different species. In an ongoing effort to assess the diversity of *Mentha* germplasm, studies were carried out at interspecific level at Central Institute of Medicinal and Aromatic Plants, Lucknow, India.[4,5,8]

DNA fingerprinting methods are widely used in plant genome research, such as in variability studies, phylogenetic analysis, synteny mapping, marker-assisted selection of superior genotypes, etc.[6,7] Random amplified polymorphic DNA (RAPD) technique has been used in several laboratories for variability analysis and identification of markers linked to agronomically important traits.[9–14] RAPD has also been used as a tool to asses genetic uniformity of the *in vitro* regenerated plantlets.[15] The present investigation is the detailed study of the available diversity in India for the taxon *Mentha arvensis* var. *piperascens* Holmes at intraspecific level, with the aim of selecting the most diverse genotypes with variations in morpho- and chemotypic characters to be used as breeding stocks in the mint improvement programme. At the same time the selected germplasm will be of use for exploitation of genes involved in the monoterpene metabolic pathway.

Materials and Methods

Plant Material

Accessions of *Mentha arvensis* var. *piperascens* Holmes were obtained from the National Gene Bank for Medicinal and Aromatic Plants, housed at CIMAP (sponsored by the Department of Biotechnology, Government of India). These are collections from diverse places of India, mutant and seedling selections, Gene bank collections, as well as some of the released varieties of CIMAP (description provided in Table 1). Suckers of 18 accessions (CIMAP/C01 to CIMAP/C18) of *M. arvensis* were planted (five replicates each) in plots of 2.5 × 2.5 m size in the normal cropping season in a CIMAP farm field. Standard agronomy practices were followed.[16]

*Correspondence to: A. K. Shasany, Genetic Resources and Biotechnology Division, Central Institute of Medicinal and Aromatic Plants, Lucknow 226012, India. E-mail: akshasany@yahoo.com

Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow 226015, India

Table 1. Menthol mint *Mentha arvensis* var. *piperascens* Holmes accessions taken in the study

Serial no.	Accession no.	Source/origin	Reference
1	CIMAP/C01	Gene bank collection	Gene bank*
2	CIMAP/C02	Gene bank collection	Gene bank*
3	CIMAP/C03	Gene bank collection	Gene bank*
4	CIMAP/C04	Gene bank collection	Gene bank*
5	CIMAP/C05	Gene bank collection	Gene bank*
6	CIMAP/C06	Gene bank collection	Gene bank*
7	CIMAP/C07	Mutant generated by seed irradiation of CIMAP/C03	Gene bank*
8	CIMAP/C08	Gene bank collection	Gene bank*
9	CIMAP/C09	Seedling selection of CIMAP/C03, open pollinated	Gene bank*
10	CIMAP/C10	Seedling selection of CIMAP/C03, open pollinated	Gene bank*
11	CIMAP/C11	Seedling selection of CIMAP/C03, open pollinated	Gene bank*
12	CIMAP/C12	Released variety of CIMAP, 'Gomti', seedling variant from the seeds of CIMAP/C18	US Patent PP 10935
13	CIMAP/C13	Seedling selection of CIMAP/C18	Gene bank*
14	CIMAP/C14	Released variety of CIMAP, 'Himalaya', hybrid of 'Gomti' and 'Kalka'	24, US Patent PP 10935
15	CIMAP/C15	Released variety of CIMAP, 'Kosi'	25, US Patent PP 12426
16	CIMAP/C16	Released variety of CIMAP, 'MAS-1', somatic variant of CIMAP/C03	Gene bank*
17	CIMAP/C17	Released variety of CIMAP, 'Kalka' (HY77), selection of 'MAS-1'	US Patent PP 10935, PP 12426
18	CIMAP/C18	Released variety of CIMAP, 'Shivalik'	Gene bank*

* As provided by Dr J. R. Bahl, breeder in charge of maintenance of the *Mentha* germplasm.

Morphochemotypic Analysis

Fresh leaves were collected in the month of May from the field-grown plants of various accessions and oil was extracted from 300 g plant samples in triplicate by hydrodistillation, using a Clevenger's apparatus. Oil yield (%) was calculated as the mean of three samples. The collected oil was subjected to gas–liquid chromatography (GLC) analysis on a Varian CX-3400, using a 30 m × 0.32 mm (0.25 μm film thickness) BP21 column. The injector and detector temperatures were maintained at 220°C and 240°C, respectively. The oven temperature was programmed from 50°C to 220°C at a rate of 6°C/min, with an initial hold of 2 min. Hydrogen was used as the carrier gas at rate of 30 ml/min and a 0.1 μl sample was injected with a split ratio of 1 : 100. Data were processed by a chromatography data station (AIMIL Ltd, India), using Winacds software, and the identification was based on the retention times of authentic samples and retention index calculations.[17]

Morphological characters in terms of plant height and leaf : stem ratios (weight of leaf : weight of stem) of individual accessions were noted from field-grown plants 110 days after planting (average of three replicates per accession). Hierarchical cluster analysis was carried out based on these morphological characters and essential oil components present in different accessions taken together. Standard Euclidian distance was generated and clusters were made through the average linkage method, using KYPLOT software (Koichi Yoshioka: http://www.qualest.co.jp).

PCR Amplification and RAPD Analysis

The pooled leaves from the five replicates of each accession constituted the material for DNA isolation. The protocol of Khanuja *et al*. (1999) was employed to isolate DNA from fresh leaf tissue.[18] Polymerase chain reaction (PCR) was carried out in 25 μl volume. A reaction tube contained 25 ng DNA, 0.2 U Taq DNA polymerase, 2.5 μl 10 × buffer, 100 μM each of dNTPs, 1.5 mM $MgCl_2$ and 5 pM decanucleotide primers. Three sets of 60 primers each were used to assess the genetic variability among the 18 accessions. The first set of 20 decanucleotide primers were custom-synthesized in the laboratory at CIMAP, using an ABI Model 392 synthesizer (Applied Biosystems). The sequences of these primers, designated as MAP 01–MAP 20, were AAATCGGAGC, GTCCTACTCG, GTCCTTAGCG, TGCGCGATCG, AACGTACGCG, GCACGCCGGA, CACCCTGCGC, CTATCGCCGC, CGGGATCCGC, GCGAATTCCG, CCCTGCAGGC, CCAAGCTTGC, GTGCAATGAG, AGGATACGTG, AAGATAGCGG, GGATCTGAAC, TTGTCTCAGG, CATCCCGAAC, GGACTCCACG and AGCCTGACGC, respectively. The other two sets of 20 primers each, OPJ 01–OPJ20 and OPT 01–OPT20, were obtained from Operon Technologies (USA). RAPD profiling was repeated twice and the consistent loci were scored for further analysis.

RAPD profiles were analysed by scoring the presence and absence of fragments of different accessions and the similarity indices were generated using Nei and Li's coefficient.[19] The average similarity matrix was used to generate a tree through the average linkage method, using KYPLOT software. The distances between individuals calculated with molecular and morphochemical data were compared through a Mantel test (Program for Mantel test, version 1.19, by Mauro J. Cavalcanti, Centro de Ciências Biológicas, Universidade Santa Úrsula: ftp://life.bio.sunysb.edu /morphmet/mantel32.exe).

Results and Discussion

Morphochemotypic Variability

Average performances of these accessions in terms of morphochemical characters are listed in Table 2. Accession CIMAP/C12 showed the highest plant height of 92.6 cm and CIMAP/C06 exhibited dwarf characteristics (47.6 cm). The accessions CIMAP/C15 (showing the highest leaf : stem ratio) also yielded the

Table 2. Morphological characters, oil content and major essential oil components of various accessions of *Mentha arvensis*

Accession no.	Morphological characters		Oil content (%)	Oil components (Percentage)					
	Plant height (cm)	Leaf stem ratio		Menthone (%)	Isomenthone (%)	Menthyl acetate (%)	Neo-menthol (%)	Menthol (%)	Others (%)
CIMAP/C01	84.0 ± 0.5	0.95 ± 0.01	0.55 ± 0.05	42.78 ± 0.07	2.57 ± 0.12	1.48 ± 0.15	0.18 ± 0.07	25.37 ± 0.21	~28
CIMAP/C02	62.2 ± 0.94	0.94 ± 0.01	0.42 ± 0.02	6.63 ± 0.20	1.75 ± 0.25	18.27 ± 0.10	1.13 ± 0.10	63.83 ± 0.11	~8
CIMAP/C03	83.5 ± 0.86	0.94 ± 0.01	0.33 ± 0.02	16.36 ± 0.05	4.92 ± 0.07	12.03 ± 0.12	1.16 ± 0.07	58.37 ± 0.08	~8
CIMAP/C04	72.7 ± 0.66	0.95 ± 0.01	0.46 ± 0.02	11.66 ± 0.22	3.88 ± 0.13	6.61 ± 0.20	1.79 ± 0.17	70.52 ± 0.26	~6
CIMAP/C05	60.5 ± 1.00	1.15 ± 0.01	0.38 ± 0.02	4.73 ± 0.11	1.70 ± 0.20	2.22 ± 0.11	3.08 ± 0.06	30.79 ± 0.13	~57
CIMAP/C06	47.6 ± 1.01	1.17 ± 0.01	0.62 ± 0.02	5.26 ± 0.17	2.08 ± 0.15	1.64 ± 0.16	2.45 ± 0.11	79.78 ± 0.18	~9
CIMAP/C07	78.2 ± 0.68	0.94 ± 0.01	0.52 ± 0.02	71.65 ± 0.19	3.42 ± 0.14	0.60 ± 0.08	2.18 ± 0.10	11.63 ± 0.21	~11
CIMAP/C08	69.3 ± 0.32	0.88 ± 0.01	0.68 ± 0.02	9.32 ± 0.10	4.21 ± 0.11	6.37 ± 0.11	2.00 ± 0.13	70.76 ± 0.17	~7
CIMAP/C09	81.0 ± 1.32	1.00 ± 0.09	0.37 ± 0.02	15.81 ± 0.20	4.44 ± 0.15	13.66 ± 0.13	1.90 ± 0.22	58.17 ± 0.15	~6
CIMAP/C10	81.4 ± 0.56	0.88 ± 0.06	0.28 ± 0.02	11.42 ± 0.11	3.45 ± 0.13	4.45 ± 0.22	1.81 ± 0.12	66.89 ± 0.08	~12
CIMAP/C11	85.7 ± 0.92	0.94 ± 0.03	0.38 ± 0.02	9.46 ± 0.13	3.90 ± 0.15	12.01 ± 0.12	1.24 ± 0.15	68.05 ± 0.28	~5
CIMAP/C12	92.6 ± 1.04	1.01 ± 0.04	0.38 ± 0.02	11.73 ± 0.08	3.52 ± 0.11	5.86 ± 0.12	1.26 ± 0.05	72.34 ± 0.16	~5
CIMAP/C13	83.0 ± 0.5	1.41 ± 0.03	0.68 ± 0.02	10.14 ± 0.19	2.95 ± 0.13	1.63 ± 0.21	2.14 ± 0.11	72.56 ± 0.18	~11
CIMAP/C14	89.8 ± 1.25	1.22 ± 0.02	0.80 ± 0.05	4.35 ± 0.20	3.00 ± 0.09	2.82 ± 0.22	1.29 ± 0.05	82.52 ± 0.33	~6
CIMAP/C15	83.6 ± 0.72	1.43 ± 0.04	0.83 ± 0.02	8.79 ± 0.12	3.85 ± 0.18	4.34 ± 0.10	1.59 ± 0.14	75.97 ± 0.20	~5
CIMAP/C16	54.6 ± 0.40	1.07 ± 0.04	0.63 ± 0.02	4.75 ± 0.12	2.02 ± 0.08	1.97 ± 0.13	1.33 ± 0.07	83.47 ± 0.14	~6
CIMAP/C17	70.6 ± 1.19	1.01 ± 0.04	0.65 ± 0.05	3.99 ± 0.20	2.56 ± 0.12	2.37 ± 0.11	1.27 ± 0.10	83.76 ± 0.10	~6
CIMAP/C18	83.7 ± 0.68	0.95 ± 0.01	0.57 ± 0.02	6.84 ± 0.06	4.19 ± 0.13	4.60 ± 0.15	1.46 ± 0.10	77.48 ± 0.21	~5

highest amount of essential oil (0.83%) and CIMAP/C10 (showing the lowest leaf stem ratio) was the lowest oil producer (0.28%). This was expected, as essential oil is synthesized and stored in special structures called glandular trichomes that are distributed abundantly on the leaf surfaces and scarcely found on the stem.[20,21] The accumulation of oil is chiefly governed by its rate of synthesis, whereas neither monoterpene catabolism nor monoterpene volatilization has any significant role to play in the levels of the stored pool of products.[22] For this reason, the content of essential oil in CIMAP/C08 (0.68%) is high in spite of having the lowest leaf : stem ratio. This also suggests enhanced levels of biosynthetic activity of the trichomes or their probable presence in higher numbers. In the essential oil biosynthesis pathway in *Mentha* (as depicted in Figure 1), pulegone is converted to isomenthone and menthone. Menthone is further converted to neomenthol and menthol and the latter, through acetylation, forms menthyl acetate. On comparative assessment of the oil constituents of these accessions, CIMAP/C07 showed the highest accumulation of menthone (71.65%) and the lowest percentage of menthol (11.63%) and menthyl acetate (0.60%) in its essential oil. The genetic and regulatory machinery in this accession is efficient enough to convert pulegone to menthone and the lowest percentage of menthol indicates a metabolic block in the menthone-to-menthol conversion in accession CIMAP/C01. The accession CIMAP/C17 showed the lowest percentage of menthone (3.99%) and the highest percentage of menthol (83.76%) in its essential oil, indicating a higher conversion rate of menthone to menthol. The other accession showing a relatively higher conversion of menthone to menthol is CIMAP/C14. This is a released variety and a hybrid between CIMAP/C12 and CIMAP/C17, retaining the monoterpene pattern of CIMAP/C17.[23] The varying amounts of oil constituents may be due to differential efficiencies of these accessions to convert one component into the other in the essential oil biosynthesis pathway. Accession CIMAP/C03 had the highest percentage of isomenthone, and menthyl acetate in its essential oil. The accession CIMAP/C05 shows the least content of isomenthone, which is a 'desired trait', but has the highest content of neo-menthol and quite low menthol content. This again indicates diversions in the metabolic pathway leading to a low level of menthol. The essential oil profile of CIMAP/C05 is quite different from the rest, as the five main components taken in the study constitute 43% of the total essential oil, compared to approximately 90% in most of the accessions. The menthyl acetate content was seen to be highest in accession CIMAP/C02, followed by CIMAP/C09, CIMAP/C11 and CIMAP/C03, indicating an active conversion of menthol to menthyl acetate. These accessions, as a result, accumulated average levels of menthol in their essential oil (58–68%).

Clustering on the Basis of Morphochemotypic Characters

The average dissimilarity matrix (Table 3) depicts the relationship among the accessions based on morphochemical characters. In cluster analysis using morphological and essential oil constituent data (Figure 2), the accession CIMAP/C05 outgrouped from the rest, followed by CIMAP/C01 and CIMAP/C07, which formed a separate cluster together. The accession CIMAP/C05 showed uniqueness in the composition of its essential oil, with low percentages of menthone, isomenthone and menthol and the highest percentage of neomenthol. The accessions CIMAP/C01 and CIMAP/C07 accumulate high menthone in the essential oil, with low menthol and menthyl acetate, indicating a lower

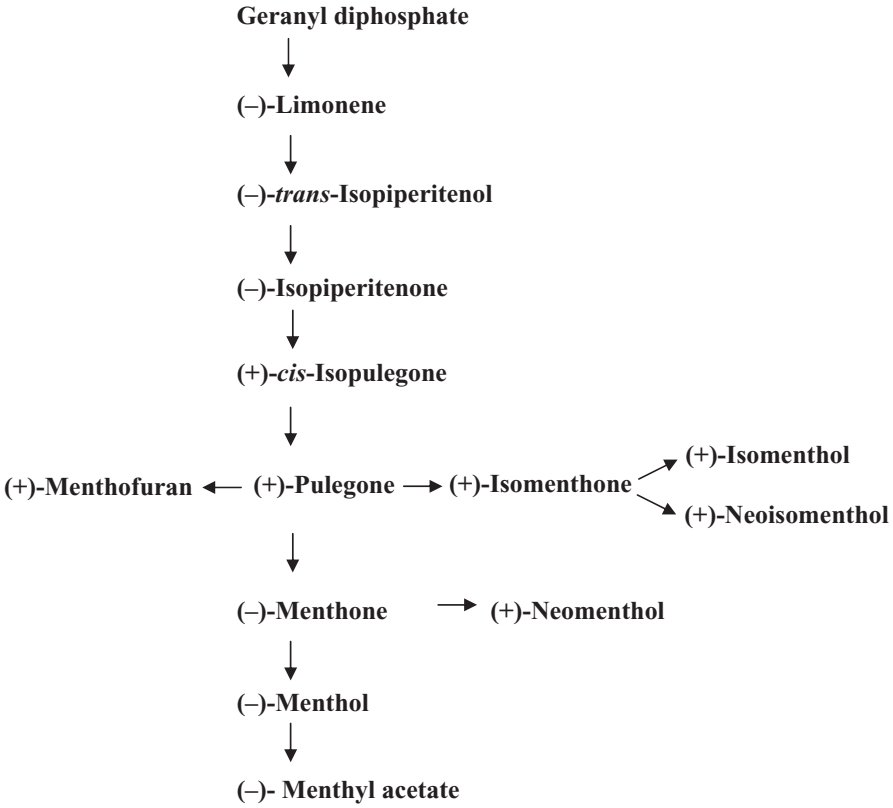

Figure 1. Metabolic pathway of menthol biosynthesis in *Mentha arvensis*

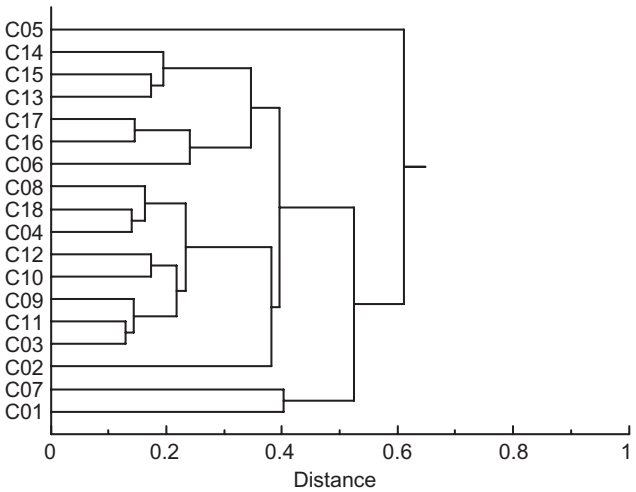

Figure 2. Distance among *M. arvensis* accessions, based on morphochemical characters

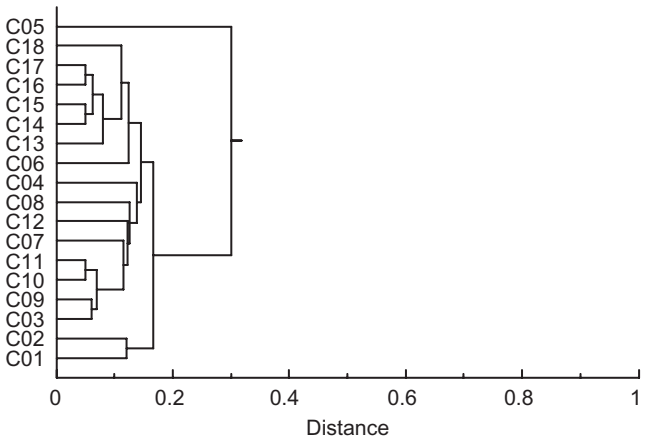

Figure 3. Genetic clustering among accessions of *M. arvensis* by RAPD analysis

conversion of menthone. Interestingly, accession CIMAP/C01 accumulates the lowest percentage of neomenthol but the accession CIMAP/C07 accumulates high neomenthol in the essential oil. All the other accessions were grouped into two major clusters. One cluster comprised accessions CIMAP/C13, CIMAP/C14 and CIMAP/C15 closely related to each other (grouped together) and the other had accessions CIMAP/C06, CIMAP/C16 and CIMAP/C17 together. The other cluster subdivided into three subclusters, with accessions CIMAP/C04, CIMAP/C08 and CIMAP/C18 in one group, CIMAP/C03, CIMAP/C09, CIMAP/C11 in the second group and CIMAP/C10 and CIMAP/C12 forming the third group. Accession CIMAP/C02 outgrouped from other accessions of this cluster. The twig of accession CIMAP/C05 is being depicted in Figure 4 along with the twigs of CIMAP/C17 and CIMAP/C14.

Genetic Variability and Cluster Analysis on the Basis of RAPD Markers

The average dissimilarity matrix (Table 4) depicts the genetic divergence among the accessions (5–43%). In the cluster analysis

Table 3. Dissimilarity matrix among different accessions of *M.arvensis* based on morphochemical characters

	CIMAP/ C01	CIMAP/ C02	CIMAP/ C03	CIMAP/ C04	CIMAP/ C05	CIMAP/ C06	CIMAP/ C07	CIMAP/ C08	CIMAP/ C09	CIMAP/ C10	CIMAP/ C11	CIMAP/ C12	CIMAP/ C13	CIMAP/ C14	CIMAP/ C15	CIMAP/ C16	CIMAP/ C17	CIMAP/ C18
CIMAP/C01	0.00																	
CIMAP/C02	0.53	0.00																
CIMAP/C03	0.47	0.40	0.00															
CIMAP/C04	0.46	0.35	0.23	0.00														
CIMAP/C05	0.62	0.62	0.68	0.57	0.00													
CIMAP/C06	0.61	0.46	0.56	0.36	0.49	0.00												
CIMAP/C07	0.40	0.64	0.53	0.48	0.63	0.61	0.00											
CIMAP/C08	0.51	0.41	0.32	0.15	0.60	0.36	0.51	0.00										
CIMAP/C09	0.51	0.36	0.14	0.19	0.62	0.49	0.50	0.28	0.00									
CIMAP/C10	0.45	0.39	0.25	0.16	0.54	0.43	0.48	0.28	0.24	0.00								
CIMAP/C11	0.47	0.32	0.13	0.18	0.66	0.50	0.55	0.28	0.15	0.21	0.00							
CIMAP/C12	0.43	0.40	0.22	0.19	0.64	0.48	0.52	0.31	0.24	0.17	0.15	0.00						
CIMAP/C13	0.54	0.53	0.49	0.35	0.55	0.34	0.56	0.38	0.43	0.41	0.44	0.36	0.00					
CIMAP/C14	0.49	0.50	0.45	0.34	0.67	0.41	0.61	0.33	0.43	0.41	0.38	0.31	0.21	0.00				
CIMAP/C15	0.56	0.56	0.48	0.38	0.68	0.42	0.62	0.37	0.44	0.48	0.44	0.39	0.17	0.18	0.00			
CIMAP/C16	0.51	0.38	0.49	0.30	0.58	0.20	0.60	0.32	0.46	0.38	0.42	0.39	0.35	0.33	0.40	0.00		
CIMAP/C17	0.46	0.38	0.41	0.23	0.60	0.29	0.57	0.25	0.39	0.30	0.33	0.28	0.31	0.22	0.33	0.14	0.00	
CIMAP/C18	0.46	0.43	0.25	0.14	0.64	0.42	0.53	0.17	0.26	0.22	0.20	0.17	0.34	0.26	0.33	0.34	0.22	0.00

Table 4. Dissimilarity matrix among different accessions of *M. arvensis* based on RAPD analysis

	CIMAP/ C01	CIMAP/ C02	CIMAP/ C03	CIMAP/ C04	CIMAP/ C05	CIMAP/ C06	CIMAP/ C07	CIMAP/ C80	CIMAP/ C09	CIMAP/ C10	CIMAP/ C11	CIMAP/ C12	CIMAP/ C13	CIMAP/ C14	CIMAP/ C15	CIMAP/ C16	CIMAP/ C17	CIMAP/ C18
CIMAP/C01	0																	
CIMAP/C02	0.12	0																
CIMAP/C03	0.15	0.12	0															
CIMAP/C04	0.18	0.13	0.13	0														
CIMAP/C05	0.33	0.31	0.28	0.3	0													
CIMAP/C06	0.21	0.12	0.14	0.17	0.3	0												
CIMAP/C07	0.19	0.13	0.1	0.14	0.3	0.19	0											
CIMAP/C08	0.21	0.33	0.12	0.14	0.3	0.18	0.13	0										
CIMAP/C09	0.17	0.11	0.06	0.14	0.24	0.14	0.13	0.12	0									
CIMAP/C10	0.19	0.14	0.08	0.15	0.3	0.2	0.11	0.12	0.06	0								
CIMAP/C11	0.17	0.13	0.08	0.12	0.29	0.14	0.12	0.13	0.06	0.05	0							
CIMAP/C12	0.18	0.15	0.15	0.15	0.31	0.14	0.15	0.13	0.11	0.11	0.09	0						
CIMAP/C13	0.2	0.15	0.15	0.17	0.31	0.12	0.15	0.16	0.15	0.16	0.12	0.1	0					
CIMAP/C14	0.18	0.16	0.13	0.14	0.32	0.16	0.14	0.14	0.14	0.17	0.11	0.43	0.07	0				
CIMAP/C15	0.16	0.13	0.12	0.15	0.3	0.12	0.14	0.17	0.14	0.14	0.1	0.1	0.08	0.05	0			
CIMAP/C16	0.18	0.14	0.15	0.17	0.31	0.09	0.13	0.08	0.13	0.12	0.11	0.1	0.09	0.07	0.07	0		
CIMAP/C17	0.18	0.13	0.12	0.14	0.31	0.1	0.11	0.15	0.13	0.12	0.12	0.1	0.08	0.05	0.06	0.05	0	
CIMAP/C18	0.21	0.17	0.15	0.17	0.31	0.15	0.16	0.17	0.14	0.16	0.15	0.13	0.13	0.09	0.11	0.13	0.1	0

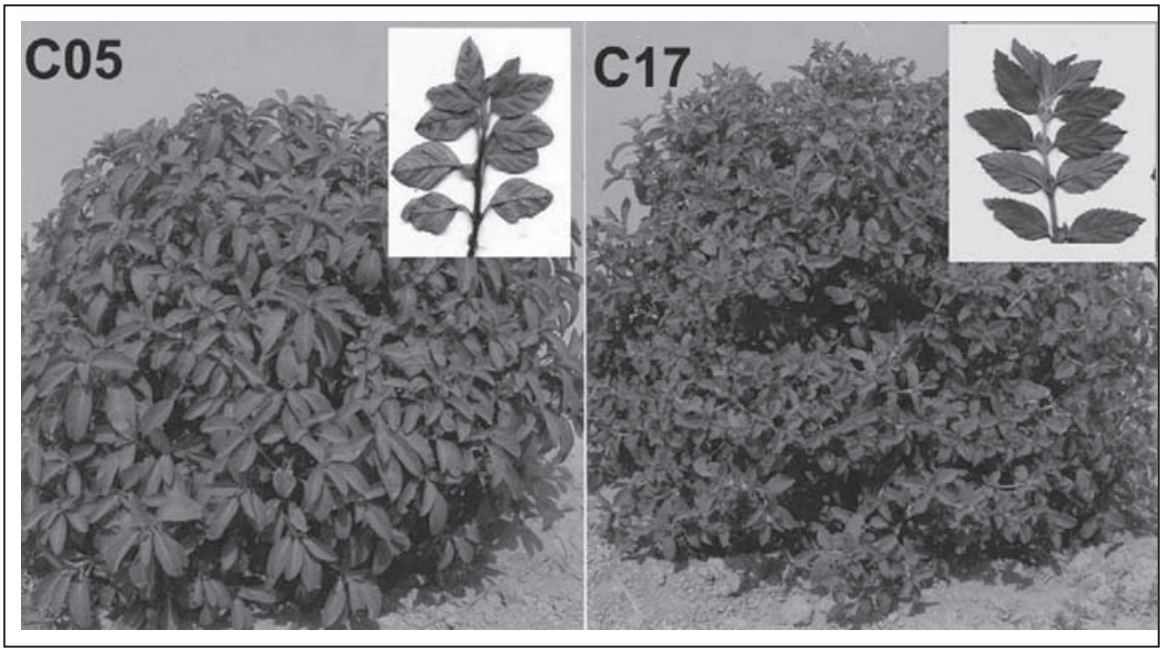

Figure 4. Accessions C05 and C17 growing in the field (inset: twigs of the plants)

(Figure 3), the accession CIMAP/C05 was found to be the most diverse genotype, showing a divergence of 28–33%. In addition, the two accessions clustering together, CIMAP/C01 and CIMAP/C02, outgrouped from the rest of the accessions. The rest of the accessions generated a bigger cluster. Accessions CIMAP/C14 and CIMAP/C15 were closely related (95% similarity). Accession CIMAP/C14 is a hybrid between CIMAP/C17 and CIMAP/C12, whereas the other accession, CIMAP/C15, is a half-sib progeny of CIMAP/C17.[24] These two accessions (CIMAP/C14 and CIMAP/C15) share a common progenitor, CIMAP/C17. In the same subcluster, CIMAP/C16 and CIMAP/C17 formed a separate group. CIMAP/C17 is a selection of accession CIMAP/C16. These four genotypes (CIMAP/C14, CIMAP/C15, CIMAP/C16 and CIMAP/C17) are from the same linage (CIMAP/C03, considering CIMAP/C16 as the somatic variant of CIMAP/C03). CIMAP/C03 showed a divergence of 12–15% from the other four genotypes mentioned above. The similarity among the four accessions (CIMAP/C14, CIMAP/C15, CIMAP/C16 and CIMAP/C17) is higher (90–95%) compared to the similarity of these accessions with CIMAP/C03. CIMAP/C12 (the progenitor of CIMAP/C14) is the seedling selection of CIMAP/C18. Genetically, CIMAP/C18 is in the same cluster as of CIMAP/C13–CIMAP/C 17. CIMAP/C13, which is a seedling selection of CIMAP/C18, clustered along with it, but the other seedling selection, CIMAP/C12, was found to be in another cluster. CIMAP/C09 was comparatively close to CIMAP/C03. CIMAP/C10 and CIMAP/C11 were found to be in same cluster, along with former two accessions. This was expected, as CIMAP/C09, CIMAP/C`10 and CIMAP/C11 are the seedling selections from the CIMAP/C03. CIMAP/C07 is a mutant derived from seed irradiation of CIMAP/C03 and present in the same cluster. All other accessions, such as CIMAP/C04, CIMAP/C05, CIMAP/C06 and CIMAP/C08 clustered separately and are gene bank collections from diverse places. The origin of the accessions is summarized in Table 1.

The comparison of distance between morphochemotypic and genetic variability showed a significant correlation value of 0.574, with a Mantel *t*-test value of 3.3359 (at $p = 0.9996$) after 10 000 random permutations. This indicates that the proportion of morphochemotypic distances correlates and shows a similar trend for the distances calculated by RAPD.

Conclusion

In this investigation, the monoterpene component analysis clearly shows the diversions and metabolic blocks in the genotypes. Except for CIMAP/C05, all other accessions were more or less similar genotypically. One correlation could be obtained for the highly deviated genotype CIMAP/C05 in the chemical components as well as genetic make-up. The close genetic relationship among the other genotypes with a high diversity in chemical components indicates that small changes in the genome for the biochemical pathway-related sequences might have led to the diversity in the chemotypes. The capability of accumulating different monoterpenes by the accessions reveals the role of differing capabilities of the intermediate enzymes in the essential oil biosynthesis pathway, thus resulting in a higher accumulation of an intermediate product (menthone) or production of other by-products (isomenthone, neomenthol) instead of menthol (main product). These accessions will serve as plant material for future study in elucidating the metabolic blocks and regulation of essential oil production in *Mentha*.

Acknowledgements

We gratefully acknowledge the Department of Biotechnology and the Council of Scientific and Industrial Research (NMITLI Cell), Government of India, for financial support.

References

1. N. Galeottia, L. D. C. Mannellia, G. Mazzantib, A. Bartolinia, C. Ghelardini. *Neurosci. Lett*. **2002**, *322*, 145.
2. K. R. Braina, D. M. Greena, P. J. Dykesb, R. Marksb, T. S. Bola. *Skin Pharmacol. Physiol.* **2006**, *19*, 17.
3. E. Guenther. *The Essential Oils.* Van Nostrand: New York, **1961**, 3.
4. S. P. S. Khanuja, A. K. Shasany, A. Srivastava, S. Kumar. *Euphytica* **2000**, *111*, 121.
5. A. K. Shasany, A. Srivastava, J. R. Bahl, S. Sharma, S. Kumar, S. P. S. Khanuja. *Plant Genet. Resour. Newslett.* **2002**, *130*, 1.
6. J. G. K. Williams, A. R. Kubelic, K. J. Livak, J. A. Rafalski, S. V. Tingey. *Nucleic Acids Res.* **1990**, *18*, 6531.
7. M. Mohan, S. Nair, A. Bhagwad, T. G. Krishna, M. Yano, C. R. Bhatia, T. Sasaki. *Mol. Breeding* **1997**, *3*, 87.
8. A. K. Shasany, S. Gupta, M. K. Gupta. *J. Essent. Oil. Res.* **2007**, *19*, 138.
9. J. Hu, L. F. Quiros. *Plant Cell Rep.* **1991**, *10*, 505.
10. P. Hadrick. *Nature* **1992**, *355*, 679.
11. P. J. Chandrashekhar, H. T. Nguyen. *Theor. Appl. Gene* **1993**, *38*, 602.
12. E. C. Howel, H. J. Newbury, R. L. Swennen, L. A. Withers, B. V. Ford Lloyd. *Genome* **1994**, *37*, 328.
13. A. Jain, S. Bhatia, S. S. Banga, S. Prakash, M. Laxmikumaran. *Theor. Appl. Genet.* **1994**, *88*, 116.
14. D. J. Mackill. *Crop Sci.* **1995**, *35*, 889.
15. A. K. Shasany, S. Gupta, M. K. Gupta, S. P. S. Khanuja. *J. Trop. Med. Plants* **2006**, *7*(2), 223.
16. Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India. *Menthol Mint Cultivation, Farm Bulletin*. ISBN 81-86943-73-2.
17. W. Jennings, T. Shibamoto. *Qualitative Analysis of Flavour and Fragrance Volatile by Capillary GC*. Academic Press: New York, **1980**.
18. S. P. S. Khanuja, A. K. Shasany, M. P. Darokar, S. Kumar. *Plant Mol. Biol. Rep.* **1999**, *17*, 74.
19. N. Nei, W. Li. *Proc. Natl Acad. Sci. USA* **1979**, *76*, 5269.
20. D. McCaskill, J. Gershenzon, R. Croteau. *Planta* **1992**, *187*, 445.
21. J. G. Gershenzon, D. McCaskill, J. I. M. Rajaonarivony, C. Mihaliak, F. Karp, R. Croteau. *Anal. Biochem.* **1992**, *200*, 130.
22. M. McConkey, J. Gershenzon, R. Croteau. *Plant Physiol.* **2000**, *122*, 215.
23. S. Kumar, B. R. Tyagi. *J. Med. Arom. Plant Sci.* **1997**, *19*, 729.
24. S. Kumar, N. K. Patra. *J. Med. Arom. Plant Sci.* **1999**, *21*, 56.