Multiple disease resistance in menthol mint genotypes

Mentha arvensis, popularly known as menthol mint or Japanese mint yields an economically important essential oil, which is the chief source of natural menthol. It is widely used by the pharmaceutical, perfumery and cosmetic industries. Eleven menthol mint genotypes were evaluated for rust (*Puccinia menthae*), powdery mildew (*Erysiphae cichoracearum*), leaf spot (*Corynespora cassiicola* and *Alternaria alternata*), leaf blight (*Alternaria alternata* and *Rhizoctonia solani*) and stem blackening and rot (*Botryodiplodia theobromae*) resistance sources under field condition at Central Institute of Medicinal and Aromatic Plants (CIMAP) Resource Centre, Udham Singh Nagar during 1996–99. Kosi was the only variety identified to be resistant against the five major fungal diseases taken up in the present study. This variety can be the donor parent in further breeding programmes in evolving menthol mint genotypes for multiple disease resistance.

Menthol mint (*M. arvensis* L. var. *piperascens*, family Labiatae) is commercially cultivated to obtain its oil, which has chemical constituents of economic importance, viz. menthone, menthol, menthyl acetate, terpenes, etc. These constituents are used in several pharmaceutical preparations, toothpaste, mouthwash, perfumery, cosmetics and confectionaries. At present[1], menthol mint is cultivated in around 1.5 lakh ha and annual production of oil in the country is around 16,000 tonnes. It is affected by many fungal diseases leading to considerable yield losses[2,3]. The important ones are rust, powdery mildew, leaf spot and leaf blight, and stem blackening and rot[4,5]. Therefore, it is worthwhile to evaluate the response of different menthol mint varieties/genotypes for disease resistance against major fungal pathogens, so as to identify multiple disease-resistant sources in *M. arvensis*.

Field experiments were conducted at the CIMAP Resource Centre. Six varieties (Damroo, Gomti, Himalaya, Kalka, Kosi and Shivalik) and five genotypes (MAH-3, SS-1-4, SS-15, S-10-11-45 and S-13-2-125)[6] of *M. arvensis* were included in the study. The soil type of the experimental field was clay-loam, and the standard package of practices for menthol mint recommended for tarai region of Uttar Pradesh and Uttarakhand was followed during the study[7]. Suckers of menthol mint were planted in furrows to a depth of 5–6 cm with row spacing of 60 cm, in the first week of February. The experiment was laid out in completely randomized block design with three replications. The plot size was 12 m^2. Prevalence of moderate temperature (25–30°C) and high humidity (75–85%) during the crop season (April–June) was congenial to natural infection of *P. menthae*, *E. cichoracearum*, *A. alternata*, *C. cassiicola*, *R. solani* and *B. theobromae*.

Field observations were recorded every year between April and June. Incidence of stem blackening and rot was recorded as percentage of affected plants out of the total plants in each plot. Severity of foliar diseases was recorded using 0–4 scale of Margina and Zheljazkov[8], with some modifications by Shukla *et al.*[9], where 0 represents no symptoms; 1, less than 10 min chlorotic spots with 0–25% leaf area affected; 2, 11–20 necrotic lesions with limited growth covering 26–50% leaf area; 3, more than 20 necrotic lesions with unlimited growth but no defoliation with 51–75% leaf area affected, and 4, more than 20 necrotic lesions with unlimited growth leading to defoliation. Based on scoring different types of disease symptoms on ten individual leaves per plant and 15 plants in each variety/genotype, the percentage disease index (PDI) was calculated as follows:

$$\text{PDI} = \frac{\text{Sum of the numerical grading recorded}}{\text{Number of leaves observed} \times \text{highest numerical rating}} \times 100.$$

The varieties/genotypes scoring PDI values in the range 0.1–10.0% were rated

Table 1. Response of menthol mint varieties/genotypes to rust, powdery mildew, leaf spot, leaf blight, and stem blackening and rot

	Per cent disease index[a]				
Menthol mint	Rust	Powdery mildew	Leaf spot	Leaf blight	Stem blackening and rot
Variety					
Damroo	7.3	16.6	13.6	18.3	13.9
Gomti	51.6	8.5	54.4	56.5	8.7
Himalaya	3.2	18.8	4.5	13.3	39.2
Kalka	3.8	49.6	5.6	5.2	32.4
Kosi	2.8	9.5	3.1	4.1	6.9
Shivalik	53.9	38.1	52.4	60.2	50.9
Genotype					
MAH-3	13.7	30.3	33.5	26.0	33.5
SS-1-4	28.9	28.1	37.6	36.6	26.7
SS-15	44.0	37.1	44.1	46.2	40.7
S-10-11-45	13.9	26.7	23.5	32.7	16.8
S-13-2-125	11.6	27.1	24.5	35.4	23.4
S.E. of difference[b]	1.0	1.1	1.1	0.9	1.3

[a]Presented as average of PDI measured 110 days after planting for four years (1996–99) in the field.
[b]Standard errors of differences of means statistics were used to compare means between PDIs of different varieties/genotypes.

Table 2. Reaction of menthol mint varieties/genotypes towards different fungal diseases in the field

	No. of varieties/genotypes/diseases					No. of varieties/genotypes exhibiting disease reaction to different combinations of diseases									
Reaction	1	2	3	4	5	1&2	1&3	1&4	1&5	2&3	2&4	2&5	3&4	3&5	4&5
R	4	2	3	2	2	1	3	2	1	1	1	2	1	1	1
MR	3	2	1	2	2	–	–	–	1	1	2	1	1	1	1
MS	1	3	2	1	2	1	–	–	1	2	–	2	–	1	–
S	1	3	3	4	4	1	1	1	1	2	1	3	2	2	1
HS	2	1	2	2	1	1	2	2	1	1	1	1	2	1	1

R, Resistant; MR, Moderately resistant; MS, Moderately susceptible; S, Susceptible; HS, Highly susceptible.
1, Rust; 2, Powdery mildew; 3, Leaf spot; 4, Leaf blight; 5, Stem blackening and rot.

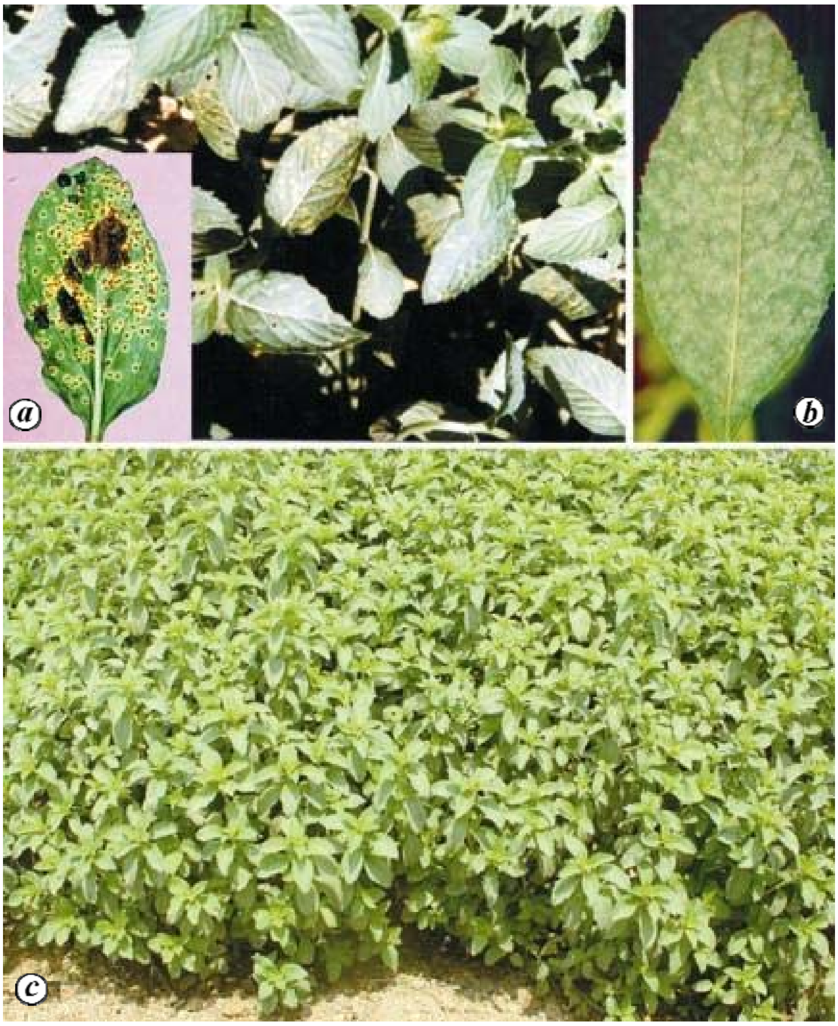

Figure 1. Menthol mint genotype showing disease reactions. ***a***, Rust susceptible cv. Shivalik. ***b***, Powdery mildew susceptible cv. Kalka. ***c***, Multiple disease-resistant cv. Kosi.

as resistant; 10.1–20% as moderately resistant; 20.1–30.0% as moderately susceptible; 30.1–50.0% as susceptible, and 50.1–100.0% as highly susceptible.

Among the varieties/genotypes tested for multiple disease resistance of menthol mint, only the variety Kosi was found to be resistant against all the five fungal diseases (Table 1). Himalaya and Damroo was resistant to rust, moderately resistant to powdery mildew and leaf blight. Genotypes MAH-3, S-10-11-45 and S-13-2-125 were moderately resistant to rust, but moderately susceptible to other diseases (Table 1). The variety Kalka was resistant to rust, leaf spot and leaf blight, but was found susceptible to powdery mildew, and stem blackening and rot. The variety Shivalik showed highly susceptible reaction to all the diseases, with more than 50% damage and complete defoliation (Figure 1 *a* and *b*).

A study of menthol mint varieties/genotypes to different combinations of diseases was also made (Table 2). Only Kosi was found resistant in different combinations of diseases (Figure 1 *c*). Two varieties, namely Himalaya and Kalka, showed resistance to rust and leaf spot diseases. Variety Damroo showed moderately resistant reaction to powdery mildew, leaf spot, leaf blight, and stem blackening and rot disease combinations. Two genotypes, S-10-11-45 and S-13-2-125, were found moderately resistant to rust and moderately susceptible to powdery mildew and leaf spot disease. SS-15 genotype showed susceptible reaction against all the diseases.

Based on the present study, variety Kosi of menthol mint was identified as resistant, showing multiple disease resistance. This variety has been granted US Patent (no. PP 12,426) on 26 February 2002. Additionally, this genotype is highly productive covering about 80% of the menthol mint-growing areas and is quantitatively commercially acceptable by the farmers and industry[10]. The result presented here has been also validated by achieving the EOAI-SOM Award 2003–05 for the development and dissemination of legendary variety Kosi of *M. arvensis*. This variety can be utilized for breeding programmes to develop multiple disease-resistant varieties against five major fungal diseases of menthol mint in India.

1. Singh, K., Singh, S., Chand, S., Anwar, M. and Patra, D. D., *Maps dew* 2007, **3**, 35–37.
2. Kalra, A., Singh, H. B., Patra, N. K., Pandey, R., Shukla, R. S. and Kumar, S., *J. Hortic. Sci. Biotechnol.*, 2001, **76**, 546–548.
3. Shukla, R. S., Chauhan, S. S., Gupta, M. L., Singh, V. P., Naqvi, A. A. and Patra, N. K., *J. Med. Aromat. Plant Sci.*, 2000, **22**, 453–455.
4. Shukla, R. S., Singh, H. B., Kalra, A. and Kumar, S., In International Conference on Integrated Plant Disease Management for Sustainable Agriculture, IARI, New Delhi, 10–15 November 1997, p. 448.
5. Shukla, R. S., Gupta, M. L. and Singh, H. N., *J. Mycol. Plant Pathol.*, 2001, **31**, 395–396.
6. Kumar, B., Singh, H. P. and Patra, N. K., *Crop Improv.*, 2007, **34**, 95–99.
7. Patra, D. D. *et al.*, *J. Med. Aromat. Plant Sci.*, 1998, **20**, 364–367.
8. Margina, A. and Zheljazkov, V., *J. Essential Oil Res.*, 1994, **6**, 607–615.
9. Shukla, R. S., Singh, H. B., Kalra, A., Singh, V. P. and Kumar, S., *J. Med. Aromat. Plant Sci.*, 1999, **21**, 311–315.
10. Singh, A. K. and Kanuja, S. P. S., *Spice India*, 2007, **20**, 14–17.

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Phylogenetic implications of hemipenial morphology in Sri Lankan agamid lizards

While useful in differentiating some of the taxa at the species level[1], the morphology of reptile hemipenes has often been considered to be of limited phylogenetic value[2,3] because of remarkable divergence even within individual genera[4–6]. Here we test for similarity between a molecular phylogeny and a phylogeny based on 15 morphological character states in the hemipenes of 17 of the 18 species (in six genera) of dragon-lizards in Sri Lanka (Squamata: Agamidae) and show that (a) molecular and morphological phylogenies show remarkable congruence, and (b) hemipenial morphology is informative in resolving supra-specific relationships among these lizards.

The Sri Lankan dragon-lizards include three endemic genera, *Lyriocephalus* (one species), *Cophotis* (two species) and *Ceratophora* (five species), and three non-endemic genera *Otocryptis* (two species), *Calotes* (seven species) and *Sitana* (one species). Although recent molecular analyses[7,8] have shown all these genera to belong to the subfamily Draconinae, some forms exhibit highly derived characters such as the rostral horns of *Ceratophora*, ovoviviparity of *Cophotis* and the highly developed extension of the canthus rostralis in *Lyriocephalus*.

Because there is no consensus on what constitutes plesiomorphic and apomorphic states in the reptile hemipenis[3], and on the relative phylogenetic importance of these characters, we gave equal weights to all 15 character states used in the morphological analysis as follows (score '1' for 'yes', and '0' for 'no', using standard hemipenial nomenclature[9]; see Table 1): (1) hemipenis divided for more than half its length; (2) flounces present; (3) apex of each lobe divided symmetrically both laterally and medially by sulcus; (4) sulcus spermaticus bifurcated; (5) a fleshy cardioid structure present at the base of the ventral sulcus; (6) lateral and medial sulcus distinct throughout the length of each lobe; (7) length of entire organ greater than its width; (8) minute denticulation present on calyces; (9) sulcus traverses apex; (10) each lobe with more than 11 flounces; (11) ventral sulcus with transverse ridges; (12) transverse ridges present along more than half of length of the ventral sulcus; (13) calyces subequal along the entire length of the organ; (14) entire length of the lateral and medial sulcus with calyces; (15) calyces present only on the lower half of both lateral and medial sulci.

We used PAUP (version 4.0 b10)[10] to construct a cladogram based on these character states under a maximum parsimony criterion (character state transformation–accelerated) and a heuristic search with stepwise addition starting tree option, random stepwise addition and TBR branch-swapping option. The tree was