Registration of a high yielding cold-tolerant menthol mint variety-CIM-Kranti of *Mentha arvensis*

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ABSTRACT

An improved variety of menthol mint (Mentha arvensis), christen as "CIM-Kranti", has been developed through half-sib progeny selection from the parent variety 'cv. Gomti'. The new variety is cold and frost tolerant and has the potential to produce higher oil (10-12%) in summer cropping season when compared to all other popular commercial cultivars of menthol mint. More importantly, during winter season (September - January) also when all other mint varieties suffer senescence due to cold and frost conditions, 'CIM-Kranti remains green in the field and grows vigorously to yield two to three times more essential oil and (100 kg/ha) along with sucker production (250-300 g/ha). This plant is distinct in terms of leafmorphology and DNA profile and can be propagated vegetatively to maintain uniformity. Hence, this newly developed variety is suitable for commercial cultivation of menthol mint to generate additional income to farmers without any additional input for cultivation during winter besides its usual cultivation as a summer crop.

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INTRODUCTION

Menthol mint, *Mentha arvensis* belonging to family — Lamiaceae, is grown for menthol production used in pharmaceutical and flavor industry. India is a leading supplier of menthol mint oil to the world and a large number of farmers in India are being benefitted by its' cultivation. Generally, the crop is cultivated during January to July, either by suckers or transplanting plantlets for production of mint oil. The crop at present is being

cultivated over an area of more than two lakh hectares, producing about 18 - 20 thousand tones of mint oil for the industry and the demand of the oil is increasing by 15-20% every year. An attempt has been made to develop a new improved clone through breeding, which could give quality mint oil during winter season along with suckers being used as planting material for raising new crop for summer cropping season.

MATERIALS AND METHODS

Origin and breeding of the variety 'CIM - Kranti'

The clone 'BB–1022', named as 'ClM-Kranti' w as developed through a half-sib progeny selection

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from several seed raised progenies of the variety 'cv.Gomti', and was multiplied by vegetative propagation for field evaluation. As the plant show ed distinct ever green morphology and growth habit consistently during the initial evaluation at Lucknow. it was further evaluated for oil yielding potential during both summer (January - July) as well as winter (August/ September - January) seasons. The oil yielding potential of clone BB-1022 was evaluated during winter season in a replicated field trial laid outwith nine released commercial varieties namely, 'MAS-1', 'Kalka', 'Shivalik', 'Gomti', 'Himalay a', 'Kushal', 'Saksham', 'Kosi' and 'CIM-Saryu' as checks in a randomized block design (RBD) at Pantnagar during 2011-12 (plot size 4m x 5m) and 2013-14 (plot size of 6m x 5m) crop seasons and at Lucknow during year 2012-13 (plot size 4m x 3.5m) keeping row to row spacing 70 cm.

RESULTS AND DISCUSSION

Pilot scale evaluation trials during winter season at Lucknow and Pantnagar

The oil yielding parameters and sucker yield of the clone BB-1022 were recorded and are presented in Tables 1 and 2. The field views of the evaluation trials during 2011-12 and 2013-14 with the selected clone are depicted in Figures 1 and 2. Senescence in all other mint varieties was observed except the clone 'BB-1022' during winter season (Figure 2). A close-up of the clone show ing distinct morphology is presented in Figure 4. The clone 'BB-1022' significantly produced highest essential oil during winter crop (August/ September to January) in the evaluation trials over three years at Pantnagar (Table 1). The sucker production yield of clone 'BB-1022' is also slightly comparable or more with respect to popular commercial varieties, 'Kosi', 'CIM-Saryu' and 'Shivalik' (Table 1, Fig. 3).

Table 1. Performance of the selected clone BB-1022 (christen as CIM-Kranti) in pilot scale trials during winter seasons at two locations (Lucknow and Pantnagar) in comparison to two check varieties cv kosi and CIM-saryu of menthol mint.

Years /Locations	Varieties	Oil content (%) ₹	Herb yield (q/ha)	Oil yield (kg <i>l</i> ha)	Sucker yield (q <i>l</i> ha)
2011-12 Pantnagar	Kosi	0.55	43.7	22.0	221.2
	CIM-Saryu	0.67	18.7	11.6	202.1
	BB-1022 (CIM-Kranti)	0.73	80.6**	53.2**	251.4
	CD at 5%	0.17 10.5		7.9	35.5
	1%	0.24	14.4	10.9	48.7
2012-13 Lucknow	Kosi	0.65	36.4	22.9	186.2
	CIM-Saryu	0.80	46.7	33.8	273.2
	BB-1022 (CIM-Kranti)	0.87	96.5**	76.1**	299.6
	CD at 5%	0.17	10.8	8.6	28.9
	1%	0.24	14.8	11.8	39.6
	Kosi	0.85	31.9	24.1	313.9
2013-14 Pantnagar	CIM-Saryu	0.85	56.9	43.3	301.9
	BB-1022 (CIM-Kranti)	0.93	152.5**	128.2**	388.9**
	CD at 5%	0.09	7.25	5.3	45.8
	1%	0.12	9.93	8.5	62.8

^{**} p < 0.01

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Fig. 1. A field view of the varietal evaluation trial at CRC Pantnagar during winter season (September - January) at harvesting stage showing senescence in all the varieties except the variety 'CIM-Kranti'

Pilot scale evaluation trials during normal summer season both at Lucknow and Pantnagar

The clone 'BB-1022' (variety 'CIM-Kranti') was also tested in evaluation trials along with nine released varieties of menthol mint during summer seasons of 2012 and 2013 at Lucknow and Pantnagar. The performance of the variety 'CIM-Kranti' along with the check varieties depicted in Table 2. The clone 'BB-1022' ('CIM-Kranti') produced higher essential oil compared to all the varieties in the evaluation trials during summer season. This was higher than the commercial variety 'CIM-Saryu' by 12% and 16% during 2012 crop season both at Lucknow and Pantnagar, respectively. How ever, it yielded 25% and 9% more oil during 2013 at

Lucknow and Pantnagar, respectively. This clearly demonstrated that the clone 'BB-1022' ('CIM-Kranti') was comparatively superior to both the earlier released varieties 'Kosi' and 'CIM-Saryu' (Table 2).

Distinguishing characteristics of the variety

The morphological features and major chemical components of the oil of the variety 'CIM-Kranti' have been described in the Table 3. The plant of CIM-Kranti is erect, ascending, with compact branches and dark green leaves, that are elliptic-ovate in shape. The essential oil conent of the variety is consistently 0.8% containing 78 – 81% menthol. The morphometric characteristics of the suckers of released varieties and 'CIM-Kranti' are

Table 2. Performance of variety 'CIM-Kranti' during summer seasons at two locations (Lucknow and Pantnagar)

Varieties/	Lucknow			Pantnagar			
Years	Oil content (%)	Herb yield (q/ha)	Oil yield (kg/ha)	Oil content (%)	Herb yield (q/ha)	Oil yield (kg/ha)	
			2012		•		
Kosi	0.90	215.2	174.6	0.80	299.7	215.8	
CIM- Saryu	0.88	230.9	183.6	0.80	258.8	186.7	
BB-1022 (CIM- Kranti)	0.87	264.2	205.6*	0.78	308.1	217.7	
CD 5%	0.09	19.19	23.96	0.02	54.3	40.5	
CD 1%	0.81	26.29	32.82	0.09	74.5	55.4	
•			2013	•			
Kosi	0.77	205.5	140.0	0.95	166.5	142.0	
CIM- Saryu	0.90	226.5	174.9	1.03	171.7	159.6	
BB-1022 (CIM- Kranti)	0.87	279.8**	218.3**	1.03	186.8	173.4*	
CD 5%	0.14	34.9	23.5	0.07	26.3	16.6	
CD 1%	0.19	47.8	32.3	0.11	36.1	22.7	

^{**} p < 0.01

provided in Table 4 show ing it's similarity w ith those of check varieties 'CIM-Saryu', 'Kosi', 'Kalka' and 'Gomti'

Incidence of viral diseases

A screening for viral incidence was also carried out against the viral diseases in different varieties. It was observed that variety CIM-Kranti was having less incidence of the diseases than the control varieties ('CIM-Saryu', 'Kosi', 'Kushal' and 'Gomti'). The viral disease incidence was 14.63% on variety 'CIM-Kranti', 29.42 on 'CIM-Saryu', 36% on 'Kosi' and 37% on 'Gomti'. This indicated that 'CIM-Kranti' is least affected by viral diseases.

Molecular characterization of improved variety CIM-Kranti

The variety 'CIM-Kranti' was compared at molecular level with other checkvarieties of *Mentha arvens* is by RAPD analysis. The genetic differentiation of the variety 'CIM-Kranti' and check varieties was established on the basis of RAPD profiles generated by using MAP series of primers (randomdecamers). A total of 135 fragments were





Fig. 2. A field view of the variety 'CIM-Kranti' and crop with suckers and herb produce during winter season in 2013-

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generated by using MAP primer series and the primer MAP 14 generated highest number of fragments (14), where as primer MAP 12 scored minimum number of fragments (3). The primer MAP17 and 13 clearly differentiated the variety 'CIM-Kranti' from other varieties. The data scored on polymorphism and band sharing was aimed at analyzing the genetic similarity index matrix obtained through multi variant analysis using Nei

and Li's coefficient (Nei and Lei 1979). The similarity coefficients were used to generate a tree for cluster analysis using UPGMA method (Fig 6). In cluster diagramme two clusters were formed having 'Saksham', 'Kosi' and 'Sambhav' in one group, whereas 'Sambhav' was out grouped. The variety 'CIM-Kranti' formed a separate group with 'Gomti' and 'Shivalik', while rest of the varieties formed separate branches in the dendogram. The

Table 3. Morphological features of the variety CIM-Kranti

Phenomic traits	Characteristic depiction in CIM-Kranti			
Plant morphology	Erect, ascending, obovate shape' compact branches			
Plant height	78.6 cm			
Stem	Hard, hairy, green and purplish at base			
Leaves	Elliptic - ovate, margin serrate, apex acute, sub-cuneate at base, hairy			
Leaf texture	Rough			
Leaf colour	Dark green			
Leaf size and area	Medium, 8.59 cm ²			
Flower colour	Purplish white			
Sucker hardness & colour	Soft, fibrous, white			
Oil content	0.80 - 0.85 %			
Chemical composition of oil	% of oil			
Menthone	2.26			
lso-menthone	1.96			
Menthyl acetate	7.69			
Neo-menthol	1.77			
Menthol	81.17			

Table 4. Morphometric characteristics of suckers of different menthol mint varieties compared to the variety 'CIM-Kranti' Incidence of viral diseases

Varieties	Color	Hardiness	Weight per 10 suckers (g)	Mean length (cm)	Average thickness (cm)	No. of nodes	Internode length (cm)
MAS-1	White	Hard, brittle	21.80	14.44	1.77	9.55	1.62
Kalka	White	Hard, brittle	41.77	20.40	2.16	11.20	1.96
Shivalik	White	Semi-hard, brittle	37.00	18.60	1.84	8.60	2.07
Gomti	White	Hard, brittle	37.00	17.55	2.13	10.55	1.92
Saksham	White	Semi-hard, brittle	28.88	14.20	1.90	8.40	1.47
Kushal	White	Semi-hard, brittle	31.00	17.50	1.80	7.80	1.64
Kosi	White	Soft, fibrous	43.50	23.10	1.90	10.60	2.10
CIM-Saryu	White	Semi-hard, fibrous	52.00	20.00	1.99	8.90	2.73
Himalaya	White	Hard, brittle	48.73	17.50	2.43	10.00	1.80
BB1022 (CIM-Kranti)	White	Soft, fibrous	38.80	21.40	2.13	11.10	2.06

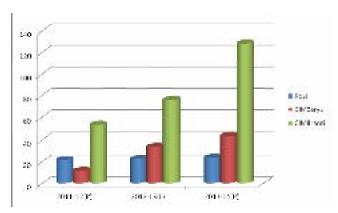


Fig. 3. Comparative oil yield of 'CIM-Kranti' as compared to check varieties ('Kosi' and 'CIM-Saryu') during winter sucker producing crop.



Fig 4. A close up view of the variety 'CIM-Kranti's howing morhological features .

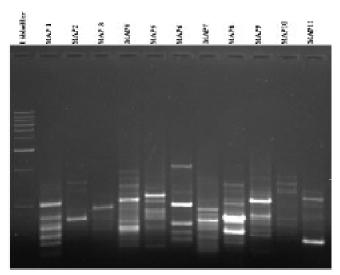


Figure 5: Characteristic profile of novel variety CIM-Kranti' of *M. arvensis* by using MAP primers

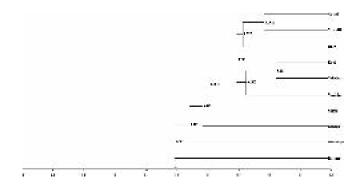


Figure 6. Phylogentic tree showing relatedness of variety 'CIM-Kranti' with other varieties

developed RAPD profiles of 'CIM-Kranti' and other nine released varieties, demonstrated the distinctness of the new variety. The calculation of genetic distances based on the number of polymorphic bands show ed that the new accession was related to 'Shivalik' and 'Gomti' with 73% similarity (Fig. 6). Further, the RAPD analysis of the variety was carried out with 'Gomti' and 'Shivalik' by OPM primer series to differentiate the new accession from 'Gomti' and 'Shivalik'. The OPM primer 9 and 15 were useful in differentiating the new variety from Gomti and Shivalik.

Statement of distinctness

The released variety 'CIM-Kranti' is erect with vigorous grow th and compact branching. The plant is tolerant to cold and frost with potential to produce higher oil in summer crop as compared to popular commercial varieties. Moreover, special feature of the variety is its potential to produce up to 100 kg/ha yield of mint oil with 79-81% menthol along with producing 250 -300 q/ha suckers as planting material during winter season. This variety has distinct morphology and DNA profile and clearly differentiated from other existing varieties.

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