Estimation Of Genetic Parameters For Agro-Economic Traits And D^2 Analysis Among The Half-Sib Progeny Of Menthol Mint (Mentha Arvensis L.)

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Abstract

Menthol mint (Mentha arvensis L.) is an important medicinal and aromatic plant, the essential oil of menthol mint most widely used in food, flavouring, pharmaceuticals, and cosmetic industries. In India, the essential oil yield of presently cultivated menthol mint varieties was reducing, and industry needs varieties having high essential oil yield with more menthol content. The objective of this experiment was to study the magnitude of genetic variability and genetic divergence in twenty-nine newly generated half-sib progeny of menthol mint. All the twenty-nine accessions were evaluated for agro-morphic traits and chemical constituents of essential oil for two consecutive years. Results revealed that, the highest genotypic coefficient of variation (GCV) was observed for menthone content (100.84%), and highest phenotypic coefficient of variation (PCV) was also for menthone content (102.84%). All the seven agro-economic characters showed high heritability (h^2) and high genetic advance as a percent of the mean (GAM). By Mahalanobis D^2 values, all the twenty-nine accessions were grouped into four different clusters. The cluster-II was the largest group which consist of 15 accessions and the clusters III, IV were smallest clusters, which consist of single accession in each. A significant amount of genetic variability was present in the accessions used in the study, and theses genetically divergent half-sib progenies can be effectively utilized for recombination breeding to get genotype with novel gene combination.

Keywords: *Genetic variability, genotypic coefficient of variation, heritability, phenotypic coefficient of variation,*

I. INTRODUCTION

The *Mentha arvensis* L. commonly known as menthol mint, corn mint, and Japanese mint widely used in food, flavoring, pharmaceuticals, and cosmetic industries[1].The cooling and soothing effect of menthol-mint oil on the skin and mucous membranes of the human body makes it a useful ingredient in pharmaceuticals and cosmetics industries [2]. The major menthol producing countries in the world are India, America, Europe, China, and Brazil. India is the leading producer, consumer, and exporter of menthol mint in the world [3]. In India, the menthol mint cultivated mainly in states of Uttar Pradesh, Bihar, Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Haryana, and Punjab [4].

The genetic potential and stability of the presently cultivated menthol mint varieties were decreasing, and industry is demanding high yielding varieties with more menthol content in essential oil. So, there is an urgent need to identify/develop genetically stable and agronomically high yielding menthol mint varieties to fulfill the industrial demand and to increase the income of farmers. The success of any crop improvement programme depends on the amount of genetic variability in the base population/ germplasms used for genetic improvement. In earlier reports, significant genotypic and phenotypic coefficient of variation, moderate to high heritability and genetic advance as a percent of the mean was reported in half-sib seed progenies of peppermint (*Mentha piperita*) [5]. Significant genetic variability was reported for agro-morphological and chemical constituents of oil in seven varieties of mentha species [3]. Multivariate analysis quantifies genetic divergence by assessing the proximity of accessions with each other and classifying them into different clusters; this will help to select genetically divergent parents for hybridization program to get the superior hybrids. Mahalanobis D^2 statistic [6] has been widely used in crop improvement programs for selection of genetically divergent parents for hybridization programmes [7]. The present study aims at the study of genetic variability and to assess the genetic divergence in newly generated half-sib progenies of menthol mint. This study will help in enhancing the efficiency of menthol mint breeding program.

II. MATERIAL AND METHODS

A. Experimental material

The planting materials for the present study consists of twenty-nine newly generated half-sib progeny (Table 1) of menthol mint (*Mentha arvensis* L.) which were derived from commercial menthol mint varieties namely Kalka, Saksham, Himalaya, CIM-Saryu, Gomti, Damroo, Kushal, Kosi, CIM-Kranti, MAS-1, and Sambhav. All the twenty-nine accessions were evaluated for two consecutive years (2016-2017 and 2017-2018) at CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar, Uttarakhand (India).

B. Experimental site

The present experiment was conducted at the experimental field of CSIR-Central Institute of Medicinal, and Aromatic Plants (CSIR-CIMAP), Research Centre, Pantnagar; Uttarakhand (India) is located between coordinates of 29°N, 79.38°E and an altitude of 243.84 m.

C. Experimental design

All the twenty-nine half-sib progeny were planted in a randomized block design (RBD) with two replications in 3 x 3m sized beds with a row to row spacing of 60 cm and plant to plant spacing of 15 cm. The standard agronomic practices were followed throughout the crop season, which includes the application of Farm Yard Manure (FYM) at the rate of 25-30 t/ha along with 90, 60, 40 kg/ha of nitrogen (N), phosphorus (P) and potassium (K), respectively. About 60 kg/ha of nitrogen was top dressed in two equal splits at 30 days and 60 days after planting. The irrigation was given as and when the crop required, and one hand weeding was carried out at the initial stage of crop growth (twenty days after planting), and second-hand weeding (Forty days after planting) and necessary plant protection measures were taken to raise the good crop stand. The morphometric data was recorded by selecting five competitive plants randomly in each accession for following traits namely plant height (cm), canopy diameter (cm), number of branches/plant, herb yield (kg/plot) and oil yield (kg/plot). Plant height was measured in centimeters from the base of the plant to the top of the main plant stem. Canopy diameter was measured in centimeters by measuring canopy width in two directions and mean canopy length was calculated. The number of branches was calculated by counting number of branches in each plant by selecting five random plants in each accession. The herb yield was calculated in kilogram per plot by taking five random plants weight and multiplying with a total number of plants in each experimental plot (3m×3m area). The essential oil yield was calculated on a plot area basis by multiplying the essential oil content in 100-gram herb with the total herb yield per plot and expressed in kilograms/ plot.

D. Essential oil extraction

About 200 g of fresh herb collected from each of twenty-nine accessions. The essential oil was hydro-distilled by using a Clevenger apparatus for about 3-4 hrs. The percent essential oil was calculated on the 100 g on a fresh weight basis. The essential oil yield calculated on plot area basis by multiplying the oil content with herb yield. The collected oil stored at 4° C in 5 ml tubes by addition of anhydrous sodium sulphate to remove the water traces.

E. Gas chromatography (GC)

Gas chromatography (GC) analysis of the essential oil samples was carried out on a DB-5 capillary column (30 m × 0.25 mm i. d., film thickness 0.25 µm) fixed inside the oven of NUCON Gas Chromatograph (model 5765) equipped with a flame ionization detector (FID). The column oven temperature was programmed from 60-230 °C, at the rate of 3 °C min^{-1}, using Hydrogen as carrier gas at a constant flow rate of 1.0 mL min^{-1}. Injector and detector (FID) temperatures were 220 °C and 230 °C, respectively. Injection size was 0.02 µL neat (syringe: Hamilton 0.5 µL capacity, Alltech USA) with a split ratio was 1:40. The major chemical constituents of essential oil, such as menthol, menthone, iso-menthone, methyl acetate, pulegone, and limonene were quantified and expressed in percent based on GC peak area (FID response) without using correction factor in all the twenty-nine half-sib progeny separately.

F. Statistical analysis

Mean data statistically analyzed by using windostat statistical software version 9.3 available at CSIR-CIMAP Research Centre, Pantnagar based on [8, 9]. The phenotypic variance (σ^2_p) and genotypic variance (σ^2_g) calculated using replicated data. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) was computed [10]. The extent of genetic advance to be expected from selecting five percent of the superior progeny was calculated [11]. The genetic divergence among the twenty-nine half-sib progeny was analyzed using Mahalanobis D^2-statistics [12] and canonical analysis. The clustering was performed based on Tocher method [6].

III. RESULTS AND DISCUSSION

The pooled mean data of agro-morphic traits of twenty-nine menthol mint accessions was presented in Table 1. The analysis of variance (ANOVA) showed significant differences among the menthol mint accessions for all the agro-

Table 1: ***Mean performance of twenty-nine half-sib progeny of menthol mint for yield and yield contributing traits in two consecutive seasons.***

Sl.No|.	Accessions	Plant height (cm)	Canopy diameter (cm)	Number of branches/plant	Herb yield (kg/plot)	Oil yield (kg/plot)
1	MASP-1	67.00	37.50	12.00	1.98	0.118
2	MASP-2	31.00	25.00	5.00	2.14	0.413
3	MASP-3	56.00	39.00	11.00	2.96	0.192
4	MASP-4	55.00	40.00	7.00	2.45	0.269
5	MASP-5	51.00	32.00	11.00	1.85	0.203
6	MASP-6	42.00	47.50	13.00	1.91	0.191
7	MASP-7	51.00	46.00	9.00	2.45	0.269
8	MASP-8	62.00	43.00	12.00	2.51	0.426
9	MASP-9	53.00	43.50	16.00	3.60	0.576
10	MASP-10	49.00	42.50	14.00	4.16	0.665
11	MASP-11	48.00	30.50	8.00	3.91	0.664
12	MASP-12	51.00	30.84	8.00	4.36	0.697
13	MASP-13	42.00	34.00	9.00	1.82	0.254
14	MASP-14	57.00	48.50	12.00	2.61	0.417
15	MASP-15	32.00	38.00	18.00	3.14	0.439
16	MASP-16	67.00	29.00	7.00	2.14	0.192
17	MASP-17	49.00	50.00	16.00	3.72	0.483
18	MASP-18	45.00	40.00	11.00	2.30	0.368
19	MASP-19	51.00	46.00	15.00	1.87	0.486
20	MASP-20	72.00	55.00	9.00	1.94	0.213
21	MASP-21	39.00	28.50	10.00	2.52	0.403
22	MASP-22	34.00	43.00	10.00	1.85	0.296
23	MASP-23	45.00	40.00	12.00	2.10	0.189
24	MASP-24	35.00	33.50	9.00	2.14	0.278
25	MASP-25	48.00	32.00	12.00	3.12	0.436
26	MASP-26	52.00	42.00	10.00	2.94	0.382
27	MASP-27	42.00	39.00	13.00	3.92	0.548
28	MASP-28	51.00	38.00	14.00	2.76	0.331
29	MASP-29	38.00	43.00	7.00	22.00	0.132
	Range	32.50-73.00	26.00-56.75	5.40-18.30	1.83-4.36	0.117-0.698
	$CD_{5\%}$	1.279	1.586	0.646-	0.021	0.003
	$CD_{1\%}$	1.726	2.139	0.871	0.028	0.004

Morphic traits studied (Table 2) which indicated the presence of ample genetic variability among the germplasm accessions used in this study.

A significant variability for chemical constituents of essential oil was also observed among menthol mint accessions (Fig. 1, Fig. 2) (Table 4). The estimates of the genotypic coefficient of variation (GCV), the phenotypic coefficient of variation (PCV), heritability, and genetic advance for agro-morphic traits studied are provided in Table 5. It was observed that the relative magnitude of PCV was marginally higher compared to the corresponding GCV for all characteristics. This indicates that, the traits were influenced by the environment to some extent

Table 2: ***Analysis of variance (ANOVA) for agronomic characters in twenty-nine newly generated half-sib of menthol mint.***

Source of variation	d.f.	Mean sum of squares (MSS)						
		Plant height	Canopy diameter	Number of branches/plant	Herb yield	Oil yield	Menthol percent	Menthone percent
Years (Y)	1	1.35	1.32	2.14	0.0001	0.0001	0.0071	14697.45
Replication	1	187.55	63.03	5.35	0.0048	0.0000	0.0000	3.10
Genotypes (G)	28	412.15*	200.75*	37.87*	2.3610*	0.1071*	4396.26*	14697.45*
G X Y	28	0.45*	0.76*	0.03	0.0004	0.0009	4.1576	1467.76
Errors	57	0.66	1.00	0.16	0.0005	0.0006	0.0021	2818.19
Total	115							

* Significant at 1% probability level;** Significant at 5% probability level; d. f.: degrees of freedom.

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Table 3: ***Variability for chemical constituents in essential oils of twenty-nine menthol mint accessions.***

Sl. No.	Accessions	Menthone (%)	Isomenthone (%)	Menthol (%)	Menthyl acetate (%)	Pulegone (%)	Limonene (%)
1	MASP-1	7.65	4.18	64.03	16.14	0.00	1.42
2	MASP-2	7.20	4.64	68.59	7.41	0.00	4.22
3	MASP-3	16.82	3.99	69.02	3.29	0.00	1.33
4	MASP-4	70.25	18.60	0.83	1.63	0.00	1.54
5	MASP-5	79.92	10.59	0.18	0.16	0.00	3.04
6	MASP-6	11.78	4.68	64.61	8.05	0.00	8.06
7	MASP-7	83.18	6.92	0.22	1.05	0.00	2.87
8	MASP-8	27.10	6.76	52.86	2.91	0.00	4.15
9	MASP-9	84.76	6.39	0.87	0.65	0.00	2.45
10	MASP-10	14.59	5.92	71.02	1.19	0.00	1.75
11	MASP-11	84.26	7.33	0.32	0.35	0.00	2.18
12	MASP-12	11.69	8.09	70.06	1.96	0.00	2.06
13	MASP-13	84.97	6.64	0.65	0.35	0.00	1.75
14	MASP-14	79.75	8.62	1.26	1.46	0.00	2.53
15	MASP-15	82.71	8.07	0.13	0.35	0.00	2.44
16	MASP-16	18.84	4.37	66.82	2.83	0.00	1.46
17	MASP-17	82.08	5.53	3.04	0.04	0.00	3.30
18	MASP-18	82.08	5.53	3.04	0.03	0.00	3.30
19	MASP-19	6.71	5.32	69.69	5.74	0.00	3.79
20	MASP-20	14.70	9.59	58.77	7.14	0.00	2.01
21	MASP-21	81.81	8.46	0.29	0.25	0.00	2.47
22	MASP-22	0.12	0.32	0.21	0.25	75.40	17.78
23	MASP-23	12.68	5.83	65.59	7.35	0.00	2.03
24	MASP-24	8.14	4.69	73.19	7.56	0.00	1.30
25	MASP-25	18.85	5.98	59.02	7.41	0.00	1.77
26	MASP-26	13.12	3.75	43.14	4.05	0.00	1.79
27	MASP-27	1.52	0.24	1.26	0.24	66.70	24.89
28	MASP-28	0.32	0.24	0.54	1.24	66.88	24.24
29	MASP-29	3.21	4.17	59.97	25.52	0.00	0.24
	Range	0.120-84.97	0.240-18.60	0.130-73.19	0.030-25.52	0.00-75.04	0.240.24.89
	CD5%	12.911	0.704	0.096	0.110	0.145	0.045
	CD1%	17.417	0.928	0.130	0.149	0.195	0.085

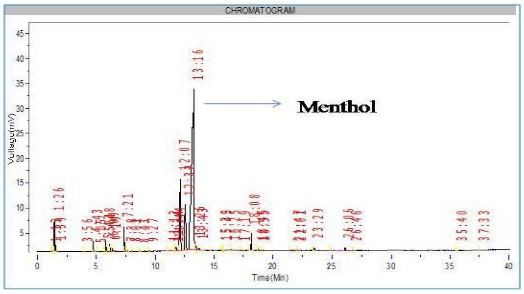

Fig. 1: ***Representative gas chromatography profile of menthol rich essential oil of half-sib progeny MASP-12.***

The genotypic variance was ranged from 0.0268-1161.9850, and phenotypic variance was ranged from 0.0268-1201.7410. The highest PCV value was observed for menthone (102.552%) content, and lowest PCV value was noticed for canopy diameter (18.022 %). Higher PCV and GCV for agro-economic traits in *Mentha piperita* L. was reported earlier [5]. The narrow difference between PCV and GCV was due to a smaller influence of environment in the expression of these traits. The proportion of genotypic variability which is transmitted from parents to progeny is reflected by heritability.The heritability estimate was found to be high (> 60%) for all characteristics. The highest heritability was reported for plant height (99 %) followed by herb yield (99 %) and oil yield (99%). The lowest heritability was reported for menthone content (96%).

The traits which showed high heritability means that traits were controlled by additive gene action, these traits can be improved by simple selection, the results are in accordance with an earlier report [5].The heritability cannot give a clear picture about the nature of inheritance of the trait. The genetic advance represents the improvement in the mean of selected families over the base population. The heritability with genetic advance as percent mean give the exact nature of inheritance of the trait. In the present study, high heritability with high genetic advance was observed for menthone percent followed by menthol content and oil yield. This suggested the major role of the additive gene with low environmental influence in governing these traits. Simple selection will help in improving these traits.

On the basis of D^2 values, all the twenty-nine menthol mint accessions were grouped into four diverse clusters (Fig.5). Cluster II was the largest group that consists of 15 genotypes followed by cluster I, consisted of 12 genotypes and cluster III and cluster IV had one genotype in each (Table 6). About nine peppermint (*Mentha piperita*) genotypes grouped into three diverse clusters by using Mahalanobis D^2-statistics [5]. The maximum intra-cluster distance was observed in cluster II (59071.56) and the minimum for cluster III (0.00) and cluster IV (0.00). The inter-cluster distance ranges from 133541.90–1955171.00. The minimum inter-cluster distance was between cluster II, and III (133541.90) and the maximum was between cluster I and III (1955171.00) (Table 7). Based on cluster mean value (Table 8) the important cluster for high oil yield was cluster III (0.48 kg/plot) along with high herb yield (3.72 kg/ plot) and menthol content (82.09%).

The cluster IV had highest mean for plant height (53.50 cm), and the cluster III had height mean for canopy diameter (51.58 cm) and number of branches (16.25). The cluster I had highest mean for menthone content (62.08%) along with low menthol content (0.43%). The genotypes within the cluster with a high degree of divergence would also produce genetically interesting genotypes upon hybridization [13]. The genotypes belong to the different cluster can be utilized for the hybridization program to get novel and desirable genotypes [14].

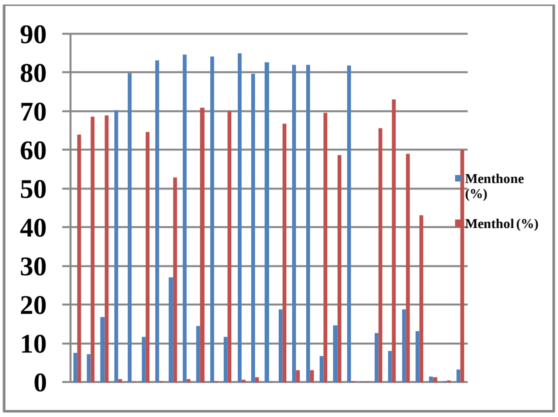

Fig.2: ***Graph representing the content of menthone (%) and menthol (%) in essential oils of the twenty-nine menthol mint accessions.***

IV. CONCLUSION

Genetic improvement of any crop requires study of genetic variability in the available germplasm accessions/populations. In the present study, we examined the magnitude of genetic variation for economic traits of twenty-nine menthol mint accessions to understand genetic variability and the genetic divergence was estimated by Mahalanobis D^2 statistics.

Table 4: ***Estimates of genetic parameters for seven economic traits of menthol mint accessions.***

Sl. No.	Character	σ^2_g	σ^2_p	GCV (%)	PCV (%)	h^2 (%)	GA	GAM (%)
1	Plant height (cm)	103.9409	104.3313	20.2854	20.3234	99	20.9627	41.7096
2	Canopy diameter (cm)	51.9986	52.598	17.9192	18.0222	98	14.7698	36.7027
3	Number of branches	9.3893	9.4888	26.9344	27.0767	98	6.2791	55.1931
4	Herb yield (kg/plot)	0.5965	0.5965	28.8843	28.8858	99	1.5909	59.4963
5	Oil yield (kg/plot)	0.0268	0.0268	44.9562	44.9578	99	0.337	92.6055
6	Menthol (%)	1099.058	1099.058	86.3005	86.3006	97.8	68.2931	177.7789
7	Menthone (%)	1161.985	1201.714	100.8426	102.9524	96	69.0506	204.2729

Table 5: ***Grouping of twenty-nine menthol mint accessions into different clusters based on seven agro-economic traits.***

Cluster number	Number of accessions	Name of the accessions
I	12	MASP-18, MASP-21, MASP-14, MASP-15, MASP-22, MASP-13, MASP-28, MASP-7, MASP-5, MASP-27, MASP-9, MASP-11.
II	15	MASP-10,MASP-12,MASP-2,MASP-19,MASP-24,MASP-4, MASP-25,MASP-16,MASP-6,MASP-23,MASP-20,MASP-1, MASP-3MASP-29, MASP-8
III	1	MASP-17
IV	1	MASP-26

Table 6: ***Estimates of inter and intra (bold) cluster distances in menthol mint accessions among four clusters.***

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Cluster 1	**15355.85**	1204582.00	1955171.00	522768.80
Cluster 2	1204582.00	**59071.56**	133541.90	160846.00
Cluster 3	1955171.00	133541.90	**0.00**	461375.20
Cluster 4	522768.80	160846.00	461375.20	**0.00**

Table 7: ***Cluster mean of seven economic traits of menthol mint accessions***

	Plant height (cm)	Canopy diameter (cm)	Number of branches	Herb yield (kg/plot)	Oil yield (kg/plot)	Menthol (%)	Menthone (%)
Cluster 1	46.88	39.44	12.01	2.74	0.40	0.43	62.08
Cluster 2	52.70	39.90	10.61	2.54	0.33	65.58	14.81
Cluster 3	51.00	51.58	16.25	3.72	0.48	82.09	0.02
Cluster 4	53.50	43.60	10.35	2.95	0.38	43.16	13.12

The Genotypic and phenotypic coefficient of variations was largest for menthone content followed by menthol content and oil yield. High heritability was reported for all the agro-economic traits studied. All the twenty-nine menthol mint accessions were grouped into four diverse clusters. Cluster II was the largest group that consists of 15 genotypes followed by cluster I, which consisted of 12 genotypes. The genetic variability available in this germplasm set can be used for selecting the superior clones, and genetically divergent genotypes/half-sib progenies can be utilized for Hybridization programs to get novel and superior hybrid clones.

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