



Trends in Phytochemical Research (TPR)

Journal Homepage: <http://tpr.iau-shahrood.ac.ir>



Original Research Article

Genetic variability, D² analysis and characters association among quantitative and qualitative traits of spearmint (*Mentha spicata* L.)

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ABSTRACT

An investigation was carried out to assess the genetic variability, genetic divergence and association of pheno-morphic and agronomic traits along with major chemical constituents of essential oil in 41 accessions of spearmint (*Mentha spicata* L.). Highly significant differences were noted for all 10 economic traits indicating the existence of considerable genetic variability among 41 accessions. High heritability (h^2) and high genetic advance was noted for herb yield (120.64%). On the basis of D² values, all 41 spearmint accessions were grouped into six diverse clusters. The cluster-I was the largest group which consisted of 20 accessions. A significant and positive correlation was observed for plant height with herb yield (0.58 * *, ** = significant at 1% probability level). The herb yield showed the highest direct effect (0.194) for oil yield. According to results, a significant genetic variability was present among 41 accessions, simple selection can be employed to improve essential oil content. More importance should be given to plant height, leaf length, leaf width and herb yield during selection to improve essential oil yield in spearmint.

ARTICLE HISTORY

Received: 20 March 2019

Revised: 15 May 2019

Accepted: 27 May 2019

ePublished: 29 June 2019

KEYWORDS

Correlation
Genetic divergence
Genotypic variance
Heritability
Mahalanobis D²

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1. Introduction

Spearmint (*Mentha spicata* L.) is a creeping rhizomatous and perennial plant having strong aroma Jirovetz et al., 2002; Almeida et al., 2019; Nazem et al., 2019; Soilhi et al., 2019). It is indigenous to England and is grown all over the world mainly in the USA and in some regions of China (Lee and Fred, 1998). It is also growing in many Southeast Asian countries (Atal and Kapur, 1982; Nemati Lafmejani et al., 2018). Spearmint is indigenously used as medicinal plant for digestive disorders and its essential oil possesses an inhibitory effect against some pathogenic bacteria (Mkaddem et al., 2009). Spearmint essential oil contains carvone with an approximate content of 60-70% of the total oil along with limonene (8.0-15.0%) (Lee and Fred, 1998). The major end use of spearmint oil is in toothpaste, mouthwash, chewing gum as well as candy

and food flavouring (Atal and Kapur, 1982; Lee and Fred, 1998). The herb is considered to be carminative and antispasmodic (Chopra et al., 1950; Reynolds, 1982; Yusuf et al., 1994). Several applications have been reported for carvone. Its use as a fragrance and flavour, food, beverages and oral hygiene products, potato sprouting inhibitor, antimicrobial agent and in medical applications increases the interest on this ketone (De Carvalho et al., 2006). Limonene found mainly applications in cosmetics, in medicine including the aromatherapy in the natural medicine (relaxing, harmonizing and stabilizing the nervous system), in the perfume industry and in the food industry (Uemura et al., 1997)

Genetic improvement of any crop requires thorough understanding of existing genetic variability among available germplasm/genotypes and knowledge of association/correlation among economic traits.

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Knowledge of the genetic nature of traits helps breeders to select the correct breeding strategy for the genetic improvement of plants. The heritability (H_b) determines the type of breeding technique and the usefulness of the selection method. Genetic advance is also very important in breeding programs. It helps breeders get a clear idea about the extent of improvement between two generations (Tahir and Razi, 2016). It is important to consider heritability along with the phenotypic and genotypic coefficients of variation to avoid bias in heritability estimates (Liu et al., 2015). 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 D² statistic (Rao, 1952) has been widely used in crop improvement programs for selection of genetically divergent parents for hybridization programmes (Ramanujam et al., 1974). The knowledge of association between yield and its contributing traits would facilitate successful development of high-yielding varieties (Mary and Gopalan, 2006). The nature of association between yield and its component traits determines the particular traits to be used as indirect selection criteria for genetic improvement of yield (Guljar and Patil, 2016). Path analysis is used to determine the magnitude of the direct and indirect effects of various variables on the dependent variable (Ahmadzadeh et al., 2012).

The majority of experiments done on spearmint have studied chemical composition of essential oil. There is no report on the genetic parameters, genetic divergence and correlation of the morpho-agronomic and chemical traits of spearmint. Hence, this study was designed to determine extent of genetic variability, genetic divergence and correlation among economic traits in spearmint accessions. This study will help to quantify genetic variability and to isolate genetically divergent parents for hybridization to develop genetically high yielding spearmint genotypes/varieties for commercial cultivation in India.

2. Experimental

2.1. Planting material

The planting materials for the present study includes 41 accessions of spearmint (Table 1).

2.2. Experimental site and design

All 41 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 (29°N, 79.38°E; altitude of 243.84 m above sea level). A randomized complete-block design (RCBD), with two replications was used. Planting was done on 3 m × 3

m beds, with a row to row spacing of 60 cm. Standard agronomic practices were followed throughout the crop season, which included an application of farm yard manure (FYM) at the rate of 10 t/ha, along with 80, 40, 20 kg/ha of nitrogen (N), phosphorus (P) and potassium (K), respectively. About 60 kg/ha of nitrogen was top-

Table 1
Genetic stocks and their origin of spearmint.

Sl. No.	Accessions	Origin
1	OPSP-1	Pantnagar, Uttarakhand, (India)
2	OPSP-2	Pantnagar, Uttarakhand, (India)
3	OPSP-3	Pantnagar, Uttarakhand, (India)
4	OPSP-4	Pantnagar, Uttarakhand, (India)
5	OPSP-5	Pantnagar, Uttarakhand, (India)
6	OPSP-6	Pantnagar, Uttarakhand, (India)
7	OPSP-7	Pantnagar, Uttarakhand, (India)
8	OPSP-8	Pantnagar, Uttarakhand, (India)
9	OPSP-9	Pantnagar, Uttarakhand, (India)
10	OPSP-10	Pantnagar, Uttarakhand, (India)
11	OPSP-11	Pantnagar, Uttarakhand, (India)
12	OPSP-12	Pantnagar, Uttarakhand, (India)
13	OPSP-13	Pantnagar, Uttarakhand, (India)
14	OPSP-14	Pantnagar, Uttarakhand, (India)
15	OPSP-15	Pantnagar, Uttarakhand, (India)
16	OPSP-16	Pantnagar, Uttarakhand, (India)
17	OPSP-17	Pantnagar, Uttarakhand, (India)
18	OPSP-18	Pantnagar, Uttarakhand, (India)
19	OPSP-19	Pantnagar, Uttarakhand, (India)
20	OPSP-20	Pantnagar, Uttarakhand, (India)
21	OPSP-21	Pantnagar, Uttarakhand, (India)
22	OPSP-22	Pantnagar, Uttarakhand, (India)
23	OPSP-23	Pantnagar, Uttarakhand, (India)
24	OPSP-24	Pantnagar, Uttarakhand, (India)
25	OPSP-25	Pantnagar, Uttarakhand, (India)
26	OPSP-26	Pantnagar, Uttarakhand, (India)
27	OPSP-27	Pantnagar, Uttarakhand, (India)
28	OPSP-28	Pantnagar, Uttarakhand, (India)
29	OPSP-29	Pantnagar, Uttarakhand, (India)
30	OPSP-30	Pantnagar, Uttarakhand, (India)
31	OPSP-31	Pantnagar, Uttarakhand, (India)
32	OPSP-32	Pantnagar, Uttarakhand, (India)
33	OPSP-33	Pantnagar, Uttarakhand, (India)
34	OPSP-34	Pantnagar, Uttarakhand, (India)
35	OPSP-35	Pantnagar, Uttarakhand, (India)
36	OPSP-36	Pantnagar, Uttarakhand, (India)
37	OPSP-37	Pantnagar, Uttarakhand, (India)
38	OPSP-38	Pantnagar, Uttarakhand, (India)
39	OPSP-39	Pantnagar, Uttarakhand, (India)
40	OPSP-40	Pantnagar, Uttarakhand, (India)
41	OPSP-41	Pantnagar, Uttarakhand, (India)

dressed in two equal splits at 30 days and 60 days after planting. Plots were irrigated when needed. Manual weeding was done once at the initial stage of crop growth (20 days after planting), and a second hand-weeding was done at 40 days after planting. Necessary plant protection measures were taken to raise a good crop. Data were recorded on five competitive, randomly selected plants per plot for the following traits: plant height (cm), L(Leaf):S (Stem) ratio, number of sprouts, leaf length (cm), leaf width (cm), herb yield (g/plant) and oil yield (g/plant). Plant height was measured in centimeters from the base of the plant to the top of the last leaf. The L:S ratio was calculated by separating leaf and stem from total herb and weighed separately and converted into ratio individually. The leaf length was measured in centimetres from base of the leaf. The leaf width was measured in centimetres. The herb yield was calculated in gram on per plant basis by recording herb weight in individual plants. The essential oil yield was calculated on a per plant basis by multiplying the oil content in 100-grams herb with the total herb yield per plant.

2.3. Essential oil extraction

About 200 g of fresh green herb harvested separately from each of the 41 accessions. Essential oil was extracted from individual accessions by hydrodistillation for about 3-4 h using a Clevenger apparatus. Percent essential oil content was calculated (on a 100-g basis) and essential oil yield was calculated on per plant basis on fresh weight basis by multiplying the oil yield/100 g herb with total herb yield/plant. Anhydrous sodium sulfate was added to extracted essential oil to remove water traces and stored at 4 °C until needed for further analysis.

2.4. Gas chromatography (GC)

Oil samples were analyzed on a Nucon gas chromatograph model 5765 equipped with a flame ionization detector (FID) and two stationary phases of different polarity, viz., BP-20 (30 m length × 0.25 mm internal diameter × 0.25 μm film thickness) and DB-5 (30 m length × 0.32 mm internal diameter × 0.25 μm film coating) fused silica capillary columns. Hydrogen gas used as carrier at 1.0 ml/min and temperature programming was done from 70 °C to 230 °C at 4 °C/min with an initial and final hold time of 2 min (for BP-20) and from 70 °C to 250 °C at 3 °C/min (for DB-5). The injector and detector temperatures were 210 °C and 230 °C, respectively. Split ratio was 1:30. Chemical constituents of essential oil were quantified and expressed in percent.

2.5. Statistical analyses

The pooled mean data were statistically analyzed

by using Windostat statistical software 9.3 version available at CSIR-CIMAP Research Centre, Pantnagar based on Singh and Chaudhary (1979) and Panse and Sukhatme (1976). The mean, standard error, ranges were determined based on Singh and Chaudhary (1979). Analysis of variance was carried out by following the procedure given by Panse and Sukhatme (1976). Genetic divergence was calculated by using Mahalanobis D²-statistics (Mahalanobis, 1936) and canonical analysis. The clustering was done on the basis of Tocher's methods (Rao, 1952). The mean values of all the traits were subjected to correlation and path coefficient analyses (Dewey and Lu, 1959). Genetic parameters, including broad sense heritability (h² bs), genetic advance (GA), and genotypic and environmental coefficient of variation (GCV and ECV) were estimated from the components of variance by using the following formulas.

$$\text{Environmental variance (MSe)} = \sigma_e^2 \quad (\text{Eqn. 1})$$

$$\text{Genotypic variance } (\sigma_g^2) = (MS_g - MS_{y_g})/ry \quad (\text{Eqn. 2})$$

$$\text{Phenotypic variance } (\sigma_p^2) = \sigma_g^2 + \sigma_e^2 \quad (\text{Eqn. 3})$$

$$\text{Broad sense heritability } (h^2_{bs}) = \sigma_g^2 / \sigma_p^2 \times 100 \quad (\text{Eqn. 4})$$

$$\text{Genetic advances (GA)} = h^2_{(bs)} \times \sigma_p \times k \quad (\text{Eqn. 5})$$

$h^2_{(bs)}$ = Heritability in broad sense

σ_p = Phenotypic standard deviation of the trait

k = Standard selection differential which is 2.06 at 5 per cent selection intensity

$$\text{Phenotypic coefficient of variation} = \sqrt{\text{phenotypic variance}} / (\text{grand mean}) \times 100 \quad (\text{Eqn. 6})$$

$$\text{Genotypic coefficient of variation} = \sqrt{\text{genotypic variance}} / (\text{grand mean}) \times 100 \quad (\text{Eqn. 7})$$

$$\text{Environmental coefficient of variation} = \sqrt{\text{environmental variance}} / (\text{grand mean}) \times 100 \quad (\text{Eqn. 8})$$

$$\text{Genetic advance as percent mean} = (\text{genetic advance}) / (\text{grand mean}) \times 100 \quad (\text{Eqn. 9})$$

3. Results and Discussion

3.1. Genetic variability

Variation among the pooled mean of 41 accessions for ten economic traits was highly significant (Table 2). The meticulous study of analysis of variance, means and critical differences (C.D.) revealed significant differences among 41 accessions for all ten economic traits studied thereby indicating the existence of considerable genetic

Table 2

Estimates of genetic parameters for eleven traits of spearmint accessions.

Sl. No.	Character	σ^2_g	σ^2_p	GCV (%)	PCV (%)	h^2 (%)	GA	GAM (%)
1	Plant height(cm)	201.10	203.80	23.16	24.13	98.68	0.32	64.70
2	L:S ratio	0.07	0.07	8.92	9.69	95.53	0.54	40.56
3	Herb yield (g/plant)	33441.45	33447.01	57.27	58.56	82.35	376.74	120.64
4	Canopy diameter(cm)	811.45	813.96	29.65	31.38	96.58	58.77	64.64
5	No. of sprouts	122.66	124.78	48.51	49.30	93.45	23.01	101.56
6	Leaf length (cm)	0.72	0.72	16.49	17.71	95.67	1.75	36.47
7	Leaf width (cm)	0.26	0.26	17.21	17.92	93.45	1.06	36.91
8	Oil yield (ml/plant)	0.02	0.02	30.41	31.25	84.25	0.32	64.70
9	Oil percent (%)	0.02	0.02	28.22	29.28	83.49	0.29	60.31
10	Carvone (%)	553.27	558.14	42.57	44.63	99.26	48.67	91.94
11	Limonene (%)	74.16	77.14	53.27	55.83	99.47	18.09	115.02

Table 3

Estimates of inter and intra cluster distances in spearmint accessions among six clusters.

	Cluster -1	Cluster -2	Cluster -3	Cluster -4	Cluster -5	Cluster -6
Cluster- 1	4346.75	21992.76	69291.49	60692.58	14188.07	184156.90
Cluster-2	21992.76	5224.23	155527.80	15226.93	54995.29	314688.40
Cluster- 3	69291.49	155527.80	2951.82	248528.50	35552.51	31728.26
Cluster- 4	60692.58	15226.93	248528.50	2603.80	110493.00	441998.80
Cluster- 5	14188.07	54995.29	35552.51	110493.00	7963.88	119901.70
Cluster- 6	184156.90	314688.40	31728.26	441998.80	119901.70	7192.90

variability among the accessions. The statistical and genetic parameters for the heritable and non-heritable components of variation were computed. Maximum amount of genotypic coefficient of variation (GCV) was observed for herb yield (57.27%) followed by limonene percent (53.27%) suggesting effective selection for these traits for genetic improvement. The highest phenotypic coefficient of variation (PCV) was noticed for herb yield (58.56%) and the lowest PCV was observed for L: S ratio (9.69%) (Table 2). Phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all the traits studied. This clearly indicates that observed variation was not only due to genotype alone but also due to environment (Kumar et al., 2014; Gupta et al., 2017).

A high heritability estimate (h^2_{BS}) with corresponding high genetic advance (GA) is more reliable for selection than that with low genetic advance (GA). This indicated that simple selection will help in genetic improvement of these traits. A high heritability (82.35%) with high genetic advance (376.74%) was perceived for herb yield/plant. High genetic advance as a percent of mean was observed for herb yield/plant (120.64%) followed by limonene percent (115.02%); this indicated effective selection will improve these traits (Lal et al., 2001), herb yield per plant as high GCV (57.27) and PCV (58.56) along with high heritability (82.35%). Carvone percent was also as high GCV and PCV; therefore priority should be given to above these traits in spearmint breeding programme.

On the basis of D^2 values, all 41 accessions were

grouped into six diverse clusters (Fig. 1). The cluster I was largest which consist of 20 accessions (OPSP-26, OPSP-32, OPSP-6, OPSP-11, OPSP-15, OPSP-33, OPSP-27, OPSP-38, OPSP-13, OPSP-17, OPSP-37, OPSP-14, OPSP-23, OPSP-3, OPSP-5, OPSP-19, OPSP-21, OPSP-16, OPSP-24 and OPSP-28) and smallest cluster was cluster IV (OPSP-4 and OPSP-25). Maximum intra-cluster distance was observed in cluster V (7963.88) and the minimum intra-cluster distance was observed in cluster IV (2603.80) (Table 3). The accessions belong to same cluster has narrow genetic variability and accessions

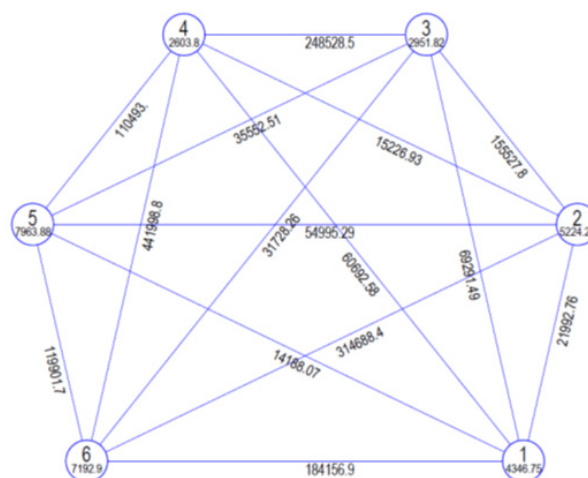


Fig. 1. Mahalanobis euclidean distance depicting inter and intra cluster distance among six clusters of 41 spearmint accessions by Tocher method.

Table 4

Cluster mean of eleven economic traits of spearmint accessions.

	Plant height (cm)	L:S ratio	Herb yield (g/plant)	Canopy diameter(cm)	No. of sprouts	Leaf length (cm)	Leaf width (cm)	Oil yield (ml/plant)	Oil percent (%)	Carvone (%)	Limonene (%)
Cluster 1	63.44	1.38	312.22	95.06	23.11	5.07	2.88	0.49	0.49	62.26	16.95
Cluster 2	56.31	1.37	181.31	74.94	16.15	4.51	2.68	0.53	0.52	64.40	15.50
Cluster 3	72.50	1.25	568.17	115.92	29.00	4.95	2.89	0.51	0.51	54.93	21.02
Cluster 4	37.00	1.36	77.20	55.60	13.80	4.24	3.00	0.32	0.36	51.09	13.01
Cluster 5	62.83	1.28	396.17	106.08	30.67	4.95	3.12	0.46	0.46	21.27	11.31
Cluster 6	63.00	1.27	732.50	144.00	42.00	5.75	2.90	0.65	0.45	30.02	15.96

Table 5

Genotypic correlation coefficients among eleven traits of spearmint accessions.

Variables	Plant height	L:S ratio	Herb yield	Canopy diameter	No. of sprouts	Leaf length	Leaf width	Oil yield	Oil percent	Carvone	Limonene
Plant height	1.00										
L:S ratio	0.01	1.00									
Herb yield	0.58 **	-0.15	1.00								
Canopy diameter	0.70 **	0.02	0.82 **	1.00							
No. of sprouts	0.09	-0.03	0.63 **	0.41 **	1.00						
Leaf length	0.54 **	-0.14	0.39 *	0.50 **	-0.01	1.00					
Leaf width	0.04	0.15	0.11	0.05	0.17	0.28	1.00				
Oil yield	0.44 **	-0.05	0.24	0.27	-0.01	0.12	-0.18	1.00			
Oil percent	0.38 *	0.00	0.05	0.09	-0.13	0.00	-0.26	0.90 **	1.00		
Carvone	-0.14	-0.01	-0.30	-0.40 **	-0.22	-0.14	-0.15	0.15	0.33 *	1.00	
Limonene	0.02	0.09	0.22	0.11	0.12	0.01	0.26	-0.23	-0.28	-0.09	1.00

Residual effect = 0.37, * P < 0.05, ** P < 0.01

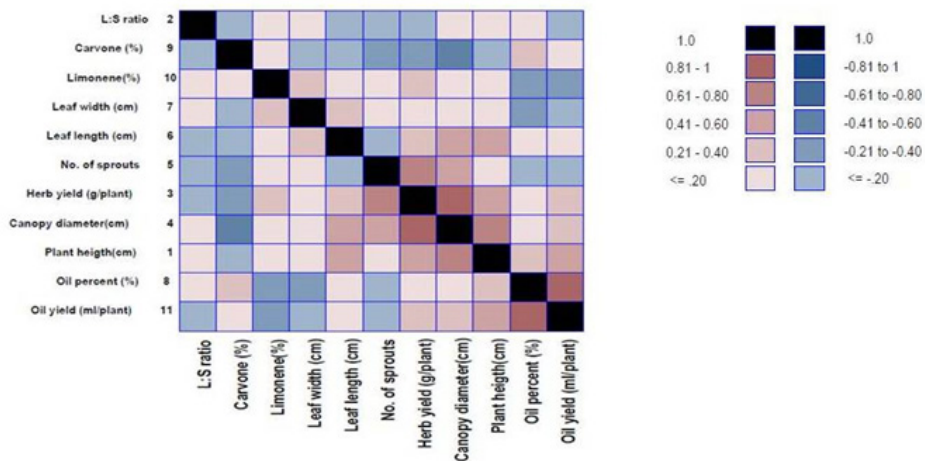


Fig. 2. Shaded genotypic correlation matrix to identify relationship among eleven traits in 41 spearmint accessions.

which belongs to different cluster has broader genetic variability. Members from two diverse clusters should be included in the hybridization programme to get more transgressive segregants in segregating generation (Ramanujam et al., 1974; Singh et al., 1995; Singh et al., 1996; Lal et al., 2001; Shukla et al., 2010). Based on the cluster mean (Table 4), the important cluster for high essential oil yield was cluster VI that possesses higher oil yield (0.65%) because of high herb yield (732.50%) and high sprout number (42.00%). The maximum divergence was observed in cluster VI.

3.2. Genotypic correlation

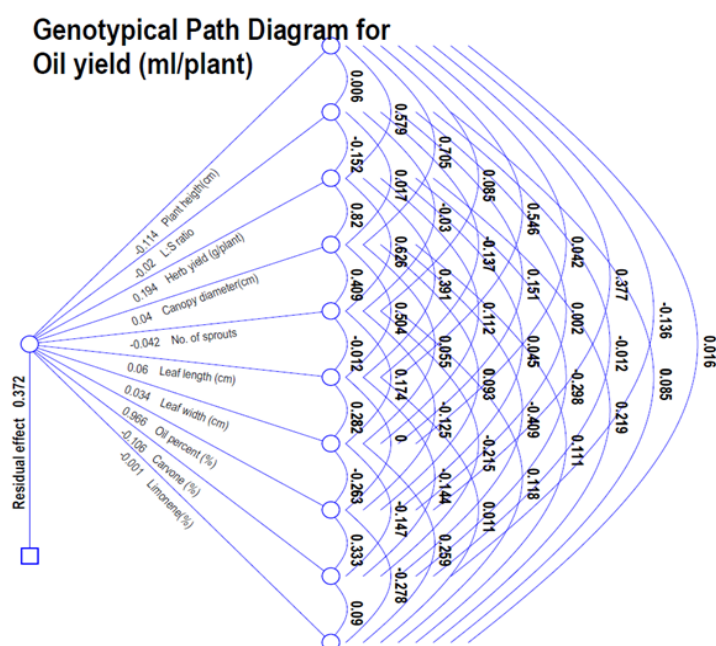
Study of association among the traits gives an idea to improve a particular character. The estimates of correlation coefficients are represented in Table 5. The results of genetic associations studies revealed that the plant height was positively and significantly correlated with herb yield/plant (0.58**), leaf length (0.54**) and oil yield/plant (0.44**) (Fig. 2). Thus, tall plants with longer leaves and more number of leaves have high herb yield and relatively high oil content in comparison with dwarf plants with less number of leaves. Hence, taller plants

Table 6

Direct (bold) and indirect effects on eleven traits related to path analysis in spearmint accessions.

	Plant height(cm)	L:S ratio	Herb yield (g/plant)	Canopy diameter(cm)	No. of sprouts	Leaf length (cm)	Leaf width (cm)	Oil percent (%)	Carvone (%)	Limonene (%)
Plant height(cm)	-0.114	-0.001	-0.066	-0.080	-0.010	-0.062	-0.005	-0.043	0.016	-0.002
L:S ratio	0.000	-0.020	0.003	0.000	0.001	0.003	-0.003	0.000	0.000	-0.002
Herb yield (g/plant)	0.112	-0.030	0.194	0.159	0.122	0.076	0.022	0.009	-0.058	0.043
Canopy diameter(cm)	0.029	0.001	0.033	0.041	0.017	0.020	0.002	0.004	-0.017	0.005
No. of sprouts	-0.004	0.001	-0.027	-0.017	-0.042	0.001	-0.007	0.005	0.009	-0.005
Leaf length (cm)	0.033	-0.008	0.023	0.030	-0.001	0.060	0.017	0.000	-0.009	0.001
Leaf width (cm)	0.001	0.005	0.004	0.002	0.006	0.010	0.034	-0.009	-0.005	0.009
Oil percent (%)	0.364	0.002	0.044	0.090	-0.121	0.000	-0.254	0.966	0.321	-0.269
Carvone (%)	0.014	0.001	0.032	0.043	0.023	0.015	0.016	-0.035	-0.106	-0.010
Limonene (%)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-0.001

Residual effect=0.37

**Fig. 3.** Path diagram showing the values of direct and indirect contribution of independent variable on oil yield in spearmint accessions.

with longer and broader leaves are included in selection of parents/genetic stocks for higher oil yield (Kukreja et al., 1992; Gravois and Mcnew, 1993; Kumar et al., 2014; Gupta et al., 2017).

3.3. Path analysis

The path analysis was estimated to study the direct and indirect effects of different traits on oil yield. The herb yield showed the highest direct effect (0.194) for oil yield followed by leaf length (0.060) (Table 6). Direct contribution of other five traits, namely plant height, L:S ratio, number of sprouts, carvone and limonene was negative but their indirect contribution was relatively large via plant height (Fig. 3). Limonene was negatively related to carvone, during biosynthesis, and limonene was converted to carvone during maturity. The residual effect of 0.37 revealed that 90% of oil yield was contributed by the characters studied and thus indicated the adequacy of character. Therefore, the choice of the

characters to be considered for improvement of oil yield in spearmint will be herb yield, leaf length, leaf width, number of sprouts and plant height. The genetic associations along with their mean performance could be utilized efficiently for the selection of genetic stocks/parents in future hybridization programme for the improvement of spearmint (Kang et al., 1983; Mirzaie-Nodoushan et al., 2001; Singh et al., 2014; Kumar et al., 2014; Gupta et al., 2017).

4. Concluding remarks

Availability of genetic variability is very important for development of high yielding varieties. We studied genetic variability for ten traits of 41 spearmint accession in order to understand genetic divergence, correlation coefficient and path coefficient. High genotypic and phenotypic coefficient of variation along with high genetic advance was noted for herb yield. A significant genetic variability was present among 41 accessions, on

the basis of D^2 values, all 41 accessions were grouped into six diverse clusters. The cluster I was largest which consist of 20 accessions (OPSP-26, OPSP-32, OPSP-6, OPSP-11, OPSP-15, OPSP-33, OPSP-27, OPSP-38, OPSP-13, OPSP-17, OPSP-37, OPSP-14, OPSP-23, OPSP-3, OPSP-5, OPSP-19, OPSP-21, OPSP-16, OPSP-24 and OPSP-28) and smallest cluster was cluster IV (OPSP-4 and OPSP-25). Based on the cluster mean, the important cluster for high oil yield was cluster VI, the genotypes belongs to cluster VI as high herb yield with high essential oil yield. A significant and positive correlation was observed between plant height, herb yield, and leaf length. The highest direct contribution for oil yield was herb yield. Based on this study, a significant genetic variability was prevailed among 41 accessions. Simple selection can be practiced for improving oil content in spearmint and more importance should be given to plant height, leaf length and leaf width during selection.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgement

The authors thank the Director, Central Institute of Medicinal and Aromatic Plants (CSIR - CIMAP), Lucknow, UP (India) for providing the necessary facilities.

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