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Genetic variability and character association among morpho-metric traits and essential oil constituents in eight half-sib seed progenies of peppermint (*Mentha piperita* L.)

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ABSTRACT

Menthol rich with low menthofuran peppermint (*Mentha piperita* L.) genotype MPK-5 and their eight half-sib seed progenies were evaluated for different genetic parameters, namely coefficient of variation, genetic advance, heritability, associations, and path analysis for different economic characters, viz., plant height, leaf length, leaf width, leaf: stem ratio, herb yield, essential oil content (%), and contents of various essential oil constituents (quality traits) such as sabinene, myrcene, limonene, α-pinene, β-pinene, 1,8-cineole, menthone, menthofuran, *neo*-menthol, isomenthone, menthyl acetate, menthol, and pulegone. The phenotypic coefficient of variations (PCV) was found slightly higher than the genotypic coefficient of variations (GCV) for the characters studied, indicating that the apparent variation was not only due to genetic but also influenced by the growing environment for the expression of studied traits. The highest genotypic coefficients of variation (GCV) was noted for the character pulegone, followed by menthofuran and 1,8-cineole. High heritability coupled with high genetic advance was observed for menthol followed by pulegone demonstrating that these chemical compositions might be under non-additive genetic control. The genotypic correlations were higher than the phenotypic correlations for studied traits. The significant and positive associations of β-pinene with sabinene and 1,8-cineole; α-pinene with β-pinene, sabinene, and 1,8-cineole; menthyl acetate with *neo*-menthol; and sabinene with 1,8-cineole were noted. A high direct positive effect was also recorded between menthofuran and limonene.

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1. Introduction

The aromatic herb peppermint (*Mentha piperita* L.) is widely distributed in temperate and sub-temperate climatic regions of the world (Kumar et al., 2014a). Leaves and the essential oil of peppermint are used in a wide range of applications in perfumery, cosmetics, confectionery, flavoring, food, and pharmaceutical industries (Kumar et al., 2015). The fresh herb on distillation yields an essential oil that contains a large variety of aroma chemicals in varying composition. The economic value and industrial demand of this aromatic crop invite the attention of plant breeders to develop high yielding variety to accomplish the desired market. The compound "menthofuran" is the characteristic phyto-molecule marker for differentiating peppermint oil from other mint oils (Kumar et al., 2014a). Based on the chromatographic profile, the characteristic components of the US type peppermint oil are menthol (36-46%), menthone (15-25%), isomenthone (2.0-4.5%), 1,8-cineole (4.0-6%), menthofuran (1.5-6%), *neo*-menthol (2.5-4.5%), menthyl acetate (3.0-6.5%), and pulegone (0.5-2.5%) (ISO, 2006). Due to industrial demand, a genetic improvement program was initiated for the development of peppermint genotype having lesser content menthofuran (< 5%) in its essential oil through half-sib progeny selection.

Though the number of high yielding varieties have been developed and released for commercial cultivation in due course of time, the knowledge regarding selection parameter for oil yield viz., genetic correlation, path analysis, and heritability are still unexplored (Kumar et al., 2004; Patra and Kumar, 2005). Thus, it is pertinent to assess the degree and magnitude of the character's association and path coefficient to facilitate direct and indirect selection of important traits for genetic improvement. The present study was planned with < 5%

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menthofuran containing genotypes MPK-5 (open-pollinated seed progeny of variety Kukrail) to have an idea of inter-relationships between different characters for an appropriate selection strategy in *M. piperita* L.

2. Experimental

2.1. Plant materials and experimental design

The peppermint planting material comprises of parental genotype MPK-5 (developed as open-pollinated seed progeny of variety Kukrail in the year 2000) portraying characteristics of high menthol with low menthofuran (>1.0%) content, the ability of flowering and producing viable seeds and its eight half-sib seed progenies, named as MPK-5 (1), MPK-5 (2), MPK-5 (3), MPK-5 (5), MPK-5 (6), MPK-5 (7), MPK-5 (8), and MPK-5 (9) (Fig. 1). The experiment was laid in a randomized block design (RBD) with three replicates, each block comprised of 5 rows at the space of 50 cm. This study was commenced in January and during the experiment, the soil of the experimental site was sandy-loam in texture, having a pH of 7.8. The fertilizers were applied in the ratio of 60:40:40 kg N, P_2O_5, and K_2O/ha, respectively.

2.2. Isolation and analysis of essential oil

On 110 days after planting, the fresh herbs were collected from plants of middle length row to avoid border effect. The essential oil was distilled from 200 g of fresh herb using the Clevenger type apparatus, and the relevant oil content was estimated on a weight basis. The gas chromatography (GC) insrumentation was used to estimate oil quality traits, , namely α-pinene, β-pinene, sabinene, myrcene limonene, 1,8-cineole, menthone, menthofuran, *iso*-menthone, menthyl acetate, *neo*-menthol, menthol and pulegone.

2.3. Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis

GC analysis of the essential oil was performed on Centurion scientific gas chromatograph (model CS-5800), equipped with Supelcowax 10 (30 m × 0.25 mm internal diameter, film thickness 0.50 μm) and BP-5 (30 m × 0.25 mm i.d., film thickness 0.25 μm) fused silica capillary columns and a flame ionization detector (FID). For Supelcowax 10 column, the oven temperature was programmed from 70 °C to 170 °C with a ramp of 4 °C/ min , then programmed to 240 °C with a ramp of 5 °C/ min with initial and final hold times of 5 and 15 min, respectively. However, for BP-5 column oven temperature was programmed from 60 °C to 240 °C at the ramp of 3 °C/min, with a final hold time of 10 min. Nitrogen was used as the carrier gas at 1.0 mL/min. The injector and detector temperatures were adjusted at 240 °C and 250 °C, respectively. The sample of 0.2 μL was injected in the split mode (1:70).

Clarus 680 GC interfaced with a Clarus SQ 8C Quadrupole mass spectrometer of PerkinElmer fitted involving Elite-5 MS fused-silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) were employed in GC-MS analysis of the peppermint oil. The temperature of the oven was programmed between 60 to 240 °C, at 3 °C/min, and programmed to 270 °C at 5 °C/ min; the temperature of injector was 250 °C; the adjusted temperature for transfer line and source temperatures was 220 °C; injection size 0.03 μL neat; 1:50 was the split ratio; He at 1.0 mL/min was the carrier gas; 70 eV was the ionization energy, and the mass scan varied over the range 40-450 amu. The retention index was employed for identification of the constituents in the essential oil, using a homologous series of *n*-alkanes (C_7-C_{30} hydrocarbons, Supelco Bellefonte, PA USA), MS Library search (NIST and WILEY), correlating with the literature published (Adams, 2007). GC peak area (FID response) determines the relative percentage of individual components in the essential oil.

2.4. Data collection and statistical analysis

The various morpho-metric characters, namely plant height, leaf length, leaf width, leaf: stem ratio, herb yield, oil content (%), and quality attributes viz. sabinene, myrcene, limonene, α-pinene, β-pinene, 1,8-cineole, menthone, menthofuran, *neo*-menthol, isomenthone, menthyl acetate, menthol, and pulegone were recorded at the maturity of the crop, i.e. 110 days from the day of planting for every four consecutive years (2011-2015). The pooled mean data were evaluated for correlation and path analysis following Dewey and Lu (1959) method. Variability was evaluated for different qualitative traits. The heritability and genetic advance were estimated pursuing standard protocols. All the statistical parameters were analyzed through SPAR-1 software (IASRI, New Delhi).

3. Results and Discussion

3.1. Variance components and coefficients of variation

The quantified values (as a percentage) of different chemical constituents analyzed through gas chromatography are shown in Table 1. The analysis of variance (ANOVA) revealed that the mean square of seed progenies was found to be highly significant ($P<0.01$) for all the studied quantitative and qualitative traits except α-pinene, which was significant at 0.05 probability level (Table 2). Similarly, a significant variability among the agronomic traits and chemical constituents of essential oil have been previously studied among accessions/ genotypes of different crops including *M. piperita* (Kumar et al., 2014b), *Mentha arvensis* L. (Venkatesha et al., 2019b), *Gaultheria procumbens* L. (Nikolic et al., 2013), and *Satureja horvatii* (Bukvicki et al., 2014). The range of magnitudes for quantitative and qualitative traits was considerably more extensive, demonstrating the potential of exploring the variability in seed progenies for promoting genetic enhancement. The extent of the phenotypic coefficient of variation (PCV) was in-variably larger than genotypic coefficient of variation (GCV), indicating that the apparent variation was not only due to

Fig. 1. Morphological variation among eight half sib seed progenies of MPK-5 (A) MPK-5 (open pollinated seed progeny of variety Kukrail); (B) MPK-5 (1); (C) MPK-5 (2); (D) MPK-5 (3); (E) MPK-5 (5); (F) MPK-5 (6); (G) MPK-5 (7); (H) MPK-5 (8); (I) MPK-5 (9).

genetic but are also influenced by the impact of environment for the expression of qualitative and quantitative traits, while for qualitative traits the same values of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) indicated strict genetic control over these traits (Table 3). The finding of the higher phenotypic coefficient of variation (PCV) than the genotypic coefficient of variation (GCV) for quantitative and qualitative traits is supported by previous studies on *Mentha* spp. (Kumar et al., 2014b; Mishra et al., 2016; Venkatesha et al., 2019a; Venkatesha et al., 2019b).

Heritability alone does not indicate the amount of genetic improvement that would result in the selection of individual genotype. Hence, knowledge on heritability coupled with genetic advance helps predict gain under selection than heritability alone. The heritability and genetic advance estimation were found to be superior for qualitative traits except for oil content and α-pinene, demonstrating that these traits might be under non-additive genetic control.

3.2. Association among characters

Awareness about character association plays a vital role in deploying an indirect selection of the traits and for

Table 1
Percentage composition (%±SD) and chemical constituents of essential oil in eight half-sib seed progeny of MPK-5 genotype of *Mentha piperita* L.

Compound	RI†	RI††	Content (%)								
			MPK-5	MPK-5 (1)	MPK-5 (2)	MPK-5 (3)	MPK-5 (5)	MPK-5 (6)	MPK-5 (7)	MPK-5 (8)	MPK-5 (9)
α-Pinene	1034	932	0.391±0.006	0.362±0.004	0.384±0.004	0.384±0.006	0.345±0.004	0.385±0.004	0.616±0.005	0.433±0.009	0.407±0.004
β-Pinene	1126	978	0.508±0.005	0.515±0.003	0.485±0.004	0.472±0.005	0.47±0.005	0.481±0.004	1.004±0.003	0.648±0.004	0.579±0.005
Sabinene	1137	971	0.394±0.003	0.357±0.005	0.261±0.006	0.308±0.005	0.333±0.006	0.337±0.005	0.973±0.006	0.557±0.010	0.469±0.005
Myrcene	1172	990	0.528±0.008	0.925±0.051	0.351±0.001	0.288±0.004	0.583±0.003	0.626±0.002	0.967±0.004	2.171±0.005	1.055±0.059
Limonene	1217	1026	2.566±0.009	2.254±0.005	1.872±0.006	2.181±0.010	2.278±0.011	3.694±0.006	5.01±0.009	8.056±0.009	2.968±0.007
1,8-Cineole	1230	1028	0.919±0.002	1.576±0.007	0.241±0.004	0.461±0.009	1.199±0.006	0.921±0.007	7.572±0.006	4.548±0.016	1.477±0.006
Menthone	1501	1150	13.377±0.068	10.058±0.014	10.078±0.069	10.265±0.022	11.824±0.152	6.166±0.027	3.778±0.010	15.071±0.529	1.916±0.016
Menthofuran	1517	1160	0.639±0.009	0.297±0.009	0.234±0.004	0.185±0.003	0.387±0.008	6.088±0.004	2.836±0.007	3.096±0.007	0.374±0.007
Isomenthone	1532	1160	1.057±0.007	1.597±0.009	2.414±0.011	1.748±0.062	1.809±0.007	1.765±0.014	2.72±0.008	0.712±0.011	2.678±0.022
Menthyl acetate	1590	1298	3.253±0.014	3.913±0.037	2.94±0.016	2.781±0.024	3.678±0.060	3.844±0.024	3.62±0.065	0.345±0.007	0.404±0.007
neo-menthol	1621	1161	2.829±0.009	2.871±0.006	2.905±0.016	2.98±0.0140	2.918±0.015	3.353±0.051	6.651±0.143	5.457±0.069	8.549±0.031
Menthol	1665	1176	51.076±0.720	61.016±0.514	72.772±0.552	71.569±1.128	61.389±0.403	60.104±0.763	25.566±0.100	44.444±0.682	20.603±0.133
Pulegone	1695	1236	5.088±0.010	4.879±0.010	0.448±0.016	2.142±0.062	3.816±0.082	9.424±0.063	27.557±0.609	3.626±0.043	46.247±0.765

RI: Retention index; †Supelcowax-10 column; ††BP-5 column.

Table 2
Analysis of variance (MSS) of nineteen traits in eight half-sib seed progeny of *Mentha piperita* L.

Sources	DF	Plant Height	Leaf Length	Leaf Width	L/S ratio	Oil content	Herb yield	α-Pinene	β-Pinene	Sabinene
Replication	2	8.441	0.107	0.013	0.005	0.002	0.270	0.002	0.008	0.016
Treatment	8	64.666**	0.6329**	0.3542**	0.0559**	0.0175**	24.4354**	0.0194*	0.0886**	0.1417**
Error	16	5.528	0.078	0.043	0.008	0.004	0.150	0.006	0.004	0.003
Myrcene	**Limonene**	**1,8-Cineole**	**Menthone**	**Menthofuran**	**Isomenthone**	**Menthyl acetate**	**neo-Menthol**	**Pulegone**	**Menthol**	
0.002	0.000	0.000	0.028	0.195	0.001	0.001	0.007	0.232	0.098	
0.973**	11.8583**	17.3766**	57.4073**	12.5087**	1.412**	5.9033**	13.3569**	706.9617**	1047.001**	
0.001	0.000	0.000	0.036	0.106	0.001	0.001	0.003	0.093	0.044	

low heritability exhibiting traits.The estimates of the combinational situation and correlation may often be deceptive due to mutual cancellation of component traits; therefore, it is essential to study the path analysis, associations and other selections parameters which will authenticate the causal relationship along with the degree of relationship (Vir and Gupta, 2001; Bhargava et al., 2003).

The genotypic correlations were higher than corresponding phenotypic correlations for most of the character pairs studied (Table 4), demonstrating the influence of changing environment condition on relationships with traits at genetic/phenotypic level (Singh and Khanna, 1993; Singh et al., 2003; Kumar et al., 2005). A similar observation has been obtained while studying the correlation coefficients among the menthofuran rich half-sib seed progenies of *M. piperita* L. (Kumar et al., 2014b). The correlation coefficients between genotypic and phenotypic level among 19 characters described a highly significant and positive association between β-pinene with sabinene and 1,8-cineole; α-pinene with β-pinene, sabinene, and 1,8-cineole, whereas negative and highly significant genotypic associations were observed between menthyl acetate and *neo*-menthol with pulegone; and menthone with menthofuran (Table 4). The plastidial biosynthesis of peppermint followed the methyl erythritol phosphate (MEP) pathway through the availability of C_5 isoprenoid precursors, namely *iso*-pentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) (Eisenreich et al., 1997). The different magnitude and orientation of the correlation of major chemical constituents can be explained based on their biosynthetic pathway (Fig. 2). The product compounds which depend on the same precursor molecule might show negative association as they compete for the same precursor.

3.3. Path coefficient analysis

Path coefficient analysis and partitions trait association into direct and indirect effects were evaluated for deriving a genotypic correlation between the essential oil quality characters with menthofuran (Table 4). The path coefficient is an efficient selection strategy applied to study the direct/indirect association in the variables, further permitting/validating the components that affect the correlation variable (Shipley, 1997; Scheiner et al., 2000). The path coefficient analyses for menthofuran content revealed that limonene expressed the positive maximum direct effect on menthofuran content (1.068) followed by menthyl acetate (0.602) and *neo*-menthol (0.467) (Fig. 3, Table 5). It suggests that the selection for limonene content would simultaneously enhance the menthofuran content. While on the other side, pulegone had the highest direct negative effect (-2.073) continued by menthone (-1.936), menthol (-0.643), and 1,8-cineole (-0.630) on menthofuran content. The menthone (-1.936) and menthol (-0.643) have negative direct effect and genotypic correlation with menthofuran in addition to negative indirect effect with leaf width, oil content, α-pinene,

Table 3
Estimates of the genetic parameter in eight half-sib progeny of *Mentha piperita* L.

Character	Range	Mean ± SE	Gen.var	Phe. Var	GCV	PCV	GA	HER(B)
Plant Height	56-68	60.88 ± 1.357	19.713	25.241	7.292	8.251	7.143	78.09
Leaf Length	3.93-5.40	4.80 ± 0.161	0.185	0.263	8.951	10.678	0.622	70.25
Leaf Width	2.23-3.23	285 ± 0.119	0.104	0.146	11.28	13.411	0.469	70.74
L/S ratio	01-1.40	1.15 ± 0.052	0.016	0.024	10.955	13.47	0.172	66.14
Oil content	0.23-0.48	0.34 ± 0.037	0.004	0.008	19.304	27.013	0.069	51.06
Herb yield kg/m^2	3.1-11.86	9.56 ± 0.223	8.095	8.245	29.729	30.003	5.755	98.18
α-Pinene	0.34-0.61	0.41 ± 0.045	0.004	0.011	16.135	24.999	0.057	41.65
β-Pinene	0.46-1.00	0.57 ± 0.037	0.028	0.032	29.256	31.373	0.3	86.95
Sabinene	0.26-0.97	0.44 ± 0.028	0.046	0.048	48.544	49.811	0.421	94.97
Myrcene	0.28-2.17	0.83 ± 0.013	0.324	0.325	68.371	68.431	1.171	99.82
Limonene	1.87-8.05	3.43 ± 0.004	3.953	3.953	57.945	57.946	4.095	99.99
1,8-Cineole	0.24-7.57	2.10 ± 0.004	5.792	5.792	114.515	114.516	4.957	99.99
Menthone	1.91-15.07	9.17 ± 0.109	19.124	19.16	47.686	47.731	8.996	99.69
Menthofuran	0.18-6.08	1.57 ± 0.187	4.134	4.24	129.424	131.069	4.084	97.5
Isomenthone	0.71-2.72	1.83 ± 0.012	0.471	0.471	37.414	37.433	1.412	99.89
Menthyl acetate	0.34-3.91	2.75 ± 0.020	1.967	1.969	50.946	50.962	2.887	99.93
neo-Menthol	2.82-8.54	4.27 ± 0.030	4.451	4.454	49.303	49.318	4.344	99.93
Menthol	20.60-72.77	52.06 ± 0.381	348.855	349.292	35.877	35.899	38.427	99.87
Pulegone	0.44-46.24	11.47 ± 0.175	235.623	235.715	133.831	133.857	31.608	99.96

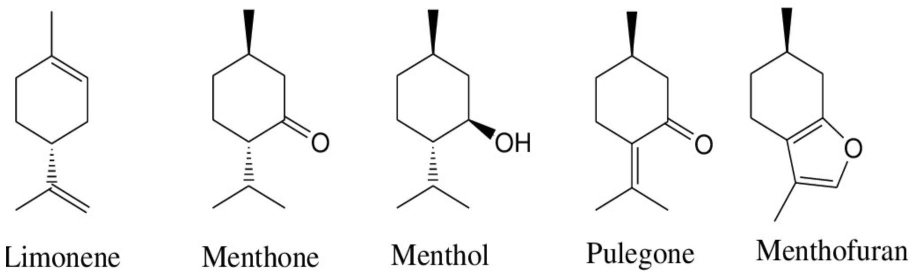

Fig. 2. Major constituents of the essential oils of peppermint (*Mentha piperita* L.) accessions.

sabinene, *neo*-menthol, menthol and plant height, leaf length, leaf width, oil content, herb yield, α-pinene, sabinene, myrcene, limonene, menthol, and *neo*-menthol. The limonene had a maximum direct positive effect (1.068) and displayed a negative indirect effect with chemical constituents (qualitative traits) involving 1,8-cineole (-0.454), menthyl acetate (-0.313), menthone (-0.301), pulegone (-0.199) and β-pinene (-0.135). Careful examination of quality traits with the negative direct effect is needed for the selection strategies in the improvement of menthofuran content in the essential oil. In the present investigation, the half-sib breeding approach was explored to elevate the quality, quantity, and productivity in the *M. piperita*. Previous studies demonstrated that genetic variability remains to hinder in the vegetatively propagated crops, further open-pollinated seed progenies (OPSP), hybridization and mutation breeding which could be considered as an approach for exploring variability among genotypes/varieties (Kumar et al., 2004; Patra and Kumar, 2005). The aforementioned authors reported that the essential oil content of OPSPs of variety Shivalik varies from 0.37 to 1.08. In the plant breeding strategies, the judicious selection is the primary objective practiced for higher yields and better quality attributes. Genotypic coefficient of variation (GCV), genetic advance, and broad-sense heritability for quantity and quality attributes are presented in (Table 3). The genotypic coefficient of variation (GCV) of pulegone has been as high as 133.83%; selecting such characters offers an excellent opportunity for choosing desired progenies of MPK-5. The genotypic coefficient of variation (GCV) determines the nature and amount of variations, but the level of heritability is not determined based on these estimates. According to Burton and de Vane (1953), the estimates of the genotypic coefficient of variation concerning heritability provide a piece of better knowledge on the amount of genetic advance than via phenotypic selection. Here, the herb yield is

Table 4

Genotypic (G) and Phenotypic (P) correlation among nineteen traits in eight half-sib seed progeny of *Mentha piperita* L.

Characters		Leaf length	Leaf width	L/S ratio	Oil content	Herb yield	α- Pinene	β-Pinene	Sabinene	Myrcene	Limonene	1,8-Cineole	Menthone	Menthofuran	Isomenthone	Menthyl acetate	*neo*-Menthol	Pulegone	Menthol
P. Height	P	0.135	-0.114	0.519	0.363	0.294	-0.302	-0.43	-0.448	-0.539*	-0.388	-0.501*	-0.246	0.299	0.194	-0.249	-0.115	0.456	0.184
	G	0.167	-0.239	0.546	0.573*	0.337	-0.405	-0.497*	-0.536*	-0.611**	-0.44	-0.568*	-0.278	0.338	0.221	-0.28	-0.129	0.518*	0.229
L. Length	P		0.637**	-0.041	0.189	0.628**	-0.129	-0.178	-0.014	-0.107	0.015	-0.019	0.087	-0.097	-0.082	-0.006	0.032	-0.081	0.223
	G		0.679**	-0.195	-0.029	0.780**	-0.164	-0.223	-0.144	-0.121	0.018	-0.225	0.1	-0.115	-0.969	-0.007	0.04	-0.1	0.247
L. Width	P			-0.142	-0.133	0.314	0.139	0.249	0.362	0.368	0.461	0.394	-0.056	-0.032	-0.109	0.303	0.22	-0.423	0.480*
	G			-0.39	-0.337	0.39	0.45	0.407	0.445	0.443	0.549*	0.468*	-0.07	-0.044	-0.128	0.359	0.259	-0.508*	0.555*
L/S ratio	P				0.600**	0.358	-0.073	-0.153	-0.154	0.096	0.241	-0.103	0.122	-0.109	-0.376	0.001	-0.184	0.26	0.142
	G				0.759**	0.468*	-0.095	-0.184	-0.21	0.118	0.296	-0.128	0.142	-0.145	-0.457	0.006	-0.226	0.312	0.193
Oil	P					0.333	-0.153	-0.234	-0.203	-0.274	-0.052	-0.177	0.399	-0.177	0.1	-0.426	-0.523*	0.533*	-0.091
	G					0.441	-0.15	-0.275	-0.343	-0.372	-0.073	-0.249	0.556*	-0.255	0.144	-0.590**	-0.732**	0.738**	-0.076
Herb yield	P						-0.13	-0.238	-0.163	-0.003	0.117	-0.232	0.101	-0.142	-0.470*	0.175	0.116	-0.102	0.05
	G						-0.242	-0.267	-0.18	-0.003	0.118	-0.234	0.102	-0.142	-0.476*	0.176	0.116	-0.101	0.059
α-Pinene	P							0.896**	0.761**	0.219	0.394	0.711**	-0.337	0.294	-0.026	0.449	0.357	-0.516*	0.281
	G							0.996**	0.988**	0.359	0.611**	0.989**	-0.528*	0.476*	-0.034	0.692**	0.557*	-0.516*	0.349
β-Pinene	P								0.938**	0.384	0.527*	0.917**	-0.385	0.33	-0.064	0.584*	0.458	-0.676**	0.272
	G								0.998**	0.414	0.565*	0.984**	-0.413	0.362	-0.067	0.628**	0.493*	-0.717**	0.267
Sabinene	P									0.438	0.581*	0.950**	-0.378	0.28	-0.106	0.633**	0.504*	-0.747**	0.282
	G									0.453	0.596**	0.974**	-0.391	0.289	-0.107	0.650**	0.518*	-0.764**	0.302
Myrcene	P										0.896**	0.587*	0.165	-0.381	-0.662**	0.526*	0.193	-0.519*	0.305
	G										0.897**	0.588*	0.165	-0.382	-0.663**	0.526*	0.192	-0.520*	0.311
Limonene	P											0.720**	0.155	-0.368	-0.519*	0.453	0.096	-0.463	0.558*
	G											0.720**	0.156	-0.368	-0.519*	0.454	0.096	-0.463	0.565*
1,8-Cineole	P												-0.217	0.135	-0.126	0.558*	0.349	-0.656**	0.344
	G												-0.217	0.135	-0.126	0.558*	0.349	-0.656**	0.349
Menthone	P													-0.850**	0.014	-0.645**	-0.842**	0.567*	-0.19
	G													-0.851**	0.014	-0.646**	-0.542*	0.567*	-0.192
Menthofuran	P														0.108	0.454	0.637**	-0.324	-0.135
	G														0.108	0.454	0.637**	-0.325	-0.134
Isomenthone	P															-0.664**	-0.414	0.481*	0.079
	G															-0.665**	-0.414	0.482*	0.081
Menthyl acetate	P																0.909**	-0.923**	0.113
	G																0.909**	-0.924**	0.114
neo-Menthol	P																	-0.876**	0.034
	G																	-0.877**	0.035
Pulegone	P																		-0.156
	G																		-0.155

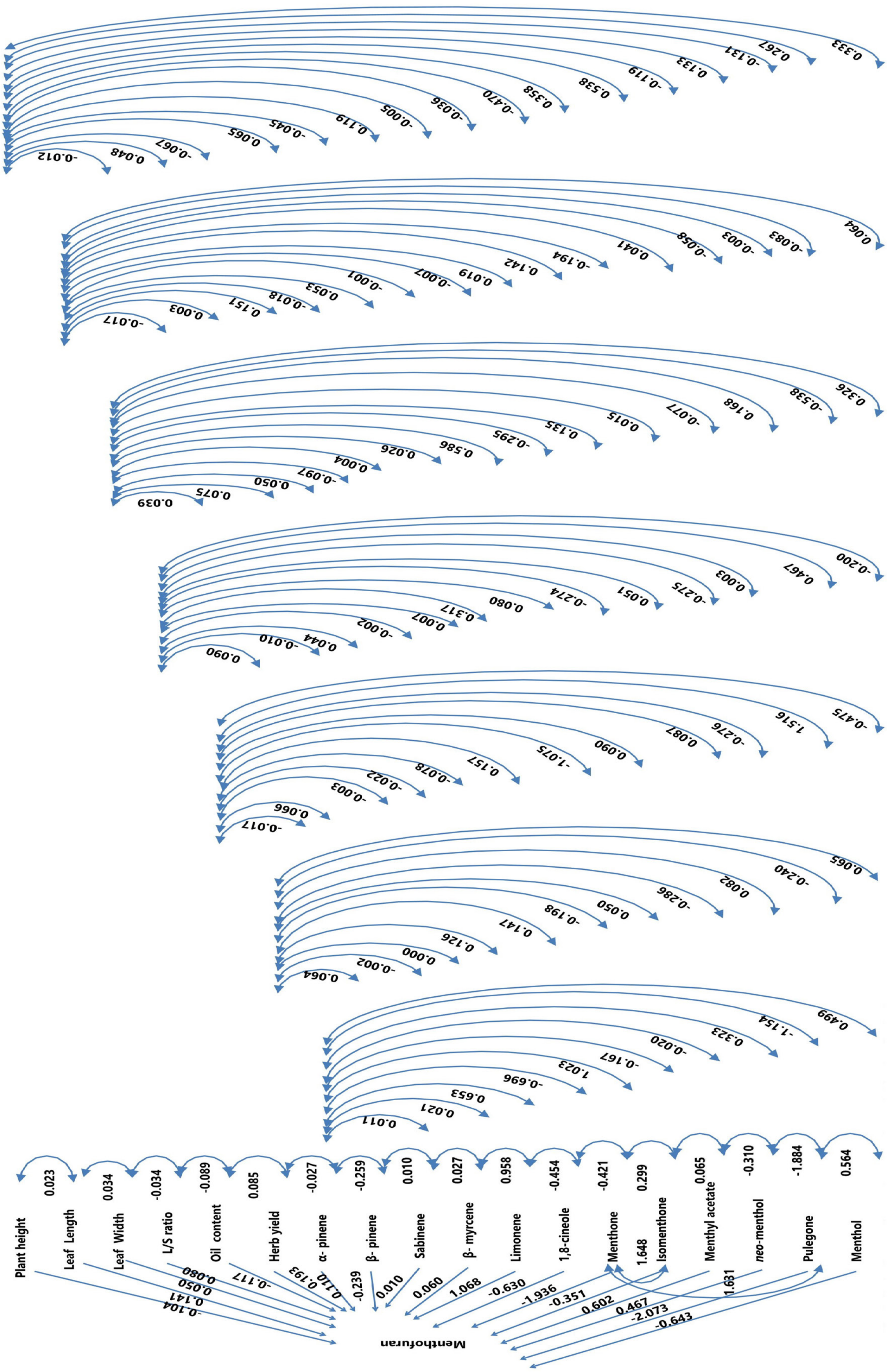

Fig. 3. Path diagram from nine accessions of *Mentha piperita* L.

Table 5
Path coefficient analysis of *Mentha piperita* L. for menthofuran content.

Character	Plant Height	Leaf Length	Leaf Width	L/S ratio	Oil content	Herb yield	α- Pinene	β- Pinene	Sabinene	Myrcene	Limonene	1,8-Cineole	Menthone	Iso mentyhone	Menthyl acetate	*neo*-Menthol	Pulegone	Menthol	rg menthofuran
Plant Height	-0.104	0.023	-0.012	0.048	-0.067	0.065	-0.045	0.119	-0.005	-0.036	-0.47	0.358	0.538	-0.119	0.133	-0.131	0.267	-0.333	0.229
Leaf Length	-0.017	0.141	0.034	-0.017	0.003	0.151	-0.018	0.053	-0.001	-0.007	0.019	0.142	-0.194	0.041	-0.058	-0.003	-0.083	0.064	0.247
Leaf Width	0.025	0.096	0.05	-0.034	0.039	0.075	0.05	-0.097	0.004	0.026	0.586	-0.295	0.135	0.015	-0.077	0.168	-0.538	0.326	0.555
L/S ratio	-0.057	-0.027	-0.019	0.088	-0.089	0.09	-0.01	0.044	-0.002	0.007	0.317	0.08	-0.274	0.051	-0.275	0.003	0.467	-0.2	0.193
Oil content	-0.06	-0.004	-0.017	0.067	-0.117	0.085	-0.017	0.066	-0.003	-0.022	-0.078	0.157	-1.075	0.09	0.087	-0.276	1.516	-0.475	-0.076
Herb yield	-0.035	0.11	0.02	0.041	-0.052	0.193	-0.027	0.064	-0.002	0	0.126	0.147	-0.198	0.05	-0.286	0.082	-0.24	0.065	0.059
α-Pinene	0.042	-0.023	0.022	-0.008	0.018	-0.047	0.11	-0.259	0.011	0.021	0.653	-0.696	1.023	-0.167	-0.02	0.323	-1.154	0.499	0.349
β-Pinene	0.052	-0.031	0.02	-0.016	0.032	-0.052	0.119	-0.239	0.01	0.025	0.604	-0.621	0.8	-0.127	-0.04	0.293	-1.022	0.461	0.267
Sabinene	0.056	-0.02	0.022	-0.018	0.04	-0.035	0.128	-0.244	0.01	0.027	0.637	-0.614	0.757	-0.101	-0.064	0.304	-1.074	0.491	0.302
Myrcene	0.064	-0.017	0.022	0.01	0.043	-0.001	0.04	-0.099	0.004	0.06	0.958	-0.37	-0.32	0.134	-0.399	0.246	-0.399	0.334	0.311
Limonene	0.046	0.003	0.027	0.026	0.009	0.023	0.067	-0.135	0.006	0.053	1.068	-0.454	-0.301	0.129	-0.313	0.212	-0.199	0.297	0.565
1,8-Cineole	0.059	-0.032	0.023	-0.011	0.029	-0.045	0.122	-0.236	0.01	0.035	0.769	-0.63	0.421	-0.047	-0.076	0.261	-0.724	0.422	0.349
Menthone	0.029	0.014	-0.003	0.012	-0.065	0.02	-0.058	0.099	-0.004	0.01	0.166	0.137	-1.936	0.299	0.008	-0.302	1.746	-0.364	-0.192
Isomenthone	-0.035	-0.016	-0.002	-0.013	0.03	-0.027	0.052	-0.087	0.003	-0.023	-0.393	-0.085	1.648	-0.351	0.065	0.212	-1.321	0.209	-0.134
Menthyl acetate	-0.023	-0.014	-0.006	-0.04	-0.017	-0.092	-0.004	0.016	-0.001	-0.04	-0.555	0.079	-0.026	-0.038	0.602	-0.31	0.858	-0.31	0.081
neo-Menthol	0.029	-0.001	0.018	0.001	0.069	0.034	0.076	-0.15	0.006	0.031	0.485	-0.352	1.25	-0.16	-0.4	0.467	-1.884	0.594	0.114
Pulegone	0.013	0.006	0.013	-0.02	0.085	0.022	0.061	-0.118	0.005	0.011	0.103	-0.22	1.631	-0.224	-0.249	0.425	-2.073	0.564	0.035
Menthol	-0.054	-0.014	-0.025	0.027	-0.086	-0.02	-0.086	0.171	-0.008	-0.031	-0.495	0.414	-1.098	0.114	0.29	-0.432	1.818	-0.643	-0.155

under additive genetic control through high heritability along with high genetic advance; a further selection of these important traits could be an effective strategy.

4. Concluding remarks

Considering together the genetic parameters, selection of pulegone, menthofuran, 1,8-cineole, and menthone could be used as promising criteria for selection in genetic improvement since these characters generally possess a high genotypic coefficient of variation, phenotypic coefficient of variation, heritability estimate, and genetic advance. Nevertheless, considering the correlations along with the path analysis for the selection of low menthofuran content (%) via indirect selection, the limonene content should be a reliable and significant parameter for the high yielding elite half-sib genotypes selection with desirable menthofuran content. As demonstrated by the results of the present study, a potent breeding technique like selection in half-sib seed progenies has been able to ensure variability and improvement in the studied quantitative and qualitative traits.

Conflict of interest

The authors declare that there is no conflict of interest.

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