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ORIGINAL RESEARCH ARTICLE

Estimation of intra-specific genetic variability and half-sib family selection using AMMI (Additive Main Effects and Multiplicative Interactions) model in menthol mint (*Mentha arvensis* L.)

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ABSTRACT

Menthol mint (Mentha arvensis L.) is an aromatic and medicinal herb that belongs to the family- Lamiaceae. Mint oil and menthol crystal are widely used in large amounts in cosmetics, perfumery, essential oil and pharmaceutical industries in India and abroad. In addition of being a popular flavoring agent for food, confectionery, and cigarettes, natural menthol has a profound cooling and soothing effect on the human skin and mucous membranes that makes it a useful ingredient in several pharmaceuticals and cosmetics preparations. Demand for natural mint oil is very high world wide. The genetic variability and essential oil yield instability are some of the common setbacks that plant breeders of menthol mint are facing towards developing widely adapted varieties with superior yield and quality of menthol oil. Hybridization between some of the Mentha species occurs frequently in nature. Based on the stability statistics, a number of mint varieties have been developed and released by CSIR-CIMAP, Lucknow (India) for their commercial farming. These include MAS-1, MAS-2, Himalaya, Kalka, Kushal, Saksham, Sambhav, Damroo, Kosi, CIM Saryu, CIM Kranti, etc.In addition, two local varieties Shivalik and Gold are also available for cultivation in India. Nevertheless, as per farmers demand, there is still scope for breeding high oil yielding varieties of menthol mint that are early maturing and have thin suckers. In order to fill this gap, the present investigation was carried out to determine the intra-specific genetic variability and estimation of stability and adaptability patterns of a set of seven half-sib selections from a seed progeny of menthol mint variety Kosi, The selections namely, Line -1, Line -2, Line -3, Line -5, Line -10, Line -11, Line -15 and two check varieties Kosi and CIM-Kranti were evaluated in multiyear/environments set up in

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India. An AMMI (Additive Main Effects and Multiplicative Interactions) model was applied that provides more authentic information on broader inferences on adaptability. Based on the AMMI model, line-15 showed the most extensive adaptability and proved to be the most stable line due to its ability to tolerate broad environmental conditions (temperature/or abiotic stress) in different years. This early maturing line 15 also registered 15% superiority in essential oil yield over variety Kosi and CIM-Kranti. Comparative performance data of these lines is presented and discussed in this communication.

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INTRODUCTION

Mentha is a genus of about 25 species of the family Lamiaceae. Amongst these, menthol mint (*Mentha arvensis* L.) is widely used in the food flavorings, pharmaceutical, and cosmetic industries. Besides being a popular flavoring agent in food, confectionery, and cigarettes, natural menthol also has a cooling and soothing effect on the skin and mucous membranes of the human body, making it a useful ingredient of several pharmaceuticals and cosmetics products. Worldwide, approximately 10,000 tonnes of natural menthol and 2,000 tonnes of synthetic menthol is used by the end user industries every year (Kang and Pham, 1991; Lal 2013). Until about 15 years ago, the bulk of the world's *Mentha arvensis* oil used to come from Brazil and China. But India subsequently overtook Brazil, and, today India is the top global producer and supplier of menthol oil in the trade (Lal 2013).

The basic information on intra specific genetic variability and wide adaptability/stability of commercially released varieties of *M. arvensis* is lacking. Among the various objectives of intra specific genetic variability and multiyear field testing for essential oil yield, the establishment of appropriate adaptation strategies in breeding programs and defining the domains for variety recommendations are important considerations. The adaptation strategy objectives focus on responses of a set of varieties help in obtaining indications that can predict future breeding material that may be produced from the available genetic base of which the tested varieties are assumed to be the representative samples. This strategy has worked very well in several aromatic and medicinal crops. (Annicchiarico, 2002; Gauch, 2007; Kang and Pham, 1991; Lal et al., 2017a, 2017b, 2017c, 2018a, 2018b, 2018c, 2018d, 2019). High yield stability usually refers to a genotype's ability to perform consistently at high or low yield levels, across a wide range of environments over years (Akcura et al., 2005; Annicchiarico, 2002; Eskridge, 1990; Finlay and Wilkinson, 1963). Several biometrical methods, including univariate and multivariate models have been developed to assess these stability parameters (Akcura et al., 2005). Among these, the most widely used ones are the regression coefficient (Finlay and Wilkinson, 1963), the environmental variance (Purchase, 1997), the Shukla's stability variance (Shukla, 1972) and Wrike's ecovalence (Wrike, 1962) models. More recently, the AMMI stability value (ASV) based on the AMMI (Additive Main Effects and Multiplicative Interactions) model's PCA1 and PCA2 (Principal Components Axis 1 and 2, respectively) scores for each cultivar/variety (Lal et al., 2017b). This AVS effectively measures the distance from the coordinate point to the origin in a two-dimensional scattergram of PCA 1 scores against PCA 2 scores (Lal et al., 2018b).

The practical interest of combining high levels of mean yield and yield stability has led to the development of the yield reliability concept (Eskridge, 1990; Lal et al., 2018a; Lal et al., 2017a) where a reliable essential oil yielding variety is characterized by the consistently high oil yield across the years/environments (Annicchiarico, 2002). The use of a yield reliability index further facilitates the variety selection or recommendation because the mean yield and the yield stability are combined into a unique measure of genotype merit (Annicchiarico, 2002).

Environment interactions (G × E) and yield stability studies are very limited in mint crop (Lal 2013). Moreover, no stability and reliability study on menthol mint varieties developed by CIMAP, Lucknow (India) has been performed so far. The objective of present study, therefore, was to evaluate the intra specific genetic variability along with estimation of stability and adaptability patterns of a set of seven half-sib selections for essential oil yield. The Seven selections, namely Line -1, Line -2, Line -3, Line -5, Line -10, Line -11, Line -15 along with two commercial varieties Kosi and CIM-Kranti as check, were evaluated in multi-years/ environments trials for determining their stability and reliability for making cultivar recommendations for commercial farming in India.

MATERIALS AND METHODS

From twenty-five seed progenies/half-sib progenies of the variety Kosi that were evaluated for herb/plant (g) and oil content (%) , twelve elite half-sib lines were selected in the year 2010-2011.These lines were designated as : Line-1, Line-2, Line-3, Line-4, Line-5, Line-6, Line-7, Line-8, Line-9, Line-10, Line-11, Line-15. These twelve elite half-sib lines, along with two best check varieties Kosi and CIM–Kranti were subsequently evaluated in Initial Evaluation Trial (RBD, 3 replications; plot size = 1.5m^2) in the year 2011-2012 at the Research Farm of CSIR- Central Institute of Medicinal and Aromatic Plants, Lucknow, (U.P.). The evaluation data is summarized in Tables 1 and 2. The seven best performimg half-sib lines for the essential oil yield of good quality, namely Line -1, 2, 3, 5, 10, 11, 15 and two check varieties Kosi and CIM-Kranti were then evaluated in the advance evaluation trials at the Research Farm of CSIR-CIMAP in the three consecutive growing seasons of 2013-14, 2014-15, and 2015-16 in a randomized complete block design with three replications and the plot size = 1.6m^2. The suckers in pieces (size = 5 cm) were planted in 40 cm rows to rows and 5 cm pieces to pieces spacing. The plants received normal intercultural operations, irrigation, and fertilizer applications (120 kg N, 80 kg P_2O_5, and 60 kg K_2O per hectare). Minimum and maximum night and day temperatures ranged from 8° – 11 °C to 15° - 17 °C, respectively during suckers planting time, and from 25° – 30 °C to 35° – 40 °C, respectively, at crop maturity. Average rainfall during the growth season was 5 to 7 mm according to weather data of the Metrological Laboratory of CSIR-CIMAP, Lucknow, India.

The essential oil was extracted from the fresh herbs by hydro-distillation using a Clevenger apparatus (Clevenger, 1928). The oil composition was analyzed by GC using Varian CX – 3400 instruments employing 30 m × 0.25 m SUPELCOWAX – 10 capillary columns with temperature program from 50° - 220 ° C @ 6°/m, Initial and final temperature holds were of 2 and 5 minutes, respectively. H_2 was used as carrier gas at 1 ml/minute. Data were processed on the AIMIL chromatography data system. Identification of constitutes was based on retention time of reference compounds.

Statistical analysis

In the IET, data were recorded for four most important economic traits: plant height (cm), No.of branches/plant, plant spread (cm^2), and fresh herb yield (g/plot) after 82 days. Another parameter i.e. essential oil content (%) was measured after 76, 82 and in 91 days and converted in to essential oil (g) yield/plot (Table 1,2). The best selected seven half-sib lines for the essential oil yield of good quality, namely Line -1, 2, 3, 5, 10, 11, 15, and two check varieties Kosi and CIM-Kranti were then evaluated in the advance evaluation trials. The statistical analyses were performed for only essential oil yield/ plot (g), using MATMODEL VERSION 3.0 program mode: fitting AMMI Model software (Gauch, 2007; Kang and Pham, 1991). That gave outputs of AMMI and joint regression models including analysis of variance, regression coefficients, as well as genotypes and environment mean and stability.

Table 1: Analysis of variance (ANOVA) of the initial evaluation trial (RBD; Rep. 3, plot size= 1.50 m²) of fourteen genotypes of menthol mint

Sources of variation	d.f.	Mean sum of squares (m.s.s.)							
		Plant Height (cm)	Branches/ plant	(Spread cm²)	Fresh herb yield (Kg/plot)	Essential oil content (%) in 76 days	Essential oil content (%) in 82 days	Essential oil content (%) in 91 days	Essential oil yield (g/plot)
Replications	2	266.66	9.74	14480.00	0.107	0.006	0.0004	0.008	0.299
Genotypes	13	155.85**	10.53**	703989.00**	2.017**	0.023**	0.0733**	0.164**	4.678**
Error	26	32.69	3.35	27110.77	0.026	0.006	0.0008	0.006	0.069
Total	41	-	-	-	-	-	-	-	-

**P<0.01

Table 2: Mean performance of half sib lines in Initial Evaluation Trial (RBD, 3 Rep., plot size=1.5 m² in methol mint

Entries/ genotypes	GEN. codes	Origin	Characters							
			Plant Height (cm)	Branches/ plant	(Spread cm²)	Fresh herb yield (Kg/Plot) in 82 days	Essential oil content (%) in 76 days	Essential oil content (%) in 82 days	Essential oil content (%) in 91 days	Essential oil yield (g/plot)
Line-1	WELL	H.S.S.	70.00	8.33	2500.00	5.10	1.20	0.89	1.30	4.26
Line-2	RKLL	H.S.S.	75.00	8.33	1043.33	3.60	1.12	1.03	1.20	3.72
Line-3	CHIP	H.S.S.	80.00	7.00	1446.67	5.13	1.10	1.35	1.53	6.93
Line-4	CIMA	H.S.S.	76.33	9.67	1233.33	4.50	1.03	1.18	0.93	5.32
Line-15	WILK	H.S.S.	79.00	10.33	1406.33	4.60	1.25	1.17	1.23	5.37
Line-6	CIMP	H.S.S.	73.67	11.00	1866.67	3.33	1.20	1.32	1.22	4.39
Line-7	PANK	H.S.S.	73.00	9.00	1800.00	4.40	1.13	1.35	1.13	5.94
Line-8	ZENU	H.S.S.	71.67	10.67	2283.33	4.40	1.20	1.45	1.77	6.38
Line-9	CHHA	H.S.S.	79.33	12.33	1666.67	4.50	1.22	1.50	0.91	6.75
Line-10	S200	H.S.S.	76.33	13.33	1733.33	3.83	1.22	1.30	1.20	4.98
Line-11	EVAN	H.S.S.	67.33	10.67	1575.00	3.73	0.95	1.20	0.97	4.48
Line-5	HODG	H.S.S.	63.00	12.00	2266.67	3.57	1.02	1.22	1.23	4.34
Kosi	CORS	CSIR CIMAP	63.33	12.33	2416.67	2.20	1.13	1.23	1.27	2.71
CIM- Kranti	TPLL	CSIR CIMAP	55.33	8.33	2506.67	3.03	1.13	1.18	1.10	3.60
CD (5%)			9.62	3.08	276.94	0.27	0.13	0.05	0.13	0.44
CD (1%)			12.98	4.16	373.74	0.36	0.17	0.06	0.18	0.59
CV (%)			7.98	17.89	8.95	2.28	6.59	2.28	6.29	5.34
SEM			3.30	1.06	95.06	0.09	0.04	0.016	0.04	0.15
SED			4.67	1.50	134.44	0.13	0.06	0.023	0.06	0.22

GEN= Genotypes; H.S.S. =Half Sib progeny Selection, CD= Critical difference; CV= Coefficient of variation; SEM= Standard error means; SED= Standard error difference

RESULTS AND DISCUSSION

The mean essential oil yield/plot (g) of the nine lines of menthol mint (*Mentha arvensis*) across three consecutive years ranged from 1.22 to 2.64/ plot (g). The differences in the rank of lines in the different years/environments indicated the presence of G × E interactions (Table 3). After critical perusal of data (Tables 4-12), some important points came out. For examples AMMI F and AMMI I picked the same winners in environment/ year 2nd or 66.67 % but they chose the different winner in the environ/year Ist or 33.33 %. AMMI F was ranking more to be trusted, the average loss from selecting AMMI I winners should be 0.21 or 10.93 % of the grand mean. AMMI I was ranking more to be trusted, the average gain from picking AMMI I winners would be 0.066 or 3.406% of the grand mean.

Table 3: ANOVA for model AMMI 1 for menthol mint lines

Source	d.f.	SS	MS	Probability
Treatments	26	31.57	1.21	0.0000144***
Genotypes (G)	8	21.57	2.70	0.0000002***
Environments/years (E)	2	2.24	1.12	0.0356029***
G × E	16	7.76	0.49	0.1204125*
IPCA 1	9	5.53	0.61	0.0641240
Residual	7	2.23	0.32	0.4351384
Error	54	17.03	0.31	
Total	80	48.61	0.61	

Grand Mean = 1.95 essential oil yield g/plant; Large residuals exceeding this by a factor of 1.960, 2.576, or 3.291 are marked with *, **, or *** respectively. Assuming normality, 5%, 1%, and 0.1% of the residuals exceed these limits.

It is imperative to point out here that the largest AMMI I gain of 0.199 or 10.217% of the grand mean occurs in the year 2nd, where AMMI F picks line/ genotype Line-5, but AMMI I chooses variety genotype Line-15 instead. The values presented in Table 9 may be useful for assessing the significance of mean separations in the above listing of data estimates. The average value of the maximum of N normal realizations is the first order statistic. This value times the standard error of the treatment means it provides a typical indication of the largest upward bias present in the data, which is most likely to affect the highest means (Table 9). The noise SS in the interaction can be estimated as G × E degree of freedom times Error MS. Accordingly, the G × E SS contains approximately: G × E pattern 2.72 or 34.97%; G × E noise 5.05 or 65.03%; G × E total 7.76. Ordinarily, there is considerable selectivity, with pattern recovered mostly in the early IPCA axis, and noise recovered mainly in the late IPCA axis. Consequently, these estimates provide a rough guide for model diagnosis by keeping the early axis that is mostly pattern and relegating the others to a discarded residual (Table 6-8, 10-11; Fig. 1-2).

Notwithstanding, the selection of varieties for yield and stability and genotype × environment interaction continues to be a challenge for plant breeders, geneticists, and other researchers who study varietal performance in field evaluation trials over the years or across diverse environments. AMMI model for genotype × environment interaction can improve the efficiency of related plant breeder's progress towards the selection of well adopted and stable varieties in mint crop also. The methods of

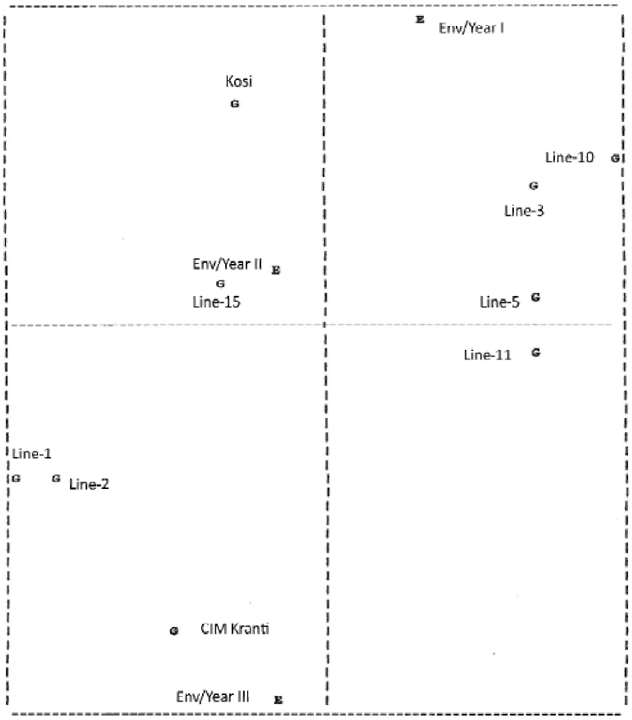

Figure 1: Matmodel version 3.0 Mega-environments for AMMI 1 Model, cultivars, switch points, including hypothetical winners in menthol mint genotypes

Genotypes	Mean	AMMI 1	Interval	Maximum	Count	Histogram
Line-11	2.448	0.081	1	0.028	6	***
Line-15	2.640	0.398	2	0.055	5	**
Line-3	2.441	-0.050	3	0.083	5	**
Line-5	2.450	0.337	4	0.110	2	*****************
Line-10	1.747	0.544	5	0.138	2	*****************
Kosi	1.314	-0.357	6	0.165	1	*********
Line-1	1.695	0.116	7	0.193	1	*********
Line-2	1.585	-0.720	8	0.220	2	*****************
CIM Kranti	1.216	-0.349	9	0.248	0	
ENV I	2.184	0.741	10	0.275	0	
ENV II	1.825	0.146	11	0.303	1	*********
ENV III	1.837	0.887	12	0.330	0	
			13	0.358	1	*********
			14	0.385	0	
			15	0.413	0	
			16	0.440	0	
			17	0.468	0	
			18	0.495	0	
			19	0.523	0	
			20	0.550	1	*********

Figure 2: AMMI1 model for data with nine genotypes, three years, three replications and a histogram for the AMMI1 residuals in the menthol mint lines

Table 4: Genotypes, codes, means, environmental means, environmental IPCA Axis 1 Scores for model AMMI I in menthol mint

	Genotypes names	GEN codes	Origin	Mean	Count	Index	Genotypes names	GEN codes	Mean	Count
	Line-11	EVAN	H.S.S.	2.45	3	2	Line-15	WILK	2.64	3
	Line-15	WILK	H.S.S.	2.64	3	4	Line-5	HODG	2.45	3
	Line-3	CHIP	H.S.S.	2.44	3	1	Line-11	EVAN	2.45	3
	Line-5	HODG	H.S.S.	2.45	3	3	Line-3	CHIP	2.44	3
	Line-10	S200	H.S.S.	1.75	3	5	Line-10	S200	1.75	3
	Kosi	CORS	CSIR CIMAP	1.31	3	7	Line-1	WELL	1.70	3
	Line-1	WELL	H.S.S.	1.70	3	8	Line-2	RKLL	1.58	3
	Line-2	RKLL	H.S.S.	1.58	3	6	Kosi	CORS	1.31	3
	CIM-Kranti	TPLL	CSIR CIMAP	1.22	3	9	CIM-Kranti	TPLL	1.22	3
ENV.	years	Score	Means	Count	Index	Score	years	Index		
1. ENV. I	2013-14	0.74	2.18	9	1	0.74	2013-14	1	2.18	9
3. ENV. III	2015-16	0.15	1.83	9	2	0.14	2015-16	3	1.84	9
2. ENV. II	2014-15	-0.89	1.84	9	3	-0.88	2014-15	2	1.83	9

Grand mean = 1.948 essential oil yield g/plant; GEN= Genotypes; H.S.S. =Half Sib progeny Selection

Table 5: Genotype IPCA Axis 1 Score for Model AMMI I in menthol mint lines

Genotypes name	GEN codes	Score	Index	Genotypes name	GEN codes	Score
Line-11	EVAN	0.080556	5	Line-10	S200	0.543794
Line-15	WILK	0.398244	2	Line-15	WILK	0.398244
Line-3	CHIP	-0.04966	4	Line-5	HODG	0.336936
Line-5	HODG	0.336936	7	Line-1	WELL	0.116198
Line-10	S200	0.543794	1	Line-11	EVAN	0.080556
Kosi	CORS	-0.35665	3	Line-3	CHIP	-0.04966
Line-1	WELL	0.116198	9	CIM-Kranti	TPLL	-0.34933
Line-2	RKLL	-0.72008	6	Kosi	CORS	-0.35665
CIM-Kranti	TPLL	-0.34933	8	Line-2	RKLL	-0.72008

Table 6: Genotypes/varieties mean for Environment/Year I in menthol mint genotypes

Genotypes	Codes of genotypes	Count	Data	AMMI 1	Residual	Rank	Index	Genotype names	Data	Index	Genotype names	AMMI 1
Line-11	EVAN	3	2.61	2.74	-0.14	1	2	WILK	3.52	2	WILK	3.17
Line-15	WILK	3	3.52	3.17	0.35*	2	4	HODG	2.75	4	HODG	2.93
Line-3	CHIP	3	2.69	2.64	0.05	3	3	CHIP	2.69	1	EVAN	2.74
Line-5	HODG	3	2.75	2.93	-0.18	4	1	EVAN	2.61	3	CHIP	2.64
Line-10	S200	3	2.34	2.39	-0.04	5	5	S200	2.34	5	S200	2.39
Kosi	CORS	3	1.21	1.29	-0.08	6	7	WELL	1.98	7	WELL	2.02
Line-1	WELL	3	1.98	2.02	-0.04	7	8	RKLL	1.38	8	RKLL	1.29
Line-2	RKLL	3	1.38	1.29	0.09	8	6	CORS	1.21	6	CORS	1.28
CIM-Kranti	TPLL	3	1.18	1.19	-0.01	9	9	TPLL	1.18	9	TPLL	1.19

Environments I and Mean 2.184, AMMI gain 0.000001; In essential oil yield g/plant the root mean square residual is 0.166. Large residuals exceeding this by a factor of 1.960, 2.576, or 3.291 are marked with *, **, or *** respectively. Assuming normality, 5%, 1%, and 0.1% of the residuals exceed these limits

Table 7: Genotypes/varieties mean for Environment/Year II in menthol mint lines

Genotypes	Codes of genotypes	Count	Data	AMMI 1	Residual	Rank	Index	Genotype names	Data	Index	Genotype names	AMMI 1
Line-11	EVAN	3	2.55	2.34	0.22	1	4	HODG	2.66	2	WILK	2.57
Line-15	WILK	3	2.02	2.57	-0.55***	2	1	EVAN	2.55	4	HODG	2.38
Line-3	CHIP	3	2.24	2.31	-0.07	3	3	CHIP	2.24	1	EVAN	2.34
Line-5	HODG	3	2.66	2.38	0.29	4	2	WILK	2.02	3	CHIP	2.31
Line-10	S200	3	1.77	1.70	0.07	5	5	S200	1.77	5	S200	1.70
Kosi	CORS	3	1.26	1.14	0.12	6	7	WELL	1.65	7	WELL	1.59
Line-1	WELL	3	1.65	1.59	0.06	7	6	CORS	1.26	8	RKLL	1.36
Line-2	RKLL	3	1.21	1.36	-0.15	8	8	RKLL	1.21	6	CORS	1.14
CIM-Kranti	TPLL	3	1.06	1.04	0.01	9	9	TPLL	1.06	9	TPLL	1.04

Environments II and Mean 1.825, AMMI gain 0.199; In essential oil yield g/plant the root mean square residual is 0.166. Large residuals exceeding this by a factor of 1.960, 2.576, or 3.291 are marked with *, **, or *** respectively. Assuming normality, 5%, 1%, and 0.1% of the residuals exceed these limits

Table 8: Genotypes/varieties mean for Environment/Year III in menthol mint lines

Genotypes	Codes of genotypes	Count	Data	AMMI 1	Residual	Rank	Index	Genotype names	Data	Index	Genotype names	AMMI 1
Line-11	EVAN	3	2.19	2.26	-0.08	1	3	CHIP	2.40	3	CHIP	2.37
Line-15	WILK	3	2.38	2.18	0.20	2	2	WILK	2.38	1	EVAN	2.26
Line-3	CHIP	3	2.40	2.37	0.03	3	1	EVAN	2.19	2	WILK	2.18
Line-5	HODG	3	1.93	2.04	-0.11	4	8	RKLL	2.17	8	RKLL	2.11
Line-10	S200	3	1.13	1.15	-0.02	5	4	HODG	1.93	4	HODG	2.04
Kosi	CORS	3	1.47	1.52	-0.05	6	6	CORS	1.47	6	CORS	1.52
Line-1	WELL	3	1.46	1.48	-0.02	7	7	WELL	1.46	7	WELL	1.48
Line-2	RKLL	3	2.17	2.11	0.05	8	9	TPLL	1.41	9	TPLL	1.41
CIM-Kranti	TPLL	3	1.41	1.41	-0.01	9	5	S200	1.13	5	S200	1.15

Environments/Years III and Mean 1.837, AMMI 1 Gain 0.000001

Table 9: Scope, count first order, typical bias values, ENV PCA Scores, ENV Linear Regressions and ANOVA for Model PCA. in mint lines

Scope	Count	First order	Typical bias	ENV/Years names	PCA 1	PCA 2	PCA 3	Mean	Slope	R-squared
Genotype within one environment/year	9	1.485	0.482	ENV. I (years 2013-14)	1.357	-0.568	-0.297	2.184	0.390	0.388
Environment/year within one genotypes	3	0.846	0.274	ENV. II (years 2014-15)	0.866	0.390	0.773	1.825	-0.031	0.004
All treatments	27	1.998	0.648	ENV.III (years 2015-16)	0.496	0.872	-0.538	1.837	-0.359	0.273

Analysis of variance (ANOVA) for Model Principal Component Analysis (PCA) and Linear Regressions.

Source	df	SS	MS	Probability	Source	df	SS	MS	Probability
TRT	26	31.572	1.214	0.0000144 ***	TRT	26	31.57	1.21	0.0000144 ***
PCA 1	11	24.145	2.195	0.0000004 ***	GEN	8	21.57	2.70	0.0000002 ***
PCA 2	9	4.574	0.508	0.1354647	ENV	2	2.24	1.12	0.0356029 *
PCA 3	7	2.853	0.408	0.2718316	G x E	16	7.76	0.49	0.1204125
rror	54	17.034	0.315		Joint Regr	1	1.60	1.60	0.0286296 *
Values for assessing the significance of mean separations in the data estimates				**Data estimates % of GM**	GEN Regrs	7	2.54	0.36	0.3454035
Unweighted grand mean without imputed data				1.948	ENV Regrs	1	0.44	0.44	0.2452707
With 54 d.f. the root error mean square				0.562	Residual	7	3.19	0.46	0.2075392
With 3 replications the standard of treatment means				0.324	Error	54	17.03	0.32	
Coefficient of variation of treatment means				16.642% of GM	Total	80	48.61	0.61	
S.E. of difference between two treatment means				0.549					
With 54 d.f. $t_{0.05}$ 0f 2.005 giving $LSD_{0.05}$				0.919					

The grand mean 1.948 is subtracted from the data before applying PCA; grand mean = 1.948 essential oil yield g/plant; Joint regression K 0.1635379D+01; In essential oil yield g/plant the root mean square residual is 0.166. Large residuals exceeding this by a factor of 1.960, 2.576, or 3.291 are marked with *, **, or *** respectively. Assuming normality, 5%, 1%, and 0.1% of the residuals exceed these limits

partitioning G × E interaction into components including regression model the attributable to each variety measure the contribution of each array to G × E interaction as well as expressed mint crop varieties (Table 10-12 Fig. 1-2). Although, a universally acceptable selection criterion for mint or other crops that considers genotype × environment interaction does not exist. Whenever an interaction is significant, the use of main effects, overall genotypes mean across years/ environments, is questionable. The stability of performance in yield trails over the years/ environments should be considered an important aspect of all breeding or variety testing program. Both stability and yield (or any other trait) must be considered simultaneously, to make the selection of varieties more precise and reliable. In mint crop varieties also the integration of stability of performance with yield through suitable measures like AMMI Model reduced the effects of genotype × environment interaction. It helped in selecting suitable varieties of mint is a more refined manner (Table 3-12). Our findings are also in agreement with other research workers in the mint and other crops (Lal, 2007; Lal, 2013; Lal et al., 2000; Leeuvner, 2005; Purchase, 1997; Wrike, 1962).

Nevertheless, based on the stability statistics, the different menthol mint lines can be classified as stable lines. The multivariate approach AMMI model (more authentic approach than others) can provide broader inferences on adaptability. In this study, attempts have been made to compare nine different lines of menthol mints using the AMMI model and with which to select the stable menthol varieties over the years/environments. There are remarkable achievements and inconsistencies. The multivariate approach, the AMMI model, is found suitable for partitioning the Genotype x Environment into the causes of variation. As a result, one of the inference is that line-15 is not only most stable for the essential oil yield but has an excellent level of quality performance as well as stable in the pilot-scale trial also (Table 13; Fig. 3-5). In the pilot-scale trial (2016-17), the line-15 produces 140 kg/ha essential oil yield of good quality with 14.25% improvement against best check variety CIM–Kranti 120kg/ha essential oil yield only (Table 13; Fig.3-5). This line could be recommended for extensive cultivation in India.

CONCLUSION

Based on the AMMI model, line-15 showed the most comprehensive adaptability (most stable line) due to its ability to tolerate broad environmental conditions, temperature/or abiotic stress in different years/environments. Therefore, this line could be recommended for extensive cultivation in mint growing areas of the country.

Table 10: Expected Values for PCA Models in menthol mint lines

ENV	S. No.	Genotypes	Genotypes codes	PCA 1	PCA 2	PCA 3
1	1	Line-11	EVAN	2.681	2.649	2.606
1	2	Line-15	WILK	3.101	3.326	3.520
1	3	Line-3	CHIP	2.653	2.613	2.685
1	4	Line-5	HODG	2.763	2.850	2.752
1	5	Line-10	S200	1.936	2.400	2.343
1	6	Kosi	CORS	1.070	1.190	1.206
1	7	Line-1	WELL	1.727	1.984	1.976
1	8	Line-2	RKLL	1.325	1.222	1.380
1	9	CIM-Kranti	TPLL	0.954	1.131	1.183
2	1	Line-11	EVAN	2.416	2.438	2.551
2	2	Line-15	WILK	2.684	2.530	2.024
2	3	Line-3	CHIP	2.398	2.426	2.237
2	4	Line-5	HODG	2.468	2.408	2.663
2	5	Line-10	S200	1.940	1.622	1.770
2	6	Kosi	CORS	1.388	1.306	1.263
2	7	Line-1	WELL	1.807	1.631	1.653
2	8	Line-2	RKLL	1.550	1.621	1.207
2	9	CIM-Kranti	TPLL	1.314	1.191	1.056
3	1	Line-11	EVAN	2.216	2.265	2.186
3	2	Line-15	WILK	2.370	2.024	2.376
3	3	Line-3	CHIP	2.206	2.268	2.400
3	4	Line-5	HODG	2.246	2.112	1.934
3	5	Line-10	S200	1.944	1.231	1.129
3	6	Kosi	CORS	1.627	1.444	1.474
3	7	Line-1	WELL	1.868	1.473	1.457
3	8	Line-2	RKLL	1.720	1.878	2.166
3	9	CIM-Kranti	TPLL	1.585	1.314	1.409
			Genotype PCA Scores			
	1	Line-11	EVAN	0.540	0.056	0.146
	2	Line-15	WILK	0.849	-0.396	-0.654
	3	Line-3	CHIP	0.519	0.071	-0.244
	4	Line-5	HODG	0.600	-0.154	0.330
	5	Line-10	S200	-0.009	-0.817	0.191
	6	Kosi	CORS	-0.647	-0.210	-0.055
	7	Line-1	WELL	-0.163	-0.453	0.029
	8	Line-2	RKLL	-0.460	0.181	-0.535
	9	CIM-Kranti	TPLL	-0.733	-0.311	-0.177

Grand Mean =1.948 essential oil yield g/plant; the grand mean is subtracted from the data before applying PCA

Table 11: Expected Values for Regression Models in menthol mint lines

ENV	GEN	Genotypes	Genotypes codes	Additive	Joint Regr	GEN Regrs	ENV Regrs	Both Regrs
1	1	Line-11	EVAN	2.68	2.87	2.60	2.88	2.60
1	2	Line-15	WILK	2.88	3.14	3.52	3.15	3.53
1	3	Line-3	CHIP	2.68	2.86	2.69	2.87	2.69
1	4	Line-5	HODG	2.69	2.88	2.74	2.88	2.74
1	5	Line-10	S200	1.98	1.90	2.33	1.90	2.33
1	6	Kosi	CORS	1.55	1.31	1.21	1.30	1.21
1	7	Line-1	WELL	1.93	1.83	1.97	1.83	1.97
1	8	Line-2	RKLL	1.82	1.68	1.40	1.68	1.39
1	9	CIM-Kranti	TPLL	1.45	1.17	1.19	1.17	1.19
2	1	Line-11	EVAN	2.32	2.22	2.37	2.31	2.45
2	2	Line-15	WILK	2.52	2.38	2.18	2.50	2.29
2	3	Line-3	CHIP	2.32	2.22	2.31	2.30	2.40
2	4	Line-5	HODG	2.33	2.23	2.30	2.31	2.38
2	5	Line-10	S200	1.62	1.66	1.44	1.63	1.41
2	6	Kosi	CORS	1.19	1.32	1.37	1.21	1.26
2	7	Line-1	WELL	1.57	1.62	1.55	1.58	1.51
2	8	Line-2	RKLL	1.46	1.53	1.68	1.47	1.62
2	9	CIM-Kranti	TPLL	1.09	1.24	1.23	1.12	1.10
3	1	Line-11	EVAN	2.34	2.24	2.38	2.16	2.29
3	2	Line-15	WILK	2.53	2.40	2.22	2.28	2.10
3	3	Line-3	CHIP	2.33	2.24	2.32	2.15	2.24
3	4	Line-5	HODG	2.34	2.25	2.31	2.16	2.22
3	5	Line-10	S200	1.64	1.67	1.47	1.71	1.51
3	6	Kosi	CORS	1.20	1.32	1.36	1.43	1.48
3	7	Line-1	WELL	1.58	1.63	1.56	1.67	1.61
3	8	Line-2	RKLL	1.47	1.54	1.67	1.60	1.74
3	9	CIM-Kranti	TPLL	1.10	1.24	1.23	1.37	1.36

Table 12: Mean performance of menthol mint varieties essential oil and quality analysis over three years in advance evaluation trial (RBD, 3 Rep. plot size=1.6m^2) in the menthol mint

Genotypes/ lines	Genotype codes	Oil yield (g/plot)	Essential oil compositions (Content in %)						
			Menthol	Menthone	Iso-menthone	Neo-menthol	Menthyl acetate	Pulegone	Limonene
Line-11	EVAN	2.45	74.66	4.88	3.50	2.50	1.15	0.15	2.48
Line-15	WILK	2.64	78.15	3.70	2.50	2.35	4.50	0.17	0.55
Line-3	CHIP	2.44	75.81	7.75	3.14	1.98	1.20	0.10	1.52
Line-5	HODG	2.45	67.50	9.50	3.35	2.50	2.70	1.50	3.45
Line-10	S200	1.75	77.10	9.80	1.89	2.33	0.50	1.45	4.35
Kosi	CORS	1.31	71.45	7.95	3.30	1.85	3.50	1.60	2.50
Line-1	WELL	1.70	75.25	6.52	3.65	2.55	3.33	0.50	0.85
Line-2	RKLL	1.58	73.30	8.50	3.00	2.88	3.89	0.32	2.50
CIM-Kranti	TPLL	1.22	77.65	10.50	3.35	2.40	0.90	0.90	2.10

Table 13: Performance of elites lines in the Pilot Scale Trial (PST) plot size=30m^2 in the methol mint

Entries/lines	Fresh herb/plot (kg)			Essential oil content (%)			Essential oil yield/plot (ml)			Fresh herb (ql/ha)	Essential oil yield/ha (kg)	SI (%) for Essential oil	Menthol (%)
	Ist Cut.	2nd cut.	Total (kg)	Ist Cut.	2nd cut.	$\bar{x}$	Ist Cut.	2nd cut.	Total yield				
Line-3	25.00	21.60	46.66	0.68	0.93	0.81	170	200	370	155.53	123.33	2.70	75.15
Line-11	20.75	31.50	52.25	0.63	0.73	0.68	130	230	360	174.17	120.00	0.00	74.30
Line-15	25.00	32.60	57.60	0.68	0.77	0.73	170	250	420	192.00	140.00	14.25	78.50
CIM Kranti	38.00	28.35	66.35	0.50	0.59	0.55	190	170	360	221.17	120.00	-	77.15

x = Mean; Bulk distillation; SI= Superiority improvement over check (%)

Figure 3: Field and Individual plant views of elite line Line-15

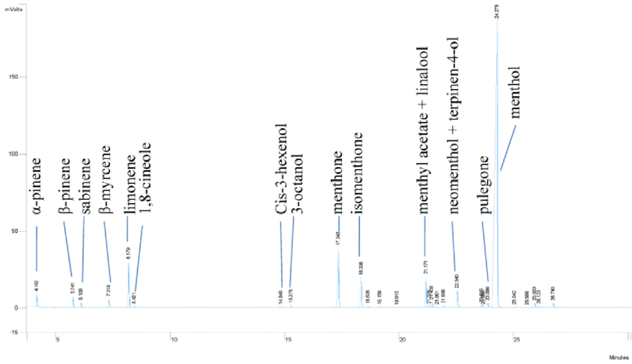

Figure 4: Chromatogram of essential oil of elite Line-15 of menthol mint

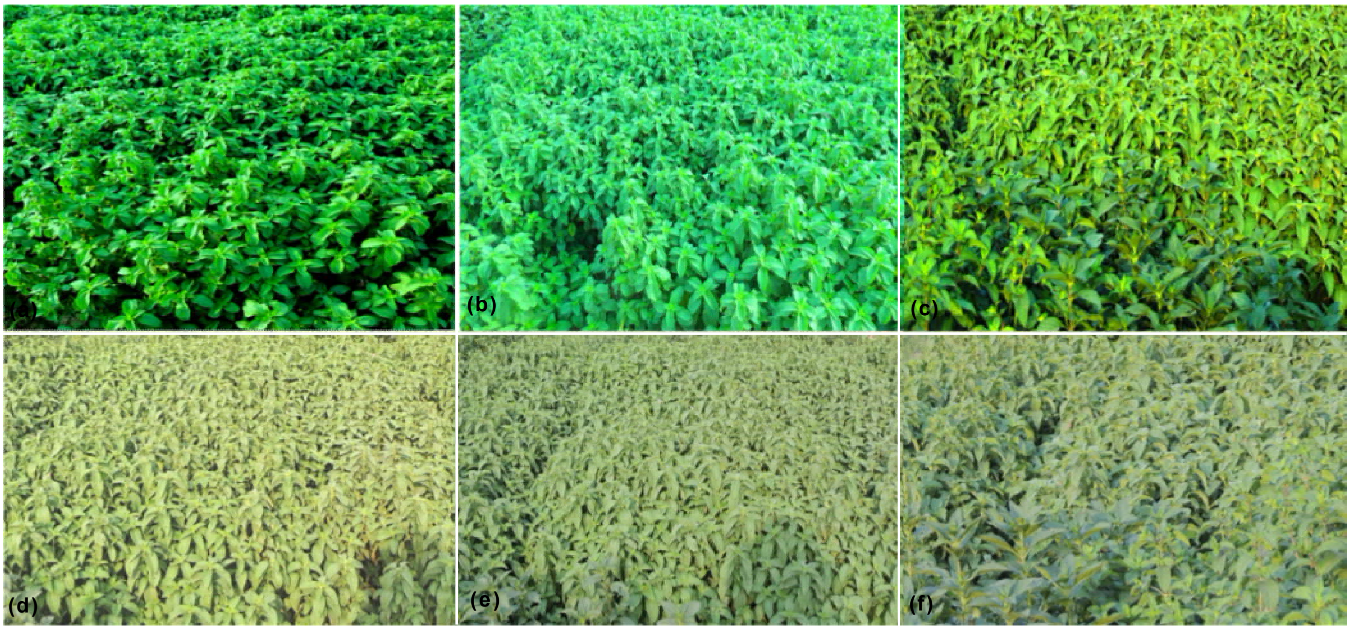

Figure 5: Field view of elite lines: (a) Line-15 (b) Line-11 (c) Line-3 (d) Line-5 (e) Kosi and (f) CIM Kranti of menthol mint

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