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ORIGINAL RESEARCH ARTICLE

Adaptability and stability pattern of a genetic character-trichome diversity in menthol mint (*Mentha arvensis* L.)

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ABSTRACT

Trichome number and their distribution patterns in menthol mint varieties are important determinants of oil yield. The purpose of this study was to monitor trichome variability in commercially used cultivars of Mentha arvensis over three consecutive years for asserting adaptation reaction to environment. The genotypes were examined in terms of quantified degree of adaptability and stability for trichome ratio i.e. glandular trichome over non secretory trichome, by multiplicative interaction (AMMI) model over 3 planting seasons. Varieties of Mentha arvensis selected for study included: Saksham, Kosi, Gomti, Sambhav, Himalaya, Damroo, Kushal, Kalka, and Shivalik. Both types of trichome and their ratio were found to be a variety-specific and proved to be a good marker for genotype selection. Based on the AMMI model, Kosi, Kalka and Himalaya varieties showed the widest adaptability for this genetic trait.

INTRODUCTION

Menthol mint (*Mentha arvensis* L.) belongs to family-Lamiaceae. The genus *Mentha* is represented by 25 species that are distributed mostly in temperate and sub temperate regions of the world (Lal, 2013).They are widely used in foods, flavors, cosmetics and pharmaceutics industries due to presence of essential oils in their foliage. The *Mentha* species show considerable chemical diversity in essential oil composition in terms of their relative monoterpenes constituents (Khanuja et al., 2000). Natural menthol 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 preparations (Mishra et al., 2016, 2017). Genetic improvements efforts in *Mentha* species are targeted towards wider adaptation, more herbage and oil yields, and quality of essential oil. Genetic variation is the basis of any plant improvement and biodiversity management enterprise. Many methods have been previously applied for genetic improvement of *M. arvensis* to create variability and improved genotypes (Mishra et al., 2017). An adaptable and stable cultivar usually refers to the cultivar's ability to perform consistently across a wide range of environments over years. The phenotypic performance of a genotype is not necessarily the same under diverse agro-ecological conditions. Several biometrical methods including univariate

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and multivariate ones have been developed to evaluate these stability parameters. However, studies dealing with assessment of G×E interactions and stability of trichome number and their distribution patterns in *M. arvensis* are meager. Though there are a few stability assessment report for the essential oil yield, but none for trichome variability parameter (Dhawan et al., 2018, 2016; Lal et al., 2013; Lal, 2013).Hence, the present investigation was conceptualized to monitor trichome ratio variability in nine commercial cultivars of menthol mint namely, Kosi, Kushal, Saksham, Kalka, Damroo, Himalaya, Shivalik, Sambhav, and Gomti over three consecutive years for analyzing the changes of adaptation reaction of these cultivars in India and to determine their stability and reliability. To recall again, an adaptable and stable cultivar usually is referring to as a cultivar's ability to perform consistently, across a wide range of years/environments. Genetic variation of the available varieties on the basis of its improvement leading to wider scope of adaptation. Several biometrical methods have been developed to assess stability. More recently, the AMMI stability value based on the AMMI (Additive Main Effects and Multiplicative Interactions) has been frequently employed in such evaluation trial data (Gauch, 2013). The present study precisely provides comparative evaluation data of *M. arvensis* genotypes for adaptability and stability of trichome ratio i.e. (glandular trichome over non secretory trichome) by multiplicative interaction (AMMI) model over 3 years.

MATERIALS AND METHODS

Plants materials

Menthol mint (*M. arvensis* L.) cultivars Kosi, Kushal, Saksham, Kalka, Himalaya, Sambhav, Damroo, Shivalik, and Gomti were obtained from CSIR-CIMAP, Lucknow, India. They were evaluated at CIMAP research farm, Lucknow in a randomized complete blocks design (RCBD) with three replications during 2011–2012, 2012–2013, and 2013–2014. In the RCBD design, each block had three rows at 75 cm distance. The suckers (~5 cm) were planted at a distance of 5 cm. The plants received normal cultivation operations, irrigation, and fertilizer applications (120 kg N_2, 80 kg P_2O_5, and 60 kg K_2O per ha). Minimum and maximum night and day temperatures ranged between 3–11°C and 12–16°C at planting and between 22–30°C and 32–41°C at harvesting, respectively. Average rainfall was 5–6 mm during the growing season (Mishra et al., 2017).

Trichome analysis

Trichome analysis of all genotypes of *M. arvensis* was done in the sixth leaf from the apical region in plants after ~100 to 105 d of transplanting. Trichomes were counted on both the surfaces of the leaf for each genotype in three microscopic fields using a stereomicroscope (Leica KL 200 LED, Schott, Germany) adjusted at 6.25mm^2 grids at its eyepiece lens at 40× magnification. Both types of trichome, i.e., glandular secretory (GST) and non-glandular non-secretory (NST), were counted for all the genotypes. Grids covering 6.26mm^2 areas were fixed at the eyepiece lens, therefore only those trichomes were counted which were present inside the grid as previously advised (Bose et al., 2013; Mishra et al., 2016).

Statistical analysis

Observations were also recorded on each cultivar for two most important economic traits: essential oil yield (kg/ha) and composition of essential oil. Calculations were performed for essential oil yield/ha only using MATMODEL version 3.0 programme mode: fitting AMMI Model software (Gauch, 2013) which gives out puts of AMMI and joint regression models including analysis of variance, regression coefficients, as well as genotypes and environment mean and stability (Lal, 2013).

RESULTS AND DISCUSSION

M.arvensis has wide diversity in relevance to morphology and genetic background. All genetic stocks of *Mentha* species showed very high genetic variability as depicted by sources of variations values in ANOVA (Table 1; Fig.1). Selection of cultivar genotypes/ variety for diverse environments is a difficult task in terms of

Figure 1: Field view of morphologically different varieties of *Mentha arvensis* used in the present investigation

Table 1: ANOVAs for different parameters in AMMI analysis

Source	df	SS	MS	Probability
TRT	26	7.69	0.295	0.9867463**
GEN	8	5.36	0.670	0.4426118**
ENV	2	0.95	0.476	0.4938927**
G × E	16	1.37	0.086	0.9999738
IPCA1	17	45.02	2.65	
Residual	15	10.05	0.67	
Error	54	35.98	0.666	
Total	80	43.68	0.546	

Grand Mean = 0.92567gst/nst; Large residuals exceeding this by a factor of 1.960, 2.576, or 3.291are marked with *, **, or *** respectively. Assuming normality, 5%, 1%, and 0.1% of the residuals exceed these limits; AMMI = Additive Main Effects and Multiplicative interactions, SS = sum of squares; MS =mean sum of squares; df = degree of freedom

Table 2: Variability observed in trichome ratio over the year and their ranking

ENV	Name	Mean	Count	Index	Name	Mean	Count
1	ENV 1	1.00744	9	1	ENV 1	1.00744	9
2	ENV 2	0.77241	9	3	ENV 3	0.99715	9
3	ENV 3	0.99715	9	2	ENV 2	0.77241	9

Table 3: Morphological characteristic and AMMI for trichome ratio in menthol mint cultivars

S. No.	Variety	Developed Salient feature	Mean	Count	Index	Mean for Index
1	Saksham	Clonal variant of Himalaya, dark green, broad leaves erect, thick suckers	0.99	3	2	1.54
2	Kosi	Half-sib progeny selection, light green leaves, bushy, thin suckers	1.54	3	8	1.01
3	Gomti	Seedling variant of Shivalik, dark green, broad leaves erect, thick sucker	0.54	3	1	0.99
4	Sambhav	Single somaclonal variant of cultivar 'Himalaya' light green leaves, thin suckers.	0.94	3	4	0.94
5	Himalaya	Hybrid of Gomti and Kalka dark green, broad leaves erect, thick suckers	0.85	3	9	0.87
6	Damroo	Selection in open pollinated seed progenies of Shivalik variety dark green, leathery broad leaves, erect, thick suckers, thick stem	0.85	3	5	0.85
7	Kushal	Somaclonal selection, dark green, broad leaves erect, thick suckers, tolerate high moisture in soil	0.70	3	6	0.85
8	Kalka	Cross-hybridization, light green leaves, thin suckers, susceptible to leaf spot disease	1.01	3	7	0.70
9	Shivalik	Introduction from China ,long, broad leaves, thick suckers	0.87	3	3	0.54

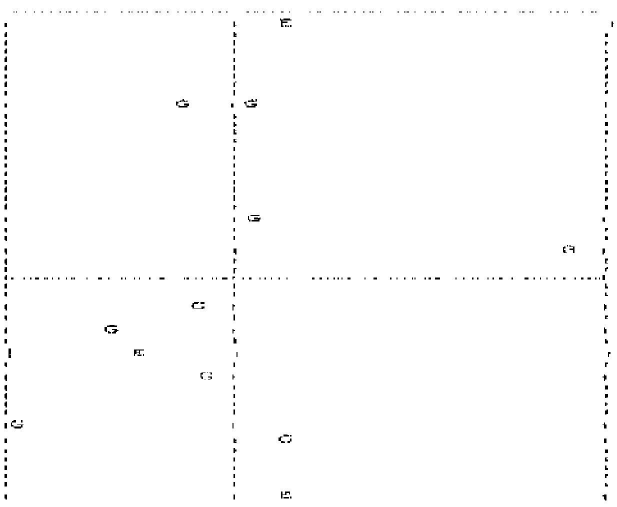

Figure 2: Biplot for genotype and environment for M arvensis cultivars

agronomic/economic yield and stability over years. Several biometrical methods including univariate, multivariate have been identified to assess adaptability/stability. Among them AMMI model for G × E interaction is a promising approach to analyze well adapted and stable genotypes/clones. In the present study, a MATMODEL Version 3.0 was used for data input process for monitoring trichome ratio in menthol mint. In basic ANOVA, elaborate root error MS was 0.816 with grand mean of 0.925. Data were found balanced and there were no missing cells. Table 2 describes the mean of varieties for trichome ratio through different years. A grand mean suggest that average ratio of glandular trichome and non glandular is equal in *Mentha arvensis* species. The environmental mean and IPCA axis scores for AMMI I and the average value of the maximum of N normal realization is the first order statistics are presented in Table 1. It was observed that there is decrease in trichome ratio in year 2 suggesting that this character is also influenced by environmental condition despite being a genetic character that influence the essential oil yield (Table 2). Further indexing for trichome ratio suggest that it was highest in Kosi followed by Kalka, Saksham, Sambhav, Shivalik, Himalaya, and least was in Gomti varieties/genotypes (Table 3).

High mean and stability usually refers to a genotype's ability to perform consistently across a wide range of years (Lal, 2013). In AMMI indexing, mean of variety suggest their interaction with the environment. In biplot analysis most of variety

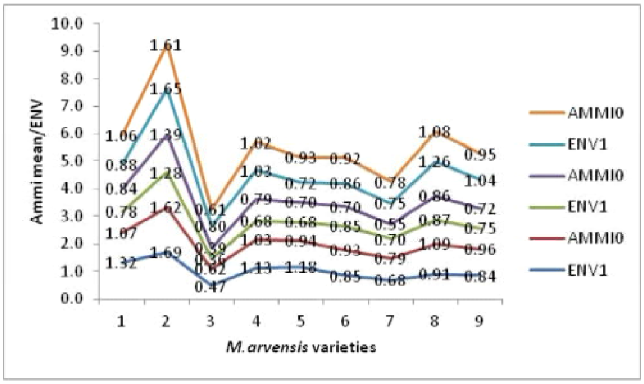

Figure 3: Mean performance and AMMI I in the three environments/ years

Interval	Maximum	Count	Histogram
1	0.01234	1	|*****************
2	0.02469	2	|**********************************
3	0.03703	3	|***
4	0.04938	1	|*****************
5	0.06172	0	|
6	0.07407	3	|***
7	0.08641	0	|
8	0.09876	2	|**********************************
9	0.11110	3	|***
10	0.12344	2	|**********************************
11	0.13579	0	|
12	0.14813	1	|*****************
13	0.16048	2	|**********************************
14	0.17282	1	|*****************
15	0.18517	3	|***
16	0.19751	0	|
17	0.20986	0	|
18	0.22220	1	|*****************
19	0.23454	0	|
20	0.24689	2	|**********************************

Figure 4: A histogram for the AMMI1 residuals for trichome ratio in mint cultivars

shows closeness except two in three consecutive years. (Fig.2). Simultaneously, in different year the performance of variety did deviate but same winners were picked by AMMI F and AMMI I in all the years/environments (Fig.3). Ordinarily there is considerable selectivity with the pattern recovered mostly in the early interaction principal component analysis (IPCA) axis and noise recovered mostly in late IPCA axis. Consequently, these estimates provide a rough guide for model diagnosis by keeping early axes that are mostly patterns and relegating the others to a discarded residual (Fig. 4).

Trichome ratio was proved to be a stable character for variety selection. Trichomes are considered to be the site of essential oil synthesis and accumulation. Trichome number per unit area is an important factor that regulates the net oil content and yield of a variety. Non glandular trichomes create a protective microenvironment for the plants but not participate in essential oil biogenesis. So ratio of glandular to non glandular trichome give a clear idea that which variety will give better essential oil yield per unit area and biomass in a given environment. The essential oil yield is highly influenced by environments and varies with cultivar to cultivar in the mint crop due to different trichome ratio. AMMI modal for genotype × environment interaction can improve the efficiency of related plant breeder's progress towards selection of well adapted and stable cultivars in mint crop also. In this study, we found that variety Kosi showed the best mean for trichome ratio over the years, followed by Kalka and Shivalik. In second year where environmental mean was low as compared to grand mean, Kosi showed maximum trichome ratio followed by Kalka and Himalaya. Therefore, these three cultivars can be recommended for mint cultivation in entire northern Indian plains.

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