PHENOTYPIC VARIATION AMONG MENTHA SPP.

Shazia Erum*, Muhammad Naeemullah and Shahid Masood**

ABSTRACT:-The aim of the present study was to investigate the morphological diversity and relationship within 17 genotypes of mentha species. Different species of Mentha were collected from different ecological zones of the world. Great variation was observed in stem length with the coefficient of variation of 198.7% and standard deviation of 14.1. Among the 17 mint collection, nine special aromas was smelled including mint gum, strong camphoraceous, mint like, spicy, lemon, pungent, musky and acrid due to the presence of diverse bioactive compounds and essential oils. The present study may help to patronize the essential oil industry on latest scientific techniques. Like other herbs Mentha have diverse aromatic properties that can be explored for utilization in the industry and this information will be helpful for exploitation and development of natural agricultural resources on scientific grounds.

Key Words: Morphology; Mentha; Diversity; Aroma; Pakistan.

INTRODUCTION

Mints are perennial aromatic herbs that are used both for medicinal and aromatic purposes. *Mentha* is distributed worldwide and is native from north temperate regions and occur in all five continents. Genus *Mentha*, a member of the family Lamiaceae and the tribe Mentheae, is divided into five sections and consists of approximately 25 species (Harley and Brighton, 1977). Within *Mentha,* it has been suggested that the five basic species *Mentha arvensis* L., *Mentha aquatica* L., *Mentha spicata* L., *Mentha longifolia* (L.) Huds, and *Mentha suaveolens* exist. Most *Mentha* species are characterized by a great morphological variation which is reflected on different taxonomic rank names attributed to mint plants during the past 200 years. Furthermore, the hybridization, that occurs frequently when the species of *Mentha* are in contact, contributes to the complex variation patterns characterizing most wild populations.Linneaus (1767) described the species of the genus based on inflorescence morphology.

Menthol is the main commercial product obtained from mint. It is produced in specialized glands present in the leaves and flowers of the plant. Moreover, *Mentha* produces secondary metabolites such as alkaloids, flavanoids, phenols, gummy polysaccharides, terpenes and quinines that are used in food, pharmaceutical, cosmetics and pesticide industries (Khanuja et al., 2000). Mint oils are mainly produced in Argentina, Angola, Australia, Brazil, Bulgaria, China, Czechoslovakia, France, Hungary, India, Italy, Paraguay, Switzerland,

* *International Islamic University, Islamabad, Pakistan.*
***Institute of Agricultural Biotechnology and Genetic Resource, National Agricultural Research Centre, Islamabad, Pakistan.*
Corresponding author: shazia_rm@yahoo.com

Thailand and USA. In India, mint species are mainly cultivated in Uttar Pradesh, Haryana and Punjab over 162800ha of agricultural land. The annual world production of *Mentha arvensis* L. and *M. piperita* L. oils are 22,000mt and 7500 mt, while India is producing 16000mt and 100 mt per year, respectively (Khanuja, et al., 2000; Patra et al., 2002). Brazil was once an important world producer and exporter of mint essential oils (Clark, 1998).

Several features have been used in the past to examine the diversity of Mentha using morphological (Malinvaud, 1880), cytological (Ruttle, 1931; Heimans, 1938; Morton, 1956; Sharma and Bhattacharyya, 1959; Harley, 1967; 1972; Harley and Brighton, 1977; Singh and Sharma, 1986) and chemical (Lawrence, 1978) markers.

Cluster analysis has been used to assess similarities among landraces in plant breeding programmes where genotypic and phenotypic repetitions of several characters were found among populations, lines, or accessions from which parents were selected for hybridization (Wilson et al., 1990; Zeinali et al., 2004).

Harley and Brighton (1977) published a critical review of the chromosome numbers in relation to the taxonomy of the genus. Natural interspecific hybridization occurs with high frequency in Mentha, both in wild and cultivated populations. Most hybrids are sterile or subfertile, but vegetative propagation enables them to persist. Complex hybrid populations may arise, and if they are subfertile, may cross with parental or nonparental species. This leads to a large diversity of chromosome numbers (24-120) and much of the taxonomy of Mentha has been complicated by hybridization, by a high morphological polymorphism, as well as polyploidy and vegetative propagation. The best known hybrids are *M. piperita* (peppermint) and *M. spicata* L. (native spearmint), which are intensively cultivated for their essential oils. *Mentha piperita* results from a cross between *M. aquatica* and *M. spicata*. Later being the hybrid between *M. suaveolens* and *M. longifolia* (Harley and Brighton, 1977).

It was noted that mints are mainly used to cure gastrointestinal disorders, but the spectrum of medical activities is broader (McKay and Blumberg, 2006). Currently, emphasis is, on conserving plant germplasm as valuable bio-resources because obscure genes from these plants may provide solutions to new diseases, insects, environmental or crop production problems (Khanuja et al., 2000; Barbara, 1999). Selection between and within accessions for a high level of essential oil content, herbage yield and other characters require an effective tool to be employed by mint breeders (Mirzaie-Nodoushan et al., 2000). Mentha must be considered an important species for the conservation of genetic resources of medicinal and aromatic plants. The collection and introduction of new genetic materials of Mentha is fundamental to obtain genetic pool adequate for further research in plant breeding for the selection of genotypes with superior quality. The objective of this work was to evaluate the morphological differences and relatedness among the mint, collected from various ecological zones of the world.

MATERIALS AND METHOD

Plant Material

In the present study, 17 genotypes collected by the scientists at Plant Genetics Research Institute, National Agricultural Research Centre, Islamabad were evaluated for various morphological and agronomical traits in an augmented design. These were planted under green house and field for multiplication and evaluation. The passport data of mint germplasm showed that collection was made from local as well as from foreign sources. Local varieties with variety codes include: Mentha local purple flower (1), Mentha local white flower (2), Peppermint / *Mentha piperita* Local (6), Lavender mint (7), Lemon mint sp. (8), *Mentha aquatica* (9), European Pennyroyal (10), White mint (12-Rawalakot), Mint Camphor (13-Rawalakot), Spear mint (16), MintBattal (17-Mansehra), *Mentha rolyana*/Pahari pudina (19). Whereas the foreign collection consist of Cool mint (11) from Canada, *Mentha arvensis* (18) from China, Nana asavi (3) and Nana maghrabi (4) belongs to Saudi Arabia.

Morphological Traits

The data/plant were recorded in triplicate. Observations documented for leaf area, thickness of leaf, thickness of stem, stem length and petiole length under quantitative category. Whereas, qualitative traits were shape of leaf blade, leaf margin, leaf apex, leaf base, leaf color, leaf arrangement, leaf venation (Figure 1), leaf odor, stem color and flower color (Ghafoor et al., 2005).

Statistical Analysis

Statistically data were analyzed by using Mstat-C and Excel software. Analysis of Variance and Duncan Multiple Range Test (DMRT) were applied for comparison among different collections of Mentha species.

Figure 1. Morphological variation in leaf among mentha genotypes

RESULTS AND DISCUSSION

In the present study 17 genotypes of Mentha species were evaluated for both qualitative and quantitative traits at National Agricultural Research Centre, Islamabad, Pakistan. Plant material under study was collected from diverse region of the world including Saudi Arabia, Japan, Canada, China and Pakistan. The quantitative traits which are mainly considered as the economic/ agronomic traits were leaf area, leaf thickness, stem thickness, stem length and petiole length for 17 genotypes of Mentha. Analysis of variance (ANOVA) and LSD at 5% probability level showed significant interaction (Table 1). Leaf area ranged from 0.76 to 7.8 cm^2 with 2.8% C. V. High variation ranging from 8.6 to 57.6 cm was observed for stem length with high degree of S.D. (14.11), indicating a good scope of selection for this trait (Table 2). however, a positive correlation was observed among thickness of leaf and stem with leaf area (Table 3).

Table 1 . Mean square values of quantitative traits

Variety	Code	Leaf area(cm^2)	Thickness of leaf (mm)	Thickness of stem(mm)	Stem length (cm)	Petiole length (cm)
Mentha local purple flower	1	2.500^{bcd}	0.1560^{de}	2.7030^{ab}	46.6700^{b}	0.23^{def}
Mentha local white flower	2	6.083^{a}	0.2230^{bcd}	2.3467^{b}	13^{efg}	0.23^{def}
Nana asavi	3	3.967^{bc}	0.2060^{bcde}	1.3900^{cdef}	37.67^{c}	0.0^{f}
Nana maghrabi	4	3.133^{bc}	0.1760^{cde}	1.4900^{cde}	57.67^{a}	0.2^{def}
Peppermint - Japan	5	3.667^{bc}	0.1530^{de}	0.9667^{f}	13.33^{efg}	0.2^{def}
Mentha piperita - Local	6	4.070^{b}	0.2200^{bcd}	1.6870^{c}	14.33^{defg}	0.6^{cd}
Lavender mint	7	1.85^{cd}	0.1860^{bcde}	1.7430^{c}	17.67^{def}	0.4^{def}
Mentha aquatica	9	2.267^{bcd}	0.200^{bcde}	1.0770^{ef}	16.67^{def}	0.2^{def}
European Pennyroyal	10	0.763^{d}	0.2467^{bc}	1.7330^{c}	16^{defg}	0.233^{def}
Cool mint	11	3.800^{bc}	0.1967^{bcde}	1.660^{c}	18^{def}	0.133^{ef}
White mint	12	1.833^{cd}	0.1200^{e}	1.050^{ef}	13.33^{efg}	0.83^{c}
Mint Camphor	13	3.4^{bc}	0.1660^{cde}	2.830^{a}	8.66^{g}	0.5^{cde}
Field mint	15	3.817^{bc}	0.1233^{e}	1.680^{c}	13^{efg}	0.433^{de}
Spear mint	16	3.817^{bc}	0.2733^{b}	0.960^{f}	20^{de}	1.83^{b}
Mint -Battal, Mansehra	17	3.033^{bc}	0.2367^{bcd}	1.4960^{cde}	38.67^{c}	0.166^{ef}
Mentha arvensis	18	7.833^{a}	0.3667^{a}	1.570^{cd}	21.33^{d}	1.8^{b}
Mentha rolyana/Pahari pudina	19	2.967^{bc}	0.1530^{de}	1.1300^{def}	40.33^{bc}	0.266^{def}
Mean ± SE		3.5 ± 0.39	0.20 ± 0.01	1.6 ± 0.12	22.6 ± 3.23	0.51±0.13
S.D.		1.70	0.06	0.53	14.10	0.58
CV (%)		2.88	0.00	0.29	198.74	0.34

Means followed by same letter (s) do not differ significantly at 0.5% probability level.

Table 2. Coefficient of Correlations among 17 genotypes for quantitative traits

	Leaf area(cm^2)	Thickness of leaf (mm)	Thickness of stem(mm)	Stem length (cm)	Petiole length (cm)
Leaf area(cm^2)	1				
Thickness of leaf (mm)	0.588878	1			
Thickness of stem(mm)	0.119067	-0.03704	1		
Stem length (cm)	0.142410	-0.05380	0.009302	1	
Petiole length (cm)	0.650775	0.534084	-0.04109	-0.30716	1

Table 3. Analysis of variance (Mean Square) for quantitative traits

Sources	Degree of freedom	Leaf area(cm^2)	Thickness of leaf (mm)	Thickness of stem(mm)	Stem length (cm)	Petiole length (cm)
Plots	2	0.922	0.000	0.980	22.330	0.021
Cultivars	18	8.704**	0.010**	0.873**	596.220**	1.265**
Error	36	1.221	0.002	0.068	15.611	0.046
CV (%)		30.940	20.380	16.320	17.430	37.610

Table 4. Qualitative traits of Mentha spp.

Code	Variety	Shape of leaf blade	Shape of leaf margin	Shape of leaf apex	Shape of leaf base	Leaf color	Leaf arrange ment	Leaf venation	Leaves Odor	Stem color	Flower color
1	*Mentha local (purple flower)*	Elliptic	Undulate	Acute	Cordate base	Light green	Opposite	Cross venulate	Mint gum	Dark reddish purple	Lilac
2	*Mentha local white flower*	Oval	Serrate	Obtuse	Cordate base	Green	Opposite	Pinnate	Mint gum	Light purplish green	Lilac
3	*Nana asavi*	Linear	Slightly serrate	Acute	Auricled	Light green	Opposite	Pinnate	Strong camphoraceous	Light green	White
4	*Nana maghrabi*	Ovate	Serrate	Obtuse	Cordate base	Dark green	Opposite	Cross venulate	Mint gum	Dark purple	Lilac
5	*Peppermint Japan*	Ovate	Serrate	Acute	Obtuse	Green	Opposite	Cross venulate	Mint gum	Dark reddish purple	Dark purple
6	*Mentha piperita Local*	Ovate	Serrate	Acute	Obtuse	Green	Opposite	Pinnate	Mint gum	Dark purple	Lilac
7	*Lavender mint*	Orbicular	Slightly serrate	Obtuse	Truncate	Purplish dark green	Rosulate	Arcuate	Spicy	Reddish purple	Lilac
9	*Mentha Aquatica*	Long elliptic	Serrate	Acute	Obtuse	Dark green	Opposite	Pinnate	Pungent	Light purplish green	Lilac
10	*European Pennyroyal*	Oval	Slightly serrate	Obtuse	Obtuse	Light green	Whorled	Pinnate	Musky	Light purple	Blue
11	*Cool mint*	Elliptic	Aerrate	Acute	Obtuse	Green	Opposite	Cross venulate	Resh mint gum	Dark Purple	Lilac
12	*White mint*	Orbicular	Serrate	Obtuse	Ordate base	Green	Opposite	Pinnate	Camphoraceous	Light Purplish green	Lilac
13	*Mint Camphor*	Ovate	Serrate	Acute	Obtuse	Light purplish green	Opposite	Arcuate	Camphoraceous	Light green	Lilac
15	*Field mint*	Orbicular	Serrate	Obtuse	Cordate base	Dark green	Opposite	Cross venulate	Mint gum	Light reddish purple	Lilac
16	*Spear mint*	Long elliptic	Crenate	Obtuse	Truncate	Light green	Opposite	Cross venulate	Acrid	Light green	Lilac
17	Mint *-Battal, Mansehra*	Ovate	Crenate	Obtuse	Truncate	Green	Whorled	Cross venulate	Mint	Brown	Lilac
18	*Mentha arvensis*	Orbicular	Crenate	Obtuse	Cordate base	Green	Opposite	Cross venulate	Lemon	Greenish purple	Blue lilac
19	Mentha *rolyana/Pahari pudina*	Linear	Slightly serrate	Acute	Obtuse	Light green	Opposite	Pinnate	Pungent	Light green	Lilac

The data depicted that leaf area ranged from 0.7 to 7.8 cm^2. However greater leaf area exhibited by *Mentha arvensis* (7.83 cm^2) from China. In contrast lowest leaf area was acquired by European pennyroyal (0.76 cm^2). *Mentha arvensis* from China again boost up with maximal leaf thickness (0.36mm) whereas White mint and Field mint showed lowest thickness of leaf. Difference among the 17 genotypes, regarding stem thickness ranged from 0.9 to 2.8 mm. But Camphor mint emerged out as a strength full stem with optimal stem thickness. Among the 17 genotypes, *Mint Maghrabi* from Saudi Arabia attained greatest stem length (57.6 cm). In contrast, Camphor mint showed dwarf/short stem length (8.6 cm). *Lemon mint officinais* (Lemon mint sp.) exhibited maximal petiole length (2.2 cm) and Mansehra mint with reduced length (0.16 cm). Exception to that sessile leaves were apparent through *Mint Maghrabi.* These results partially agree to the earlier findings of Tucakov and Savin (1967) that revealed the morphology of mint (Menth*a piperita* L.) of different origin experimentally cultivated in Belgrade. Silva et al. (2006) evaluated and conserved mint (*Mentha spp*) germplasm included 14 species and 67 accessions, which are maintained at field and greenhouse conditions in Brazil.

In the present study, great diversity was expressed within 17 genotypes for qualitative traits including shape of leaf blade, leaf margin, leaf apex, leaf base, leaf color, leaf arrangement, leaf venation, leaf odor, stem color and flower color (Table 4). Flower color of mint genotypes was found lilac in most of the cases except to Mint Asavi and *Mentha arvensis* with white and blue lilac colors respectively. However, stem color showed great variation as visible in seven different shades among the genotypes under study (dark reddish purple, light reddish purple, light purplish green, light green, brown, dark purple and light purple). Similar observations were recorded for leaf color, that are in five shades (green, light green, dark green, purplish dark green and light purplish green).

Generally, arrangement of leaf was opposite except Lemon mint spp. and Lavender mint which have rosulate arrangement. Likewise, cross venulate, pinnate, arcuate and reticulate types of leaf venation was observed in *Mentha* spp.

The odor of *Mentha* leaves is a characteristic of each genotype due to the presence of unique bioactive compounds, polyphenols (caffeic acid, rosmarinic acid, ferrulic acid, eugenol etc.) and essential oil present in them. Consequently, nine special aroma was smelled even in the 17 samples i.e., mint gum, strong camphoraceous, mint like, spicy, lemon, pungent, musky, rotten egg and acrid. Mentha species are characterized by different aroma due to the presence of bioactive compounds and essential oils evident from the literature (Zviniene et al., 1996). Moreover, shape of leaf also exemplify great variation with diverse patterns including leaf blade (> 5 types), leaf margin (>3 types), leaf apex (3 types) and four types of leaf bases were observed in the mint germplasm. Moreover, similar observations was reported by Shinwari et al. (2011) on the diversity of *Mentha* species that showed taxa maintained high levels of genetic polymorphism among species but not among populations. The polymorphism within populations depicted genotype richness, recombination and gene flow. Higher levels of diversity support the concept that mint have a long history of independent evolution.

Much phenotypic diversity among the 17 *Mentha* genotypes collected from different agro-ecological zones, and cultivated under field conditions were noticed. Relating to phenotypic diversity to origin/collecting sites of the germplasm indicated the potential for future exploration mission with maximum genetic distance to assemble broad based genetic resources of mint for future use and selection. As it can be a good source for perfumery, essential oils and condiments for flavoring and garnishing the food items as well as with great medicinal value, the diversity within the genotypes could facilitate the selection of the genotype for distinct traits.

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