

Comparative karyomorphological analyses of *in vivo* grown plants of *Mentha piperita* L. and *Mentha arvensis* L.

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Abstract

In order to distinguish between different species and populations of *Mentha*, the root, shoot, petiole length, internodal distance as well as their morphological and karyological features were taken as principle parameters. In *Mentha piperita* L. population 1 the internodal distance of the shoots was little shorter (2.8 ± 1.24 cm) than population 2 (3.5 ± 0.72 cm). The internodal lengths were nearly equal in population 1 and population 2 of *M. arvensis* L. but were longer than population 3. Leaf area was smaller in population 3 of field mint than the other two populations. However, population 2 possessed the highest leaf area (15.02 ± 0.36 cm²) among the five populations of two species studied. Flower color also varied from whitish to whitish lavender to pale purple in all the five populations. All these morphological characters clearly indicated variation among different species. The size of the chromosome varied between the species and populations with the difference in ploidy level. The genus *Mentha* regarded to be a polyploid group with $x=12$ as the basic chromosome number (Ruttle 1931). Among all the populations of these two species studied, population 1 of *M. piperita* L. contained larger chromosomes. The total chromosome length, mean chromosome length, total chromosome volume and mean chromosome volume differed among the populations. These investigations were undertaken to prepare a comparative morphological and chromosomal database within these five populations of two species of *Mentha*. All these data indicated that the five populations of *Mentha* differed in minute karyotypic details and each population contained different types of chromosomes in variable numbers, revealing uniqueness of individual populations of each species.

Keywords: chromosome, *mentha*, population, variation

Introduction

Mentha piperita L. and *Mentha arvensis* L. are two most extensively used species of the genus *Mentha* (family: Lamiaceae) which is indigenous to England and now grown and cultivated mainly in the northern part of India. It is widely found in diverse environments but wet weather with moist soil is most preferred [1]. Mint is perennial with wide spread stolons originally used as a medicinal herb. The volatile oil obtained from *Mentha* is useful for treating rheumatism, neuralgia, headache, toothache and the oil is also inhaled for treatment for bronchitis also [2]. It is also used as flavoring agent. A number of plant phenolics including rosmarinic acid are found in mint which was responsible for its medicinal properties. These plant phenolics have anti-inflammatory [3], antioxidant [4], antimutagenic and anticarcinogenic activities [5, 6]. Rosmarinic acid plays a protective role in Alzheimer's disease [7], biomembrane damage [8], as vasorelaxant [9, 10] and as a chemo preventive agent [11, 12]. Thus commercial cultivation and utilization of this plant with the application of biotechnology can be of great value. The present study deals with the collection of five populations of these two species, its morphological study and karyotypic analysis. The minute and detailed examinations of somatic chromosomes revealed that many species of *Mentha* consisted of several cytotypes with different chromosome numbers [13]. The genus *Mentha* regarded to be a polyploid group with $x=12$ as the basic chromosome number [14]. These investigations were

undertaken to prepare a comparative morphological and chromosomal database within these five populations of two species of *Mentha*.

Materials and Methods

Plant material – The plants materials selected for the study were collected from the field germplasm (Strain No IC 54537 and 54538) of the National Bureau of Plant Genetic Resources (NBPGR), New Delhi and Agri Horticultural Society of India, Kolkata. The plants were established in the experimental garden of Department of Botany, Ballygunj Science College, Kolkata.

Morphological study – Plants could be distinguished from each other by their height, characteristic leaf morphology, and flower colour. Morphological study was done by observation using metric ruler and simple microscope.

Mitotic study - Root tips of field grown plants were taken for cytological analysis following propionic orcein squash technique (Singh and Sharma 1981). The root tips were pretreated at around 9.30 am (IST) in saturated solution of paradichlorobenzene (PDB) for three hrs at 18°C to 20°C. The root tips were then washed in distilled water and fixed in Carnoy's fixative (ethanol: chloroform: acetic acid in the ratio of 6:3:1) for overnight at 18°C to 20°C. The materials were transferred to 1: 2 propionic ethanol for 15 to 18 hrs and were hydrolysed in 5(N) HCl at 18°C to 20°C for 15 min, followed by thorough washing in distilled water. A brief treatment in

45% propionic acid was done and stained in 2% propionic orcein solution for three hrs. Finally the root tips were squashed in 45% propionic acid and were observed under the microscope.

Detailed karyomorphological Study – Chromosome identification is indispensable in cytological study. Well scattered somatic metaphase plates were taken for karyotype analysis. The chromosomes at metaphase stage were drawn with the help of a drawing prism at a magnification of 2500 times by Olympus microscope under 15x eyepiece and 100x oil immersion objectives. Microphotographs were taken with Carl-Zeiss Jena microscope with the help of photographic attachment. Each photograph was suitably enlarged. The parameter analyzed for chromosome morphology were total arm length of each chromosome (long arm length + short arm length), i value $\left\{ \frac{\text{short arm}}{\text{long arm} + \text{short arm}} \right\} * 100$, and arm ratio (AR = short arm length/ long arm length). Arm ratio widely used for the classification of chromosome type has been considered empirically to be a more stable parameter of the chromosomal morphology^[16, 17]. In this study primary and secondary constrictions were analysed from the well scattered metaphase plates for karyotypic analysis. In the figure the chromosomes with secondary constrictions were drawn in outline for identification.

Results and Discussion

Morphological study

Mentha piperita L. population 1 (Fig. 1A) was found to be smaller and weaker than *M. piperita* L. population 2 (Fig. 1D). *Mentha arvensis* L. was larger in size than *M. piperita* L. Among three populations of fieldmint (*M. arvensis* L.), population 3 (Fig. 1P) was shortest and population 2 (Fig. 1K) was longest as revealed from plan height and leaf area studies (Table 1).

Mentha piperita L. Population 1 (Fig. 1A): These are aromatic herbs with a square and purplish stem. Average height is of 35.0 cm. Stems are purplish green with smooth texture and internodal distance is about 2.8 cm in average. Leaves are dark green and oppositely arranged. Average petiole length is of 1.5 cm. Leaves are glabrous, ovate, oblong in shape. Leaf length varies 3.0 cm to 5.0 cm and leaf area is 5.5 cm². Leaf margin is coarsely toothed with blunt apex. Venation is reticulate. Flowers are small, pale purple in colour, arranged in a thick terminal spike (Fig. 1B). Inflorescence is verticillaster. Calyx is pubescent five toothed. Corolla is pale purple in colour, bilabiate and glabrous. Upper lip is single lobed where as lower lip is three lobed. Lobes are subequal. Stamens are four in number and exerted. Filaments are white. Style is exerted and stigma is two lobed. Roots are white, long, very thin, slender and 2.5 to 3.0 cm in length.

Mentha piperita L. Population 2 (Fig. 1D): These are aromatic herbs with a square greenish stem. Average height is of 40 cm. Stems are dark green with smooth texture and internodal distance with an average of 3.5 cm. Leaves are dark green, oppositely arranged, glabrous and oblong in shape. Leaf length ranges from 4.0 cm to 6.0 cm and leaf area is 8.76 cm² in average. Leaf margin is broadly serrated with an acute

apex. Venation is reticulate. Average petiole length is of 2.1 cm. Flowers are small, pale purple in colour and arranged in thick terminal spike (Fig. 1E). Inflorescence is verticillaster. Calyx is pubescent and five toothed. Corolla is pale purple in colour, bilabiate and glabrous. Upper lip is single lobed and lower lip is three lobed. Lobes are subequal. Stamens are four in number and exerted. Filaments are white. Style is exerted and stigma is two lobed. Roots are white, long, very thin, slender and 2.0 to 3.0 cm in length.

Mentha arvensis L. population 1 (Fig. 1G): These are aromatic herb with erect and hairy quadrangular stem. Average plant height is of 32.0 cm. Stems are green pubescent with average internodal distance of 2.5 cm. Leaves are green, oppositely arranged, pubescent and ovate in shape. Leaf length varies from 4.0 to 6.0 cm. Average leaf area is of 12.96 cm². Leaf margin is broadly serrated with a rounded apex. Average petiole length is of 1.6 cm. Flowers are small, lavender in colour and present in axillary verticillaster inflorescences (Fig. 1H). Terminal spike is completely absent. Calyx is pubescent and five toothed. Corolla is pale purple in colour, bilabiate and glabrous. Upper lip is single lobed and lower lip is three lobed. Lobes are subequal. Stamens are four in number and exerted. Filaments are white. Style is exerted and stigma is two lobed. Roots are white, medium, very thin, slender and of 1.5 to 2.5 cm in length.

Mentha arvensis L. Population 2 (Fig. 1K): These are aromatic herb, with erect quadrangular stem. Average plant height is of 38.0 cm. Stems are brownish green, sparsely pubescent and with average internodal distance of 3.0 cm. Leaves are green, oppositely arranged, sparsely pubescent, ovate and oblong in shape. Leaf length is of 4.0 cm to 5.0 cm. Average leaf area is of 15.02 cm². Leaf margin is serrated with an acute apex. Average petiole length is of 1.8 cm. Flowers are small and arranged in a verticillaster inflorescence (Fig. 1M). Terminal spike is absent. Calyx is pubescent and five toothed. Corolla is whitish in color, bilabiate and glabrous. Upper lip is single lobed and lower lip is three lobed. Lobes are subequal. Stamens are four in number and exerted. Filaments are white. Style is exerted and stigma is two lobed. Roots are white, medium, very thin, slender and of 1.4 to 2.6 cm in length.

Mentha arvensis L. population 3 (Fig. 1P): These are aromatic herb with erect and square stem. Average plant height is of 28.0 cm. Stems are green, sparsely pubescent with average internodal distance of 1.5 cm. leaves are green, oppositely arranged, sparsely hairy and ovate in shape. Leaf length is of 3.5 cm to 4.5 cm. Average leaf area is of 7.02 cm². Leaf margin is broadly serrated with an acute apex. Average petiole length is of 1.0 cm. Flowers are small and arranged in a verticillaster inflorescence (Fig. 1Q). Terminal spike is absent. Calyx is pubescent and five toothed. Corolla is whitish lavender in color, bilabiate and glabrous. Upper lip is single lobed and lower lip is three lobed. Lobes are subequal. Stamens are four in number and exerted. Filaments are white. Style is exerted and stigma is two lobed. Seeds are nutlets. Roots are white, medium, very thin, slender and of 1.6 to 2.8 cm in length.

Table 1: Morphological characters of different populations of *Mentha piperita* L. and *Mentha arvensis* L.

Plant Material	Characters					
	Height (cm)*	Petiole length (cm)*	Leaf texture	Leaf area (cm ²)*	Internodal distance (cm)*	Flower
<i>M. piperita</i> L. population1	35±0.88	1.5±1.34	Glabrous	5.5±0.94	2.8±1.24	Pale purple
<i>M. piperita</i> L. population 2	40±1.56	2.1±0.22	Glabrous	8.76±1.56	3.5±0.72	Pale purple
<i>M. arvensis</i> L. population1	32±0.44	1.6±1.37	Pubescent	12.96±1.86	2.5±1.38	Lavender
<i>M. arvensis</i> L. population 2	38±0.68	1.8±0.72	Pubescent	15.02±0.36	3.0±1.42	Whitish
<i>M. arvensis</i> L. population 3	28±1.44	1.0±0.83	Sparsely pubescent	7.02±0.24	1.5±0.96	Whitish lavender

*Data represented as Mean±SE from five replicates.

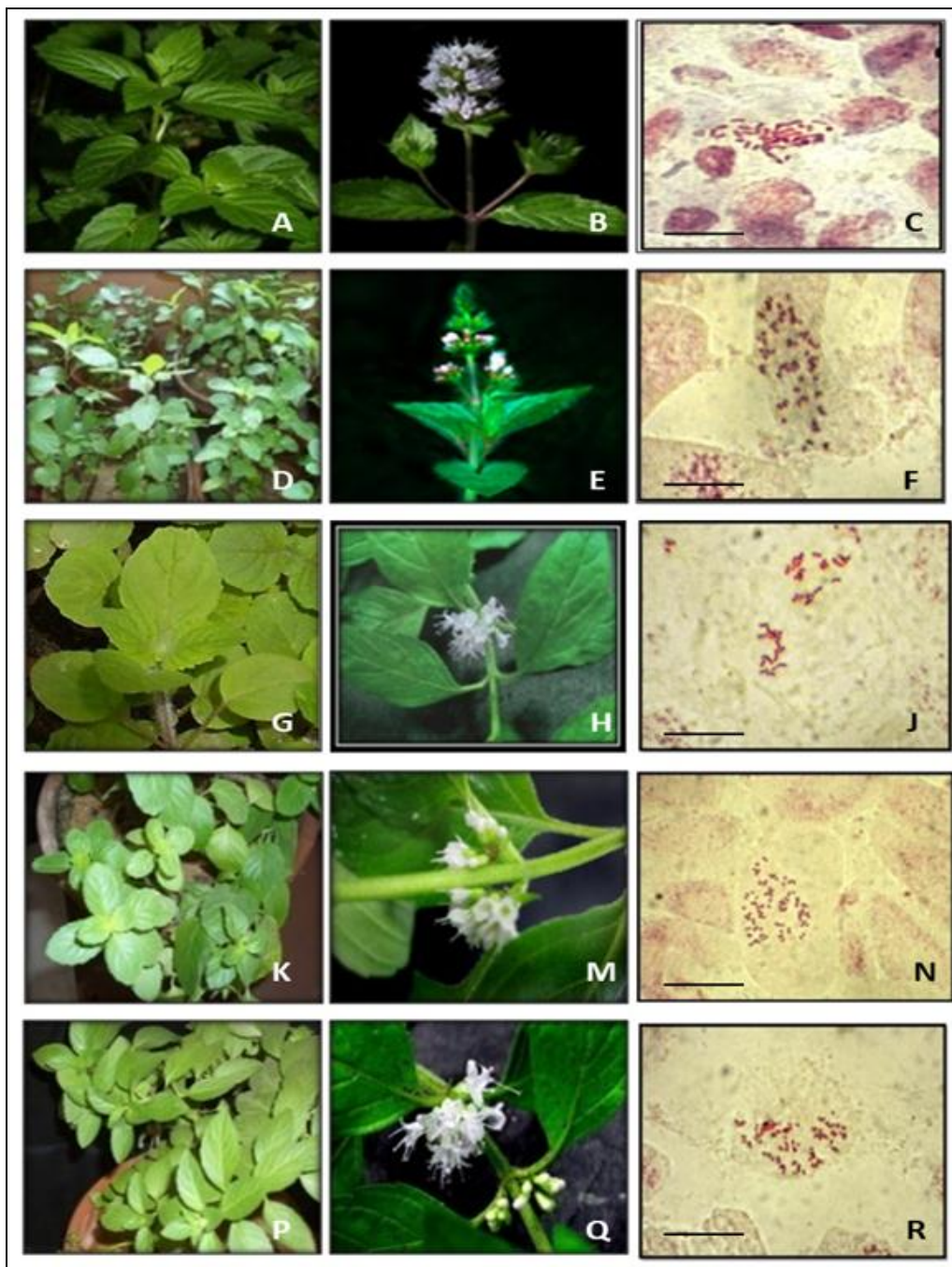


Fig 1: (A, B) Plant and flower of *Mentha piperita* L. population 1. (C) Somatic metaphase plate of *Mentha piperita* L. population 1. (D, E) Plant and flower of *Mentha piperita* L. population 2. (F) Somatic metaphase plate of *Mentha piperita* L. population 2. (G, H) Plant and flower of *Mentha arvensis* L. population 1. (J) Somatic metaphase plate of *Mentha arvensis* L. population 1. (K, M) Plant and flower of *Mentha arvensis* L. population 2. (N) Somatic metaphase plate of *Mentha arvensis* L. population 2. (P, Q) Plant and flower of *Mentha arvensis* L. population 3. (R) Somatic metaphase plate of *Mentha arvensis* L. population 3.

Karyotype analysis

Comparative diploid karyotype analysis of *in vivo* plants of five populations of two species of *Mentha*: Detailed analysis of numerical data suggests that the karyotype can be distinguished mainly based on mean chromosome length, mean chromosome volume and karyotype formula. Karyomorphological features of chromosomes are summarized in Table 2.

Karyotype of *Mentha piperita* L. population 1 (Fig. 2A, B): – Chromosomal analysis of *in vivo* plants revealed the presence of $2n = 36$ chromosomes with symmetrical karyotype: Karyotype formula is $2St (SAT) + 2St (SAT)/m (SAT) + 26Sm + 6m$. One pair chromosome with $St (SAT)$ is $2.4 \mu m$ in length and one pair chromosome with $St (SAT)/m (SAT)$ is $2.2 \mu m$ in length. Thirteen pairs of Sm chromosomes range from $0.9 \mu m$ to $1.9 \mu m$ and three pairs of m chromosomes range in length from $1.3 \mu m$ to $1.4 \mu m$. Mean chromosome length was $1.69 \mu m$ and mean chromosome volume was $85.06 \mu m^3$ (Table 2).

Karyotype of *Mentha piperita* L. population 2 (Fig. 2C, D): – Chromosomal analysis of *in vivo* plants revealed the presence of $2n = 60$ chromosomes with symmetrical karyotype: Karyotype formula is $2St (SAT)/m (SAT) + 2St (SAT) + 40Sm + 12m + 4St$. One pair chromosome with $St (SAT)/m (SAT)$ is $2.2 \mu m$ in length and one pair chromosome with $St (SAT)$ is $2.0 \mu m$ in length. Twenty pairs of Sm chromosomes range from $0.8 \mu m$ to $1.6 \mu m$, six pairs of m chromosomes range in length from $1.0 \mu m$ to $1.3 \mu m$ and two pairs of St chromosomes range in length from $0.8 \mu m$ to $1.0 \mu m$. Mean chromosome length was $1.32 \mu m$ and mean chromosome volume was $57.02 \mu m^3$ (Table 2).

Karyotype of *Mentha arvensis* L. population 1 (Fig. 2E, F): – Chromosomal analysis of *in vivo* plants revealed the presence of $2n = 36$ chromosomes with symmetrical karyotype. Karyotype formula is $4St (SAT)/m (SAT) + 20m + 12Sm$. Two pairs of SAT chromosomes range in length from $2.0 \mu m$ to $2.6 \mu m$. Ten pairs of m chromosome length varies in between $0.9 \mu m$ to $1.9 \mu m$ and six pairs of Sm chromosomes range in length from $0.6 \mu m$ to $1.5 \mu m$. Mean chromosome length was $1.66 \mu m$ and mean chromosome volume was $83.18 \mu m^3$ (Table 2).

Karyotype of *Mentha arvensis* L. population 2 (Fig. 2G, H): – Symmetrical karyotype with $2n = 72$ chromosomes: Karyotype formula is $2St (SAT)/Sm (SAT) + 2St (SAT)/m (SAT) + 26m + 32Sm + 10 St$. Two pairs of SAT chromosomes range in length from $1.5 \mu m$ to $1.7 \mu m$. 13 pairs of m chromosome length varies in between $0.7 \mu m$ to $1.4 \mu m$, 16 pairs of Sm chromosomes range in length from $0.6 \mu m$ to $1.4 \mu m$ and five pairs of St chromosome range in length from $0.8 \mu m$ to $1.0 \mu m$. Mean chromosome length was $1.13 \mu m$ and mean chromosome volume was $31.79 \mu m^3$ (Table 2).

Karyotype of *Mentha arvensis* L. population 3 (Fig. 2J, K): – Symmetrical karyotype with $2n = 72$ chromosomes.

Karyotype formula is $4m (SAT)/Sm (SAT) + 18m + 34Sm + 14St + 2M$. Two pairs of SAT chromosomes were $2.2 \mu m$ to $2.3 \mu m$ long. Nine pairs of m chromosome length varies in between $0.9 \mu m$ to $1.5 \mu m$. Length of 17 pairs of Sm chromosomes range in length from $0.6 \mu m$ to $1.4 \mu m$ and one pair of M chromosome was $0.8 \mu m$ long. Mean chromosome length was $1.18 \mu m$ and mean chromosome volume was $45.36 \mu m^3$ (Table 2).

In order to distinguish between different species and populations of *Mentha*, the root, shoot, petiole length, internodal distance as well as their morphological features were taken as principle parameters. The plants of the same age growing under identical conditions had only been considered to avoid errors. The plant height and other characters in the two species, *M. piperita* L. and *M. arvensis* L. differed to a lesser extent. The two populations of *M. piperita* L. differed in respect of their morphological features. In population 1 the internodal distance of the shoots was little shorter (2.8 ± 1.24 cm) than population 2 (3.5 ± 0.72 cm). The internodal lengths were nearly equal in population 1 (2.5 ± 1.38 cm) and population 2 (3.0 ± 1.42 cm) of *M. arvensis* L. but were longer than population 3 (1.5 ± 0.96 cm). Leaf area was smaller in population 3 of field mint (7.02 ± 0.24 cm²) than the other two populations. However, population 2 possessed the highest leaf area (15.02 ± 0.36 cm²) among the five populations of two species studied. Flower color also varied from whitish to whitish lavender to pale purple in all the five populations. All these morphological characters clearly indicated variation among different species. The possible genetic control of these characters was exhibited by their constancy within the species of all the individuals.

The present cytological investigation of two different species and populations of *M. piperita* L. and *M. arvensis* L. revealed the occurrence up to hexaploid constitution of chromosome complement. Two populations of *M. piperita* L. showed $2n=36$ and $2n=60$ chromosomes, where three populations of *M. arvensis* L. showed $2n=72$ and $2n=36$ chromosomes. The chromosome number of this genus, so far recorded, showed multiples of twelve chromosomes indicating twelve is the basic number for this genus [14, 18, 19, 20]. The general chromosome morphology studied in these populations and species of *Mentha* was quite distinct with symmetrical karyotype. However, the species and populations differed from each other in minute karyotypic details (Table 2). These differences were principally the number and combinations of different chromosome types including those with secondary constrictions. The length of chromosomes ranged from short ($0.6 \mu m$) to medium long ($2.6 \mu m$) in size. The primary constrictions were mostly median region (m) to sub median region (Sm). The number of chromosomes with secondary constriction varied from two to four in these two species.

Table 2: karyomorphological features of five populations of two species of *Mentha*.

Plant Material	2n	RTCL (µm)	CP	TCL (µm)	MCL (µm)	TCV (µm ³)	MCV (µm ³)	KF
<i>M. piperita</i> L. population 1	2n=36	0.9 – 2.04	4	60.90	1.69	3062.40	85.06	2St(SAT) + 2St(SAT)/m(SAT) + 26Sm + 6m
<i>M. piperita</i> L. population 2	2n=60	0.8- 1.8	4	79.60	1.32	3421.73	57.03	2St(SAT)/m(SAT) + 2St(SAT) + 40Sm + 12m + 4St
<i>M. arvensis</i> L. population 1	2n=36	0.6 - 2.6	4	59.60	1.66	2994.30	83.18	4St(SAT)/m(SAT) + 20m + 12Sm
<i>M. arvensis</i> L. population 2	2n=72	0.6 - 1.7	4	81.0	1.13	2289.06	31.79	2St(SAT)/Sm(SAT) + 2St(SAT)/m(SAT) + 26m + 32Sm + 10 St
<i>M. arvensis</i> L. population 3	2n=72	0.6 - 2.3	4	84.90	1.18	3265.68	45.36	4m(SAT)/Sm(SAT) + 18m + 34Sm + 14St + 2M

RTCL: Range of total chromosome length; CP: Chromosome pairs with secondary constriction;

TCL: Total chromosome length; MCL: Mean chromosome length; TCV: Total chromosome volume;

MCV: Mean chromosome volume; KF: Karyotype Formula

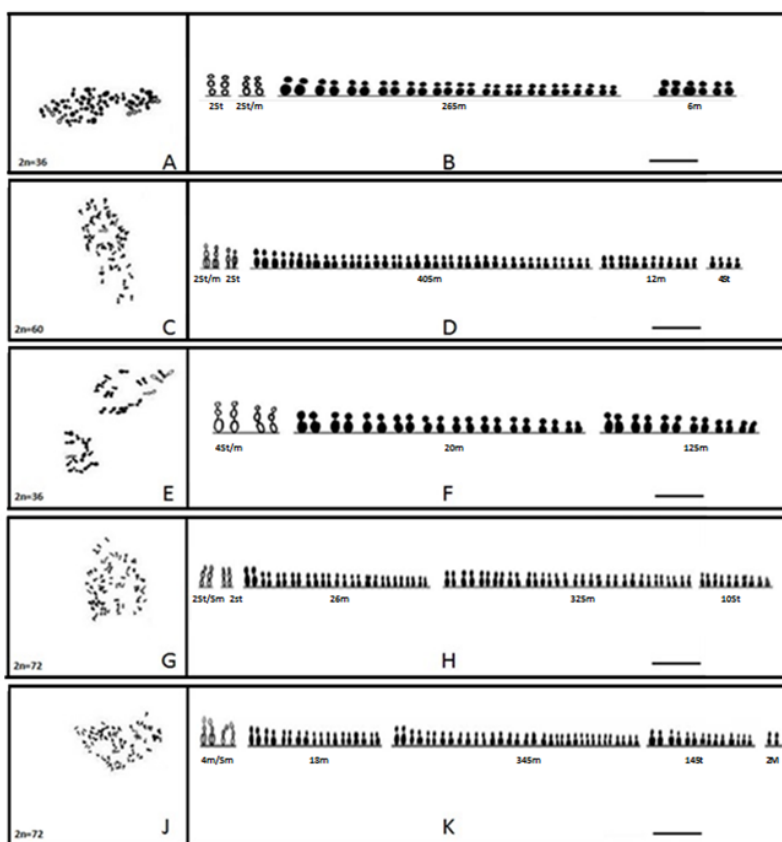


Fig 2: (A, B) Drawing prism sketch and karyogram of *Mentha piperita* L. population 1 (Ca*2500). (C, D) Drawing prism sketch and karyogram of *Mentha piperita* L. population 2 (Ca*2500). (E, F) Drawing prism sketch and karyogram of *Mentha arvensis* L. population 1 (Ca*2500). (G, H) Drawing prism sketch and karyogram of *Mentha arvensis* L. population 2 (Ca*2500). (J, K) Drawing prism sketch and karyogram of *Mentha arvensis* L. population 3 (Ca*2500).

Bar of all metaphase plates = 600 µm and karyograms = 500µm.

The size of the chromosome varied between the species and populations with the difference in ploidy level. Among all the populations of these two species studied, population 1 of *M. piperita* L. contained larger chromosomes. The total chromosome length, mean chromosome length, total chromosome volume and mean chromosome volume differed among the populations. It was interesting to note that the chromosome number variation among the populations had an

effect on total chromosome length and mean chromosome length as well as total chromosome volume and mean chromosome volume [21, 22]. All these data indicated that the five populations of *Mentha* differed in minute karyotypic details and each population contained different types of chromosomes in variable numbers, revealing uniqueness of individual populations of each species.

In several species of angiosperms a reduction in chromosome size had often been noted along with polyploidy and thus had been interpreted as due to differential condensation,

differential polynemy and other factors [23, 24]. Reports were also available where diploid and polyploids had the same chromosome size as in *Urginea indica* [25, 26]. The similarity in chromosome size might have suggested that in species of *Mentha* the condensation of chromosomes more or less remains the same in diploids and polyploids. It might also be under genetic control. The different species studied in the aforesaid investigation showed similarity in gross chromosome morphological features. It was being revealed by symmetric karyotype with chromosomes of medium to short in size. Such observations stated the fact that - Every species differs in minute details of karyotype and therefore, the role of structural changes in species evolution has been indicated [27, 28, 29, 30, 31].

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