Materials and Methods
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One elite clone each of two traditional medicinal plants, namely: (i) *Andrographis paniculata* Nees. (family: Acanthaceae), (ii) *Bacopa monnieri* (L.) Pennel (family Scrophulariaceae), two aromatic essential oil producing genera, namely: (iii) *Cymbopogon khasianus* x *pendulus* Sprengel – clone CKP-25, and (iv) *Mentha* L. (family: Lamiaceae) were used to elucidate the effect of ploidy change on micro-morphological histological characters. Owing to the range of natural polyploidy and variation in plant habit, *Mentha* spp. are examined more exhaustively covering its four important species, namely: *M. spicata* L. (2n=4x=48), *M. x piperata* L. (2n=6x=72), *M. arvensis* L (2n=8x=96) and *M. citrata* L. (2n=8x=96). Whereas, the material for laboratory observations was maintained both at the Botany Department of Lucknow University and Cytogenetics Division of Central Institute of Medicinal and Aromatic Plants, Lucknow, but for field observations and large scale experimentation the material was grown...
and maintained at the Experimental Farm of the Central Institute of Medicinal and Aromatic Plants, Lucknow, under optimum cultural conditions.

**Realization of polyploid progenies**

In all seven accessions belonging to four genera as mentioned above were used. Fast growing vegetative propagules *i.e.* growing shoots in *Bacopa monnieri* and axillary buds on stolons of *Mentha* spp., basal meristems of the leaf-buds in the slips of *Cymbopogon*, and apical meristems of *Andrographis paniculata* were immersed in 0.1% aqueous solution of colchicine for 07 hours, followed by thorough washing. Colchicine treated material was allowed to grow under optimum cultural care under potted conditions to realize polyploidy. Plants that showed stunted growth and thickened leaves in the beginning were sorted and perpetuated to recover polyploidised vegetative tissues and organs. Colchicine affected plants were scored for enlarged stomata, and screened for purity and uniformity in stomatal size. Progenies showing homogeneity in stomatal size were
isolated, cytologically tested for their ploidy status and multiplied vegetatively for further studies.

**Realization of bud-sport variants**

Vegetative propagules from both - the source diploids and realized polyploids were sown in nursery plots. Over three kilograms of suckers per 0.9 M² plot x three replications were applied to raise thick population of vegetative progenies. The emerging seedlings at 6-8 leaf stage were keenly observed to score apparent changes in plant morphology to isolate bud-sport variants. The bud-sport variants thus isolated, were transplanted in well manure plots for their growth and multiplication for detailed macro- and micro-morphological observations.

**Morpho-anatomical studies for histomorphological evaluation**

Screening and scoring of variants and autotetraploids was done on the basis of gigas effect evinced by the autotetraploids and distinctive morphological characters observed in derived variants. Criteria such as stomatal
features, frequency, stomatal index, hairiness index, gland characteristics and gland index were chosen (ref table 1, 2, 3, 4 9). Both, source diploids and their derived autopolyploids in all the four genera and their targeted species were examined for their stem and leaf anatomy with respected polyploid associated changes. Transverse section of stem, vertical section of leaf, and leaf epidermal features with respect to stomatal characteristics were studied following standard safranine – fastgreen counter-staining, and also under UV epifluorescence microscopy.

However, in order to identify the secondary metabolite storage tissue, especially citral rich essential oil storage channels in lemon grass, Cymbopogon khasianus x pendulus, the cross-sections of the leaf and stem were stained by Feulgen according to Lewinsohn et al (1998).

Fully expanded leaves at fourth node in all plants were standardized for measurements. Leaf area was measured by LOCOR’s Leaf area meter. Lower epidermal peel was taken from three leaves of 10 plants each and
three readings each were taken to record observations on stomatal guard cell length and width were recorded for equally turgid stomata, trichomes and essential oil secretary cells / storage glands.

Stomatal index - SI was determined according to Salisbury (1972) i.e. \( \text{SI} = \frac{S}{E+S} \times 100 \); where \( S \) is number of stomata and \( E \) is number of epidermal cells in microscopic field. Similarly, Gland index (Gl) was also calculated taking into account the number of glands comprising of secretary cells. Size of stomatal guard cell, secretory glands, and trichomes were measured by ocular micrometer and then converted to micron through standardization. Well scratched, unilayered epidermal peel was stained with safranin for identification of chloroplasts within guard cells and photomicrographs were taken by Zeiss Axioscope Microscope. Chloroplast number was considered as the useful criteria for recording observations on bud sport variants, source diploid and auto-tetraploid for evaluating degree of variation.
Stem sections were taken from fixed internode position in all mint plants and double staining was done. Position and number of oil glands were recorded in stem section and anatomical details of selected plants were tabulated.

For leaf and stem anatomy stains and chemicals were required

- 30%, 70%, 90% and absolute ethyl alcohol
- Rectified spirit
- Safranin stain
- Light green stain
- Glycerine as mountant

Light green is a basic dye of triphenyl methane series and safranin is chemically 3, 7-Diamino-5-phenyl phenazinium chloride. Staining was done following standard protocol and finally the leaf and stem sections were mounted in glycerine and observed under microscope.
Histo-morphology of bud-sport variants in *Mentha arvensis*

One of the major emphasis of the present study was to take stock of essential oil contributing characters in *Mentha arvensis*. Since, leaves are the main source of essential oil in *Mentha arvensis*, therefore, the study was focused on leaf morphological features including leaf / stem ratio, moisture content, and the essential oil contributing secretary cells – their frequency, distribution and relationship with leaf micro-morphology. Both leaf exomorphology vis-à-vis their frequency and distribution on stem, leaf epidermal features vis-à-vis frequency and occurrence of essential oil contributed secretary were examined exhaustively. Leaf epidermal peels were used to examine micromorphological features and record data under light and epifluorescence microscopy. For description of Leaf morphology, the standard classification on Leaf architecture by Heywood (1978) and its updated version given by Hickey (1979) was followed.
Extraction of essential oil and its qualitative evaluation

Essential oil from the freshly harvested herb was extracted by hydro-distillation at 70°C for two hours using Clevenger’s distillation unit. 200 grams of fresh herb harvested at pre-flowering stage i.e. 110 days from the day of planting, along-with 500 ml of water was applied to the two-liter capacity flask of the distillation unit for optimum distillation. Data on the essential oil so obtained were recorded to estimate essential oil concentration. Further to save oil from degradation / polymerization during storage, the water present in the fresh oil was removed by adding a pinch of sodium sulphate.

The dehydrated oil was analyzed qualitatively by Gas Liquid Chromatography according to standard protocol on a HP 5890 series 2 gas chromatograph equipped with FID using fused silica capillary column (30m x 0.5mm i.e. film thickness of 0.25μm) quoted with polydimethyl siloxane (DB-Wax). Oven temperature was programmed at 70 to 220°C at 4°C/min and held at 220°C for 4 minutes. Stationary phase used was FFAP or supelcoux and
carrier gas hydrogen at an inlet pressure of 5 psi in unsplit form was used; injector temperature 220°C and detector temperature 240°C. The total contents separated at different time value and their respective area was plotted and the major contents recorded (ref table 7, 8, and 10).

**Agronomical Parameters**

After harvesting, total plant weight, whole weight of leaves and stem were taken, once for fresh weight and once for dry weight, (after 15 days shade drying) for individual plants and over 100g and 200g sample. Leaf : stem ratio was calculated to on weight basis in fresh as well shade dried samples.

**Statistical evaluation**

For all observations Standard deviation and standard error of mean was calculated. Coefficient of variation for all parameters related to yield was estimated according to the standard formula:
STD = \sum (x-x)^2 /n  
STD is standard error of deviation

Where \( x \) is the mean value, \( x \) is variable values (values 1, 2, 3 etc) and \( n \) is the number of variables.

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CV = \frac{STD}{Mean} \times 100
\]

Whereas CV is coefficient of variation over a population

and Coefficient of Correlation (r value) as

\[
r = \frac{n(\sum xy)- (\sum x)(\sum y)}{\sqrt{[n\sum x^2-(\sum x)^2][n\sum y^2-(\sum y)^2]}}
\]

\( r \) = numerical measure of linear relationship between \( x \) and \( y \)

\( x \) = deviation of variable \( x \) measured from mean

\( y \) = deviation of variable \( y \) measured from mean

\( n \) = number of pairs of two variables