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In Vivo induction and identification of heteroploids in ginger

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Abstract

Ginger, a commercially important spice, is propagated vegetatively and genetic variations among clones are limited. The study aimed to induce heteroploidy in ginger to create genetic variations that could potentially enhance production, improve quality and disease and insect resistance was undertaken at College of Agriculture, Vellayani, Kerala. Eight superior ginger genotypes were selected and treated with colchicine (0.1%). *In vivo* induction using colchicine resulted in twenty suspected heteroploids. These genotypes were field planted and observed for morphologic, cytologic and yield characters. The morphological characters like plant height and number of leaves per plant were significantly higher in the treated genotypes compared to the control. The chromosome number counting revealed the plants T_1S_5, T_5S_1, T_8S_1 and T_8S_4 to be heteroploids with chromosome numbers 27, 68, 24 and 30 respectively. The histogram peak of the colchicine treated plants T_1S_5, T_8S_1 and T_8S_4 obtained in both channels 50 and 200, confirming heteroploidy. Among the four heteroploids developed, the heteroploid plant, T_8S_1 was found to be highly promising with respect to plant height, number of leaves plant^{-1}, fresh rhizome yield plant^{-1} and dry rhizome yield per plant. The study suggested that induced heteroploidy in ginger plants using colchicine can lead to genetic variations with potentially beneficial traits for cultivation.

Keywords

Ginger; colchicine; polyploidy; heteroploids

Introduction

Ginger (*Zingiber officinale* Rosc. 2n =22), a member of the Zingiberaceae family, is a significant spice crop valued for its pharmacological and therapeutic properties and is rich in secondary metabolites (1). It has a special combination of qualities, including antioxidant, aphrodisiac, antibacterial and anti-inflammatory effects. In European medicine, ginger was a component of most pharmaceutical preparations and it was one of the most highly prized of all mild carminatives (2).

In addition to bold rhizomes, resistance to diseases including bacterial wilt and rhizome rot, reduced fibre, high essential oil and yield are major goals for crop improvement in ginger. (2). Clonal selection is mostly used in breeding ginger because it displays a high level of sterility (3) due to chromosomal aberrations such as inversions and translocations (4,5) and is

vegetatively propagated, resulting in reduced diversity. As ginger lacks natural seed set and variability, crop development techniques like hybridization and selection are ineffective. Therefore, the earliest crop improvement programs concentrated on mutation breeding using ethyl methyl sulfonate (EMS) and gamma rays. Consequently, low yielding mutants were isolated and the impact of the mutagen treatment disappeared in later generations (6).

Ploidy has been essential to both systematic classification and evolution. Compared to their diploid relatives, polyploids exhibit more vigour and superior performance. Morphology is influenced by ploidy level and polyploid plants are found in both horticultural and agricultural crops because they frequently have better morphological traits than their diploid counterparts (7). (8) reported that it may be possible to produce improved tetraploid varieties of ginger from some of the diploid clones. Induction of autotetraploidy tried at Kerala Agricultural University in Himachal Pradesh, Maran, Nadia and Rio de Janeiro cultivars of ginger produced two autotetraploids which recorded higher rhizome yield and stomatal size and lower stomatal frequency than the corresponding diploids (9).

Chromosome number counting and flow cytometry analysis are regarded as direct methods of ploidy confirmation while indirect methods include morphological and stomatal characterization. Colchicine is used as an effective anti-mitotic agent for the induction of polyploidy and the ploidy confirmation can be done using flow cytometry analysis. Flow cytometry (FCM) is so far the most widely used method for determining the amount of nuclear DNA in plants. It makes it possible to quickly measure the fluorescence of several stained nuclei. Because of the relationship between ploidy and nuclear DNA content, the assay can be used to identify mixoploidy, determine ploidy level, and, in some cases, identify aneuploidy (10). FCM is the current method of choice since it is dependable, simple, and fast. Since the procedure typically yields samples from a few tens of milligrams of plant tissues, it is frequently termed non-destructive and appropriate for small-scale research.

In India, so far no polyploid variety has been reported for cultivation in ginger. Hence, an experiment was formulated with the objective to develop heteroploids *in vivo* from ginger using colchicine for superior yield and quality.

Materials and Methods

Eight genotypes were selected for developing heteroploids. Of this, four were the promising varieties (Athira and Aswathy released from Kerala Agricultural University and IISR Varada and IISR Mahima released from ICAR-Indian Institute of Spices Research). Remaining four genotypes (Genotype 1, 2, 3 and 4) were maintained in the Department of Plantation, Spices, Medicinal and Aromatic Crops. The rhizomes were properly cleansed to remove soil particles before being stored. The experiment was conducted in the Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Agriculture, Vellayani, Trivandrum, Kerala, India. For analysis, single bud rhizomes with a prominent bud were taken. The experiment thus had sixteen treatments involving the eight genotypes and their corresponding control plants.

Induction of ploidy

The concentration of colchicine taken was 0.1%. A hole of 3mm diameter and depth was made close to the sprouting bud using a needle and 1 ml of colchicine solution was applied into the hole. The treatment was done between 6 am to 9 am and the period of treatment was 4 hours and repeated for two consecutive days. The rhizome bits treated by colchicine were covered by cotton soaked in colchicine of corresponding percentage over the axillary bud and kept overnight on the second day after the treatment. Thereafter, each treatment was washed in sterile water and air dried (9).

The protrays (50 celled) were filled with coir pith compost containing nursery material and farm yard manure (3:1), enriched with Plant Growth Promoting Rhizobacteria or *Trichoderma* 10g/kg of mixture. The ginger sprouts were planted in protrays and partial shade was provided. The germinated plantlets were planted in polybags between one and half and two months of planting. The polybags were filled with soil, coir pith compost and farm yard manure in 2:1:1 ratio and *Trichoderma* (10g/kg) was added to the media.

Experimental design

The first experiment aimed at developing heteroploids *in vivo* was analysed using Completely Randomised Design (CRD) consisting of 16 treatments and the results were interpreted at 1% probability level using Grapes software, KAU (11). The heteroploids derived from *in vivo* method were field planted and evaluated for morphological and cytological parameters in the second generation and the ploidy level was confirmed by flow cytometry. The design of the experiment was RBD consisting of 16 treatments and 3 replications with 2 plants per replication. The plot size was 1x1 m and the crop duration was 9 months, which was carried out between January 2020 and October 2020. The experimental plot was prepared by ploughing followed by bed preparation in the garden of the Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Agriculture, Vellayani. The treated rhizome bits were planted at a spacing of 20 cm × 20 cm. The experimental site is located at 8˚28'28''N latitude and 76˚57'47''E longitude and at an altitude of 28m above mean sea level. The soil of the experimental location was red loam belonging to the Vellayani series and texturally classified as sandy clay loam. The mean relative humidity, minimum temperature, maximum temperature and rainfall were highest for the months of August (93.20 %), March (24.40 ˚C), March (33.40 ˚C) and May (12.60 mm) respectively during the field trial. The heteroploids thus screened were selected based on yield and quality parameters.

Estimation of morphological characters

Morphological characterization of the treated plants and the suspected plants were done in both the first and second

seasons. Plant height, number of tillers and number of leaves per plant of both diploids and treated plants were estimated at 90, 120 and 180 days after planting (DAP). The height of the plant was expressed in centimetre and measured from the plant's base to the tip of the main shoot's young, completely opened leaf. The number of aerial shoots and leaves produced by each observational plant was counted and mean expressed.

Estimation of anatomical characters

Anatomical parameters like stomatal frequency, length, breadth, epidermal cell number and chloroplast number were only recorded in the first season.

The leaves were collected only after exposure to enough sunlight, preferably in the noon. Using the nail varnish procedure, three samples of epidermal cells were taken. Number of stomata was counted in both control and treated plants and divided with the area to obtain frequency of stomata. The length and width of stomata was measured from five cells selected at random and stomatal size was compared. In a similar manner, the number of cells per millimetre of leaf in both the treated and control plants was used to determine the epidermal cell size. To record stomatal measurements, four leaves from the same section of the diploid and treated plants were selected. Three leaf sections from each plant were examined for chloroplast counts. Using a scalpel, the upper mesophyll tissues were removed, leaving the bottom epidermis. The number of chloroplasts in each of the three leaf samples was determined by counting five pairs of guard cells. An image analyzer (Leica) was used to measure the stomatal characteristics at 40× and 100× magnification (12).

Chromosome counting to confirm ploidy

Plants suspected as polyploids based on morphological and anatomical characters in the first season were subjected to cytological study to confirm the chromosome number. Root tips for analysis were collected from the rhizomes planted in portrays on initiation of root emergence. Between 11:00 and 11:30 a.m., actively growing root tips of 5 to 10 mm length were collected and chromosome counting was done during the mitotic metaphase stages using a Leica DMRB (Leica, Germany) microscope with a 100× objective on three metaphase plates having good chromosome counts (13).

Ploidy confirmation by flow cytometry

A single step protocol was done to prepare nuclear suspensions. A small amount of the leaf of ginger plant (typically 60 mg) was placed in the center of a plastic Petri dish. Around 1 ml of ice-cold Tris.$MgCl_2$ buffer (nuclei isolation buffer) was added to this (14). A sharp, disposable scalpel was used to quickly chop the ginger leaf tissue in the buffer. The homogenate was filtered through a 42-µm nylon mesh into a labeled sample tube. The filtrate was visually inspected to ascertain that it was free of any particles that could cause instrument clogging. Stock solution of a DNA fluorochrome was added and the mixture was gently agitated. DNA fluorochrome employed was Propidium Iodide (PI). PI stock solution was prepared at a concentration of 1 mg ml^{-1}. Preparation was carried out using double distilled water and was filtered using 0.22 µm filter. The finished product was stored at -22˚C in 1 mL aliquots. In a similar manner, 1 mg mL^{-1} of RNase stock solution was prepared. To inactivate DNases, the solution was heated at 90˚C for 15 min during preparation. PI was typically used at 50 µg mL^{-1} simultaneously with RNase at 50 µg mL^{-1}. Before analysis, the sample was incubated on ice for a few minutes to an hour, shaking it periodically. The nuclear DNA content was later examined (10). The FACS machine (BD FACSAria II) analysis was done at Rajiv Gandhi Centre for Biotechnology, Poojapura, Thiruvananthapuram.

The untreated ginger leaf samples were used as the standard (control or diploid) for standardizing the initial weight of the leaf sample to be taken and the rpm of the centrifugation process in order to achieve a suitable histogram. The diploid peak of the control sample was carefully noted. A flow cytometry histogram was acquired for every loaded sample. By contrasting the peaks seen in the treated samples and the control (diploid) samples, the number of chromosomes was deduced. The flow cytometry histogram represents Propidium Iodide- Area in the X axis and count or the number of cells that have taken up the propidium iodide dye in the Y axis. The number of cells corresponding to the ploidy of the ginger genotypes was obtained from the flow histogram.

Estimation of yield parameters

The number of days taken for yellowing and drying of the plant was noted as the maturity period. Harvesting was done eight months after planting after yellowing and drying of the plants. Yield of each plant in terms of fresh weight was recorded and expressed as (g $plant^{-1}$). The rhizomes and roots were washed and allowed to dry in hot air oven at 70+5˚C until constant weight was obtained to obtain dry rhizome and dry root weight (g $plant^{-1}$) respectively.

Results

Evaluation of first generation of plants

The colchicine treated genotypes grew slow initially and exceeded the control genotypes at later stages of growth. At 180 DAP, plant height, number of tillers and number of leaves per plant of treated plants surpassed diploid plants (Table 1). As a result, all the morphological characters recorded were higher in the control genotypes up to 180 DAP. Treated plant T_8 recorded the maximum plant height of 61.32 cm at 180 DAP. The mean plant height also showed significant difference between the treated and control genotypes which was 58.52 cm and 53.14 cm respectively at 180 DAP. At 180 DAP, the maximum number of tillers was recorded in treated plant T_8 (8.17) followed by T_5 (8.00) while the maximum number of leaves was also recorded in treated plant T_8 (75.63) followed by T_6 (74.60) although no-significant.

All the anatomical parameters recorded varied significantly among the different treated and control genotypes (Table 2). Stomatal frequency was found to be lower in the different genotypes of treated plants, the least being recorded in T_5 (54.82 mm^{-2}). The maximum stomatal length of 133.74 µm and stomatal breadth of 90.97 µm was found in the treated plant, T_5. When compared to the

Table 1. Effect of colchicine on the growth characters of ginger genotypes at 90, 120 and 180 DAP

Treatments and control	Plant height (cm)			Number of tillers			Number of leaves		
	90 DAP	120 DAP	180 DAP	90 DAP	120 DAP	180 DAP	90 DAP	120 DAP	180 DAP
T_1	27.85^{c}	35.45^{bc}	59.04^{ab}	3.19^{bcd}	5.33	7.45	12.10^{b}	23.57^{d}	72.00
T_2	26.73^{c}	39.05^{bc}	59.54^{ab}	2.60^{cde}	4.40	6.60	11.40^{b}	22.80^{d}	67.80
T_3	28.73^{c}	36.58^{bc}	60.26^{ab}	2.29^{de}	4.71	7.36	11.93^{b}	26.36^{bcd}	71.75
T_4	28.61^{c}	35.67^{bc}	59.16^{ab}	1.94^{e}	4.59	7.10	9.56^{b}	24.13^{cd}	72.30
T_5	30.90^{c}	40.05^{b}	60.30^{ab}	3.00^{bcde}	5.09	8.00	11.88^{b}	27.25^{abcd}	71.30
T_6	29.12^{c}	33.52^{c}	51.44^{de}	2.10^{de}	4.33	7.00	10.00^{b}	22.33^{d}	74.60
T_7	29.50^{c}	33.08^{c}	57.67^{abc}	2.33^{cde}	3.67	7.67	8.00^{b}	24.33^{bcd}	70.20
T_8	30.46^{c}	38.31^{bc}	61.32^{a}	3.17^{bcd}	5.18	8.17	12.75^{b}	27.11^{abcd}	75.63
C_1	41.13^{ab}	47.41^{a}	56.17^{bcd}	3.60^{abc}	6.00	6.80	24.40^{a}	30.40^{ab}	70.00
C_2	42.10^{ab}	46.77^{a}	53.95^{cde}	3.00^{bcde}	5.40	6.80	21.20^{a}	31.20^{ab}	66.40
C_3	44.85^{a}	50.37^{a}	55.66^{bcd}	4.20^{ab}	6.20	7.00	23.40^{a}	30.60^{ab}	70.00
C_4	39.28^{b}	49.09^{a}	53.69^{cde}	4.40^{a}	5.40	6.40	24.20^{a}	28.50^{abcd}	69.20
C_5	40.35^{b}	47.27^{a}	51.92^{de}	4.40^{a}	6.40	7.40	21.20^{a}	33.00^{a}	69.00
C_6	40.10^{b}	45.40^{a}	49.80^{e}	2.80^{cde}	5.80	6.60	24.20^{a}	29.40^{abc}	70.20
C_7	41.86^{ab}	47.77^{a}	51.66^{de}	3.00^{bcde}	5.40	6.40	24.80^{a}	29.00^{abc}	68.80
C_8	44.64^{a}	50.44^{a}	53.06^{cde}	3.40^{abcd}	6.20	7.80	24.80^{a}	30.20^{ab}	69.20
CD (0.05)	3.80	5.04	4.84	1.09	NS	NS	5.79	6.25	NS
SEm (±)	0.71	0.90	0.85	0.24	0.32	0.34	0.92	1.18	2.25

Table 2. Effect of colchicine on the anatomical parameters of ginger genotypes treated *in vivo* in the first generation

Treatments	Stomatal frequency (mm^{-2})	Stomatal size		Chloroplast number (mm^{-2})	Epidermal cell number (mm^{-2})
		Stomatal length (µm)	Stomatal breadth (µm)		
T_1	83.25^{d}	90.45^{bc}	79.83^{ab}	19.10^{a}	25.10^{c}
T_2	83.25^{d}	87.61^{bcd}	86.43^{ab}	13.00^{bc}	32.20^{b}
T_3	100.51^{c}	68.91^{cde}	67.59^{bc}	17.20^{ab}	29.80^{b}
T_4	102.54^{bc}	60.79^{cde}	57.93^{c}	15.71^{ab}	31.00^{b}
T_5	54.82^{e}	133.74^{a}	90.97^{a}	17.88^{a}	24.80^{c}
T_6	107.61^{abc}	32.97^{e}	30.67^{d}	17.60^{ab}	29.00^{bc}
T_7	106.60^{abc}	40.62^{de}	37.84^{d}	18.20^{a}	28.20^{bc}
T_8	83.25^{d}	103.97^{ab}	78.08^{ab}	18.77^{a}	23.80^{c}
C_1	113.71^{a}	32.70^{e}	23.60^{d}	8.20^{d}	60.60^{a}
C_2	109.64^{ab}	30.78^{e}	24.13^{d}	9.40^{cd}	61.80^{a}
C_3	111.67^{a}	31.79^{e}	23.88^{d}	10.40^{cd}	59.80^{a}
C_4	111.67^{a}	31.93^{e}	23.68^{d}	7.00^{d}	60.60^{a}
C_5	113.71^{a}	30.55^{e}	23.41^{d}	10.40^{cd}	61.20^{a}
C_6	110.66^{ab}	30.70^{e}	23.18^{d}	9.40^{cd}	61.80^{a}
C_7	107.61^{abc}	30.75^{e}	23.09^{d}	11.00^{cd}	60.00^{a}
C_8	111.67^{a}	31.92^{e}	23.13^{d}	8.00^{d}	61.80^{a}
CD (0.05)	8.02	32.96	18.67	4.77	5.33
SEm (±)	2.84	9.42	5.14	1.25	1.34

control, the treated plants had a higher number of chloroplasts, with T_1 having the highest number (19.10 mm^{-2}). Epidermal cell number was found to be higher for the control plants compared to treated genotypes and among treated plants, treatment T_8 recorded the minimum epidermal cell number (23.80 mm^{-2}).

The chromosome number counting was done using aceto-orcein stain using root tips on the suspected ginger plants which is presented in Table 3. The analysis revealed that the colchicine treated plant T_1S_5 of treatment T_1 had a chromosome number of 2n = 27 while the diploid recorded chromosome number of 2n = 22. The colchicine treated plant T_5S_1 of treatment T_5 recorded a chromosome number of 2n = 68 and the corresponding diploid had a chromosome number of 2n = 22. Two colchicine treated plants of treatment T_8 (T_8S_1 and T_8S_4) recorded chromosome number of 2n = 24 and 2n = 30 respectively while the diploid recorded chromosome number is 2n = 22. All the remaining colchicine treated plants recorded diploid chromosome number of 2n = 22.

Evaluation of second generation of plants

From the data obtained in the first year around twenty plants which were suspected to be heteroploids were carried over to the next generation for field planting to study their stability. This was necessary to ascertain the ploidy. The suspected heteroploids were screened based on morphological, cytological and yield characters in the second year. The twenty suspected heteroploids included three treated plants of T_1 (Athira), T_2 (Aswathy), T_3 (IISR Varada), T_4 (IISR Mahima), T_5 (Genotype 1), T_8 (Genotype 4)

Table 3. Effect of colchicine on the cytological parameter of ginger genotypes treated *in vivo*

Chromosome number recorded in the twenty suspected heteroploid plants			
Treatments	**Suspected heteroploid plants**		
T_1	S_1	S_5	S_{10}
	22	27	22
T_2	S_1	S_2	S_3
	22	22	22
T_3	S_1	S_2	S_3
	22	22	22
T_4	S_1	S_2	S_3
	22	22	22
T_5	S_1	S_2	S_3
	68	22	22
T_6	S_1		
	22		
T_7	S_1		
	22		
T_8	S_1	S_3	S_4
	24	22	30

* S- Suspected plant

and one each from T_6 (Genotype 2) and T_7 (Genotype 3).

The treated genotypes recorded significantly higher values for morphological characters like plant height and number of leaves per plant at 90, 120 and 180 DAP. The maximum plant height at 90 DAP was recorded in treatment T_5 (55.52 cm) which was on par with T_1 (54.27 cm), T_8 (53.01 cm), T_2 (51.13 cm) and T_4 (50.50 cm). The plant height of control plants at 90 DAP was maximum in C_8 (44.25 cm), which was on par with all other control genotypes. At 120 DAP, the maximum plant height was recorded in treated plant T_1 (69.75 cm) which was on par with the plant height of all other treated genotypes. The maximum plant height among control plants was recorded in C_5 (50.85 cm) which was on par with all other control genotypes. At 180 DAP the maximum plant height was recorded in treated plant of T_1 (80.78 cm) which was on par with the plant height of all treated genotypes and control genotype C_7 (73.30 cm). Control genotype C_4 recorded a plant height of 68.48 cm which was on par with C_8 (67.12 cm), C_6 (65.37 cm), C_2 (64.78 cm) and C_1 (60.58 cm).

Significant difference in number of tillers were recorded only at 90 and 120 DAP. The significantly higher number of tillers at 90 DAP was produced by treatments T_8 and T_5 while at 120 DAP, the number of tillers produced by the treatment T_1 was significantly higher. At 120 DAP, the maximum number of tillers was produced by the treated plant T_1 (9.44) which was on par with all other treated genotypes except T_3 (8.00). The significantly higher number

Table 4. Effect of colchicine on the plant height, number of tillers and number of leaves of heteroploid ginger genotypes

Treatments and control	**Plant height (cm)**			**Number of tillers**			**Number of leaves**		
	90 DAP	**120 DAP**	**180 DAP**	**90 DAP**	**120 DAP**	**180 DAP**	**90 DAP**	**120 DAP**	**180 DAP**
T_1	54.27^{a}	69.75^{a}	80.78^{a}	5.70^{ab}	9.44^{a}	10.90	30.30^{ab}	53.00^{a}	110.00^{a}
T_2	51.13^{abc}	68.70^{a}	73.48^{abcde}	5.67^{ab}	8.67^{ab}	9.33	29.67^{ab}	51.67^{ab}	95.67^{b}
T_3	48.75^{bcd}	66.17^{ab}	77.25^{ab}	5.33^{abc}	8.00^{b}	9.67	27.67^{abc}	49.00^{ab}	97.67^{a}
T_4	50.50^{abc}	67.63^{a}	76.39^{abc}	5.67^{ab}	8.67^{ab}	8.00	27.00^{bcd}	49.33^{ab}	100.00^{a}
T_5	55.52^{a}	66.79^{ab}	75.98^{abc}	6.00^{a}	9.33^{a}	10.00	31.00^{a}	50.00^{ab}	103.33^{a}
T_6	49.18^{bcd}	64.38^{ab}	75.00^{abcd}	5.33^{abc}	8.33^{ab}	9.00	29.00^{ab}	51.00^{ab}	99.00^{a}
T_7	47.58^{cde}	64.44^{ab}	74.93^{abcd}	5.33^{abc}	8.67^{ab}	8.00	28.67^{ab}	51.33^{ab}	92.67^{b}
T_8	53.01^{ab}	69.43^{a}	80.33^{a}	6.00^{a}	9.33^{a}	10.00	29.33^{ab}	54.67^{a}	100.00^{a}
C_1	42.00^{f}	49.12^{c}	60.58^{ghi}	4.67^{abc}	6.00^{c}	7.00	22.33^{e}	30.00^{d}	73.67^{cd}
C_2	42.00^{f}	49.17^{c}	64.78^{fghi}	4.00^{c}	5.67^{c}	7.67	24.00^{de}	32.00^{cd}	68.33^{d}
C_3	43.42^{ef}	49.50^{c}	58.10^{i}	4.33^{bc}	6.00^{c}	8.00	22.67^{e}	32.67^{cd}	73.67^{cd}
C_4	42.50^{ef}	49.43^{c}	68.48^{cdefg}	4.33^{bc}	5.67^{c}	8.00	22.33^{e}	33.67^{cd}	72.00^{cd}
C_5	42.22^{f}	50.85^{c}	59.17^{hi}	4.00^{c}	6.67^{c}	8.67	25.00^{cde}	37.67^{c}	71.33^{d}
C_6	39.22^{f}	48.82^{c}	65.37^{efghi}	4.33^{bc}	5.67^{c}	9.33	23.67^{de}	33.00^{cd}	72.00^{cd}
C_7	43.33^{f}	49.19^{c}	73.30^{abcde}	4.33^{bc}	6.33^{c}	9.00	23.00^{e}	33.67^{cd}	73.67^{cd}
C_8	44.25^{def}	49.95^{c}	67.12^{defgh}	4.00^{c}	6.00^{c}	8.00	24.00^{de}	29.00^{d}	73.67^{cd}
CD (0.05)	5.31	5.58	8.21	1.46	1.28	NS	3.53	6.17	12.45
SEm (±)	1.84	1.93	2.84	0.51	0.44	0.65	1.22	2.14	4.31

of leaves at 180 DAP was also recorded in treatment T_1 (110.00) (Table 4).

The chromosome number counting confirmed the plants T_1S_5, T_5S_1, T_8S_1 and T_8S_4 to be heteroploids with chromosome numbers 27, 68, 24 and 30 respectively. The histogram peak of the colchicine treated plants T_1S_5, T_8S_1 and T_8S_4 were obtained in both channels 50 and 200, confirming heteroploidy. The histogram peak of the treated plant genotype, T_5S_1 was obtained only in channel 200 also confirming heteroploidy.

The significantly highest fresh rhizome yield was recorded in control plant C_7 (315.17 g plant^{-1}) which was on par with colchicine treated plants of T_5 (305.00 g plant^{-1}), T_8 (301.88 g plant^{-1}), T_4 (295.00 g plant^{-1}), T_1 (290.47 g plant^{-1}), T_3 (258.17 g plant^{-1}), control genotypes of C_5 (248.67 g plant^{-1}), C_6 (237.00 g plant^{-1}) and C_8 (236.67 g plant^{-1}). The maximum dry rhizome yield was recorded in control plant of C_7 (59.42 g plant^{-1}) which was on par with treated plant T_8 (58.80 g plant^{-1}), T_4 (57.39 g plant^{-1}), T_1 (56.27 g plant^{-1}), control plant C_5 (50.21 g plant^{-1}), treated genotypes T_3 (50.07 g plant^{-1}), T_5 (48.86 g plant^{-1}), T_2 (45.00 g plant^{-1}), control genotypes C_6 (48.18 g plant^{-1}) and C_8 (47.03 g plant^{-1}).

The highest fresh rhizome yield per plot was recorded in control plant C_7 (1885.50 g plot^{-1}) followed by treated plant T_5 (1825.00 g plot^{-1}), T_8 (1815.65 g plot^{-1}) and T_4 (1775.00 g plot^{-1}). The maximum dry rhizome yield per plot was recorded in control plant C_7 (358.25 g plot^{-1}) followed by treated plant of T_8 (359.39 g plot^{-1}) T_4 (344.17 g plot^{-1}) and T_1 (329.80 g plot^{-1}). The significantly higher dry root yield was recorded in treated plant T_5 (4.35 g plant^{-1}) (Table 5).

Characterization of the heteroploids in the second year

Morphological characters

The plant height recorded in the heteroploid plants and their corresponding control plants showed significant variation at 90, 120 and 180 DAP (Table 6). The maximum plant height at 90 and 120 DAP was recorded in the heteroploid plant T_8S_4 which was 58.75 cm and 69.94 cm respectively while the maximum plant height at 180 DAP was recorded in the heteroploid plant T_8S_1 (81.18 cm). At 180 DAP, the maximum number of tillers were produced in the heteroploid plant T_1S_5 (11.00) and was on par with T_8S_1 (10.50), T_8S_4 (10.00) and T_5S_1 (9.50). The maximum number of leaves at 120 and 180 DAP was produced in the heteroploid plant T_8S_1 (54.50 and 111.00 respectively) while its corresponding diploid recorded 30.00 and 78.00 number of leaves respectively.

Yield characters

The maximum fresh rhizome yield per plant was recorded in the heteroploid plant T_8S_1 (300.00 g/plant) which was on par with T_1S_5 (285.40 g/plant), T_8S_4 (282.50 g/plant), T_5S_1 (267.50 g/plant) and control plant C_5 (249.00 g/plant). There was significant difference in dry rhizome yield among the different heteroploids and control plants and the maximum dry rhizome yield per plant was recorded in heteroploid plant T_8S_1 (57.30 g/plant).

Table 5. Effect of colchicine on yield parameters of suspected ginger plants treated *in vivo* in the second year

Treatments	Maturity period (days)	Fresh rhizome yield (g/plant)	Fresh rhizome yield (g/plot)	Dry rhizome yield (g/plant)	Dry rhizome yield (g/plot)	Dry root yield (g/plant)
T_1	234	290.47^{abc}	1741.40	56.27^{ab}	329.80	4.10^{ab}
T_2	240	226.92^{bcde}	1360.75	45.00^{abcde}	259.99	2.57^{cde}
T_3	235	258.17^{abcde}	1552.50	50.07^{abc}	294.21	2.47^{cde}
T_4	234	295.00^{abc}	1775.00	57.39^{ab}	344.17	2.55^{cde}
T_5	238	305.00^{ab}	1825.00	48.86^{abcd}	291.59	4.35^{a}
T_6	236	193.33^{e}	1170.00	38.25^{cde}	238.74	2.76^{bcde}
T_7	237	209.00^{de}	1257.00	42.84^{bcde}	263.53	3.57^{abcd}
T_8	240	301.88^{ab}	1815.65	58.80^{a}	359.39	4.03^{ab}
C_1	245	187.03^{e}	1131.10	35.77^{e}	211.31	2.27^{de}
C_2	240	200.00^{de}	1190.00	38.66^{cde}	229.99	2.04^{e}
C_3	245	211.67^{de}	1275.00	41.98^{bcde}	245.93	2.16^{e}
C_4	236	196.67^{e}	1182.00	37.95^{de}	221.85	1.76^{e}
C_5	240	248.67^{abcde}	1496.00	50.21^{abc}	300.62	2.13^{e}
C_6	244	237.00^{abcde}	1411.00	48.18^{abcd}	292.55	2.10^{e}
C_7	230	315.17^{a}	1885.50	59.42^{a}	358.25	3.04^{abcde}
C_8	243	236.67^{abcde}	1410.00	47.03^{abcd}	283.10	2.39^{cde}
CD (0.05)	NS	82.69	4.07	15.12	0.47	1.38
SEm (±)	0.55	28.63	1.35	5.24	0.15	0.48

Table 6. Morphological characters, yield parameters and chromosome number of heteroploids in the second year

Heteroploids and control	Plant height (cm)			Number of tillers			Number of leaves			Fresh rhizome yield (g/plant)	Dry rhizome yield (g/ plant)	Dry root yield per plant	Chromosome number
	90 DAP	120 DAP	180 DAP	90 DAP	120 DAP	180 DAP	90 DAP	120 DAP	180 DAP				
T_1S_5	52.56^{b}	69.38^{a}	81.07^{a}	5.50	9.50^{a}	11.00^{a}	31.00^{a}	50.50^{a}	110.00^{a}	285.40^{ab}	56.02^{ab}	4.89	27
C_1	41.00^{d}	49.61^{b}	60.22^{d}	4.50	6.00^{b}	7.50^{d}	23.00^{c}	31.00^{c}	78.50^{c}	190.00^{c}	35.83^{d}	3.00	22
T_5S_1	54.68^{b}	70.25^{a}	78.20^{b}	5.50	9.00^{a}	9.50^{abc}	30.50^{a}	50.50^{a}	100.00^{b}	267.50^{ab}	47.85^{c}	2.57	68
C_5	42.61^{cd}	50.35^{b}	58.09^{d}	4.50	6.50^{b}	8.50^{cd}	25.50^{b}	42.00^{b}	77.50^{c}	249.00^{ab}	49.25^{bc}	3.65	22
T_8S_1	54.77^{b}	69.00^{a}	81.18^{a}	6.00	9.50^{a}	10.50^{ab}	29.50^{a}	54.50^{a}	111.00^{a}	300.00^{a}	57.30^{a}	2.83	24
T_8S_4	58.75^{a}	69.94^{a}	80.62^{a}	6.00	9.00^{a}	10.00^{abc}	29.50^{a}	53.00^{a}	94.50^{b}	282.50^{ab}	53.00^{abc}	3.15	30
C_8	43.75^{c}	49.40^{b}	67.56^{c}	4.50	6.50^{b}	9.00^{bcd}	25.00^{bc}	30.00^{c}	78.00^{c}	244.00^{b}	48.00^{c}	3.21	22
CD (0.05)	2.22	2.61	2.16	NS	0.93	1.92	2.39	6.58	9.05	53.37	7.30	NS	
SEm (±)	0.64	0.76	0.63	0.58	0.27	0.56	0.69	1.90	2.61	15.42	2.11	0.47	

Discussion

Colchicine, an alkaloid substance obtained from *Colchicum autumnale* L., is the most commonly utilized antimitotic agent to induce polyploidy in numerous species. Colchicine inhibits spindle formation by binding to microscopic proteins (15). (16) observed in *Musa acuminata* that the lethal effect of colchicine interferes with the germination process and viability by interfering with the enzymes involved in the process of germination due to its toxic effect. A colchicine concentration of 0.1 % was used in this study in reference to previous studies conducted by (9) in ginger wherein she obtained two autotetraploid ginger plants using colchicine. The autotetraploid plants obtained by her were characterized by slower initial growth, increased leaf area, stomatal size, epidermal cell size and a very high pollen fertility over the control. She also obtained stability in rhizome yield in both the generations.

The morphological and cytological features of treated plants showed variations on comparison with their diploid counterparts. Therefore, the morphological features like plant height, number of tillers, number of leaves and the anatomical features like stomatal count and size and chloroplast number of the treated plants were critically examined to select putative heteroploids to be carried over to the next generation. Around twenty such plants were identified and planted in the second generation along with a few other selected plants which showed better performance along with their corresponding diploid counterparts. Screening of plants on the basis of morphological and cytological features have been discussed in some other studies in ginger (17, 18). (19) reported stomatal size to be an important indicator to detect polyploids. (20) concluded that there is a significant correlation between ploidy of plants and their stomatal number, size and chloroplast number. The efficiency of stomata size in distinguishing plants with varying ploidy levels has also been employed in many other plant species (16).

In this study the suspected plants that were carried over to the next generation showed an initial slow growth that later surpassed the control plants at 180 DAP which might be attributed to the smaller amounts of growth hormones present at the initial stage (21). This lethal effect of colchicine has been discussed in previous studies by (22) in *Vicia faba* and (18) in ginger. The suspected plants showed improved morphological features after 180 DAP. There was an increase in plant height, number of tillers and number of leaves in the suspected plants. Plant morphological characters are reported to be better indicators of ploidy than screening polyploids using stomatal characters (23). Leaf morphological features have also been used in previous studies conducted in *Platanus acerifolia* to pre-screen tetraploids (23). Enhanced morphological features have been reported in previous studies conducted by (17, 24) in ginger; (25) in parsley. Gigas characters in induced polyploids of ginger including an increase in size of plant parts like leaves and the rhizome was reported by (8).

Other anatomical characters like the stomatal frequency decreased (Figs. 5 and 6) while the stomatal size was found to increase in the suspected plants (Figs. 1 to 4). (26) reported that the stomata of polyploids are larger and exist at a lower density than diploids. Similar reports have been recorded in many other plants such as *Anise hyssop* (12). The chloroplast number was also higher in treated plants compared to the control like the stomatal size (Figs. 7 and 8) while the epidermal size reduced.

The suspected heteroploids along with their diploid counterparts were planted in the second generation in field for evaluation of the stability of these genotypes. The performance of treated genotypes in the second generation were better than the control genotypes. The slow sprouting of colchicine treated rhizomes in the first generation was not noticed in the second generation. Ginger buds require some recuperation time since the toxicity of colchicine causes sluggish sprouting. This initial delayed growth was not noticed in the subsequent generations as also reported by (18). Similar observations were also made in Hedychium (27). The plant height of the treated genotypes was better than their corresponding control at all stages of observation. There was significant difference in plant height between the different treated and control genotypes at 90, 120 and 180 DAP (Table 26). Treatments T_1, T_5 and T_8 were confirmed to be heteroploids. (28) also observed that the treated plants grew vigorously as the control plants. Similar observations on plant height of tetraploids were also recorded by (25) in parsley. Colchicine treated genotypes produced a greater number of leaves than control genotypes and the results were also significant at all stages of observation. Thicker and dark green leaves were also reported in tetraploids of *Ocimum basilicum* (20). (14)

Scale bars = 40µm, Magnification = 100 ×

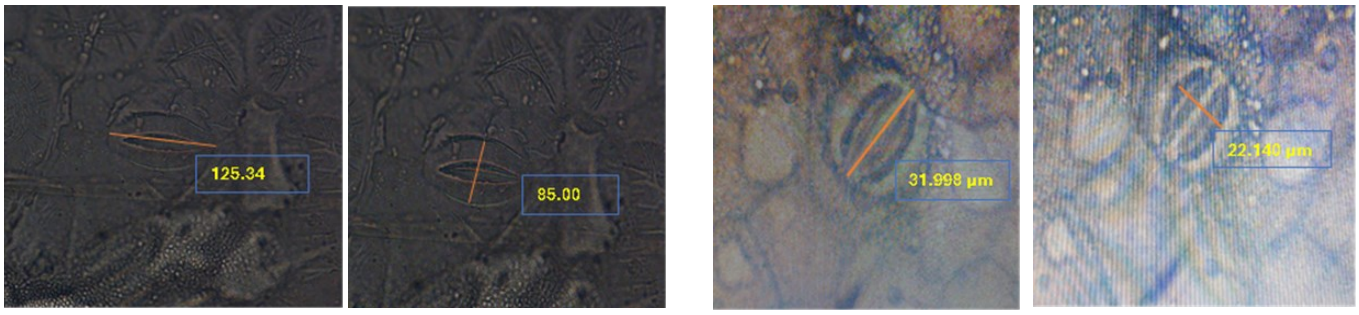


Fig. 1. (A) Stomatal length and (B) breadth of suspected heteroploid plant T_1S_5 (C) Stomatal length and (D) breadth of control C_1

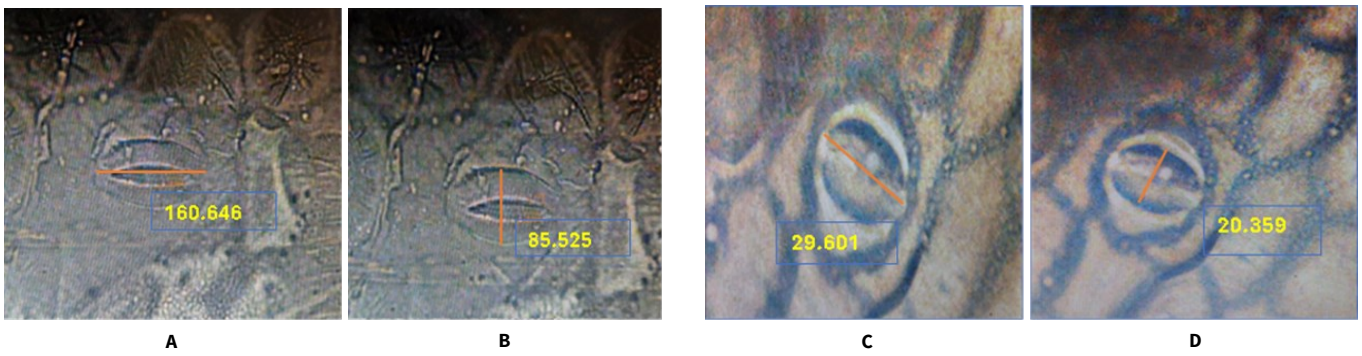


Fig. 2. (A) Stomatal length and (B) breadth of suspected heteroploid plant T_5S_1 (C) Stomatal length and (D) breadth of control C_5

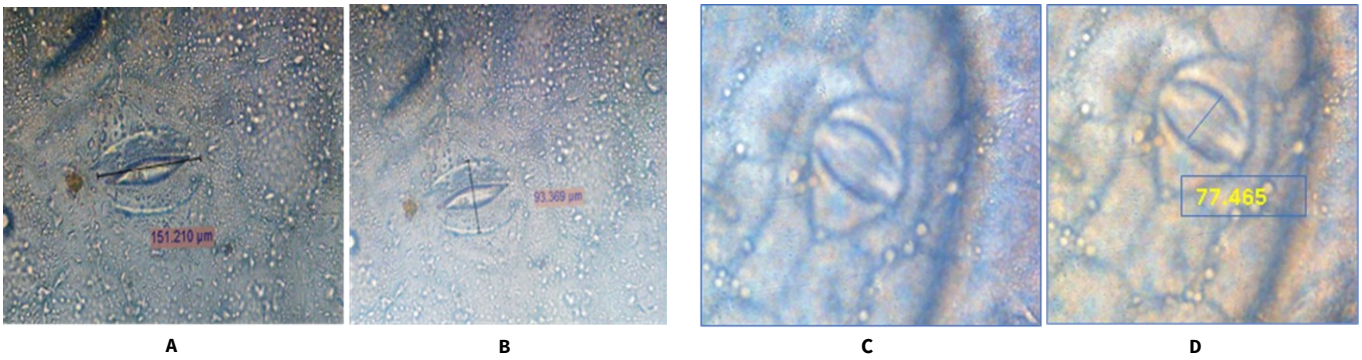


Fig. 3. Stomatal length and breadth of suspected heteroploid plants (A) & (B) T_8S_1 and (C) & (D) T_8S_4

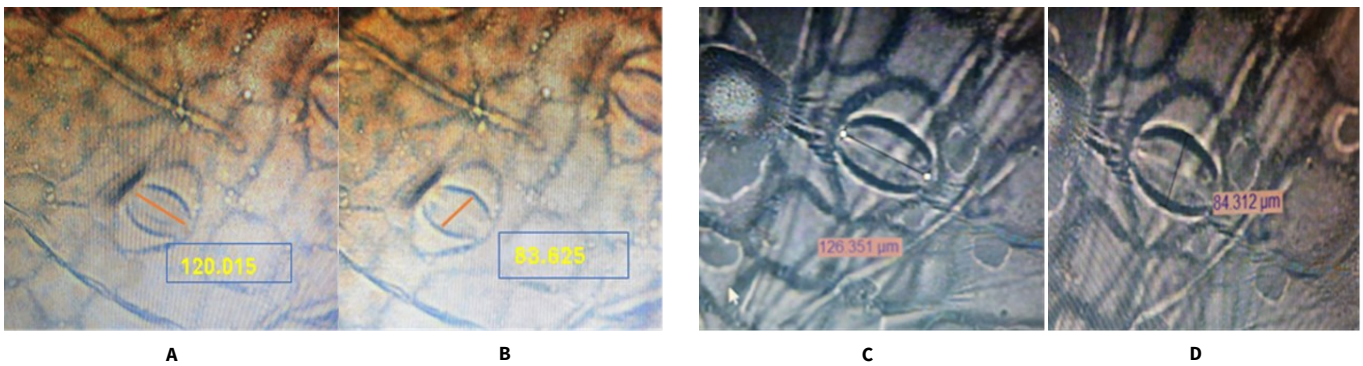
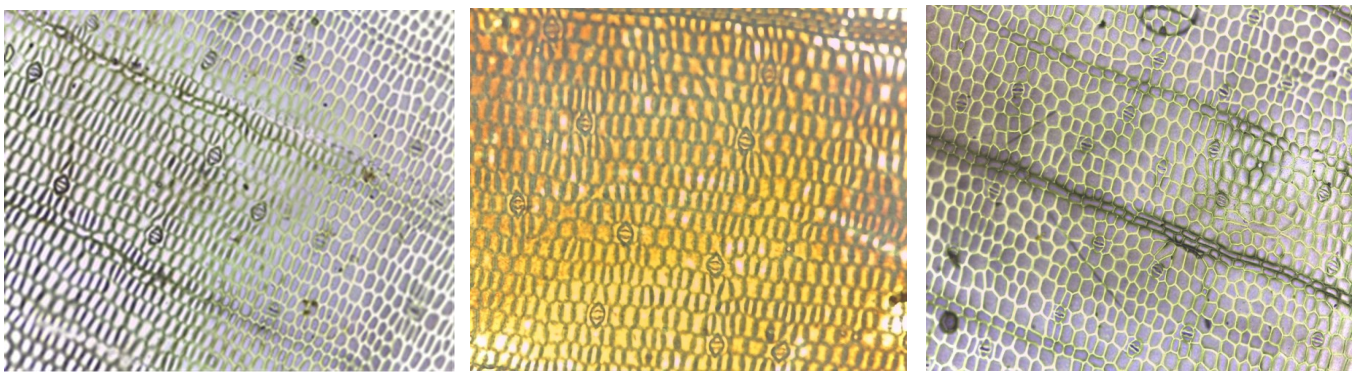


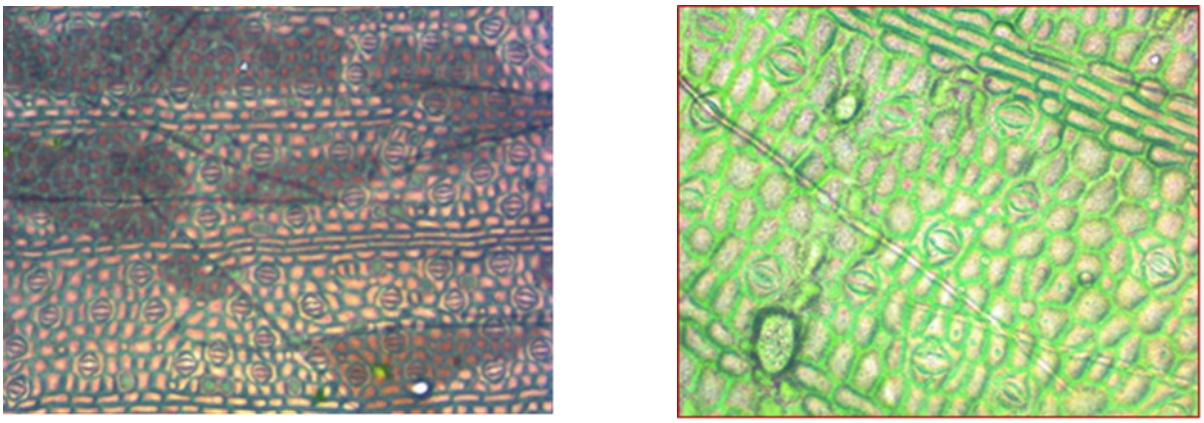
Fig. 4. Stomatal length and breadth of suspected heteroploid plants (A) & (B) T_1S_1 and (C) & (D) T_1S_{10}

Scale bars= 10µm, Magnification= 10 ×



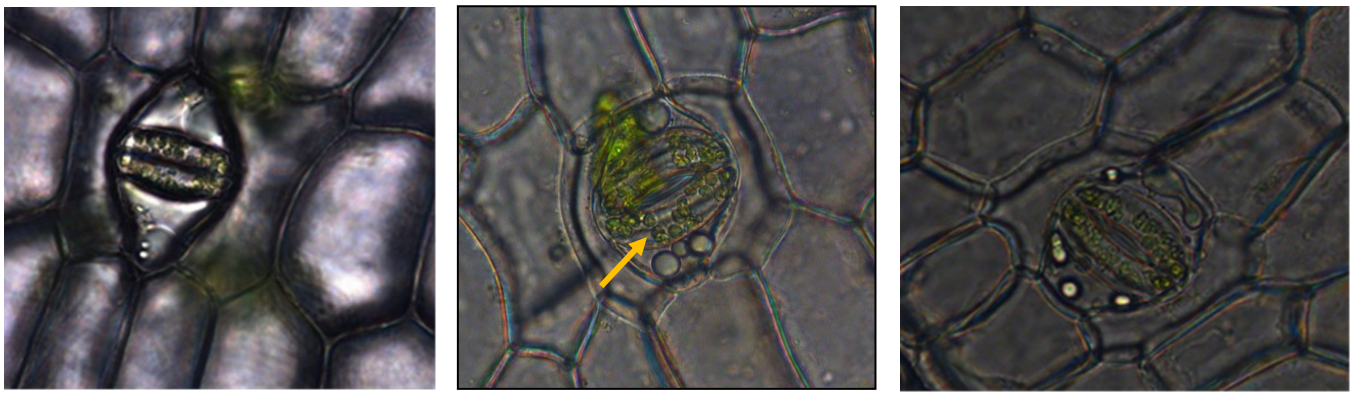
A B C

Fig. 5. Stomatal number seen in suspected heteroploid plants (A) T_5S_1 (B) T_1S_5 and (C) diploid plant



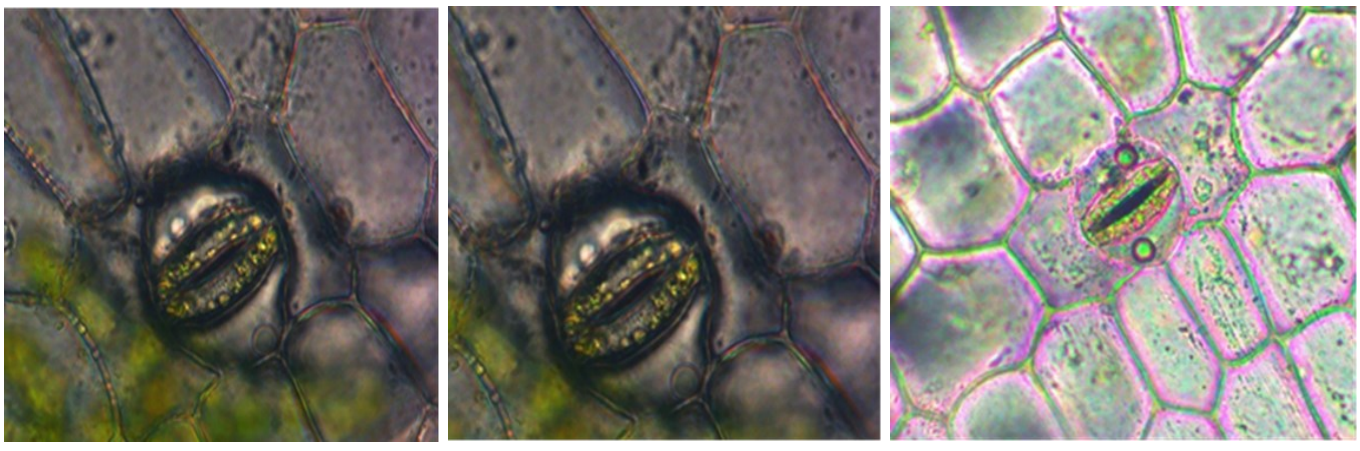
A B

Fig. 6. Stomatal number seen in suspected heteroploid plant (A) T_8S_4 and (B) diploid plant



A B C

Fig. 7. Chloroplast number in suspected heteroploid plants (A) T_8S_1 (20) (B) T_5S_1 (21) and (C) T_8S_4 (15)



A B C

Fig. 8. Chloroplast number in the diploid plants

reported that polyploidy resulted in increased cell size in genotypes which will in turn benefit their commercialization and cultivation.

The anti-mitotic properties of the chromosome doubling agent, colchicine, results in the disruption of microtubules to produce polyploids or colchiploids. The widely reported chromosome number of this rhizomatous vegetative plant is 2n = 22 with a basic chromosome number of x = 11 (29). Among the twenty suspected plants four were heteroploids as confirmed by cytological study. The four heteroploid plants (T_1S_5, T_5S_1, T_8S_1 and T_8S_4) recorded chromosome numbers of 27, 68, 24 and 30 respectively (Figs. 9 and 10). The remaining sixteen suspected heteroploids recorded somatic chromosome number of 2n = 22. (13) reported an aneuploid somatic chromosome number of 2n = 24 in an accession number 147 and chromosome numbers of 2n = 22, 2n = 30, 2n = 34 and 2n = 42 in another accession number 195. Natural occurrence of mixoploid ginger has also been reported by (30) and (13). A somatic chromosome number of 2n = 24 has been reported previously in a ginger cultivar (31).

FCM has been used to identify polyploid ginger plants induced through chromosomal doubling of diploid material. The peak position of the G_1 nuclei of a standard ginger plant was compared with the peak position of the G_1 nuclei of the known sample to estimate the ploidy level. The ploidy level of diploid and colchicine treated genotypes was satisfactorily ascertained using flow cytometry as depicted by representatives of histograms showing diploids (2x) (Fig. 11) and heteroploids. Given that younger tissues with lower concentrations of starch and other metabolites were utilized in the investigation compared to older tissues, the trustworthiness of the data was indicated by the coefficients of variation, which were generally less than 2.0 (32). Out of the twenty suspected heteroploids only one fourth of them were confirmed to be heteroploids, confirming the greater reliability of FCM analysis (23).

Chromosome counting enables assessment of the results obtained through FCM. On comparison of the results of chromosome counting and flow cytometry histogram it can be interpreted that the plant, T_5S_1 is a heteroploid with chromosome number of 2n = 68 as its histogram peak was obtained only in 200X (Fig. 12B). The remaining plants, T_1S_5,

Scale bars= 40µm, Magnification= 100 ×

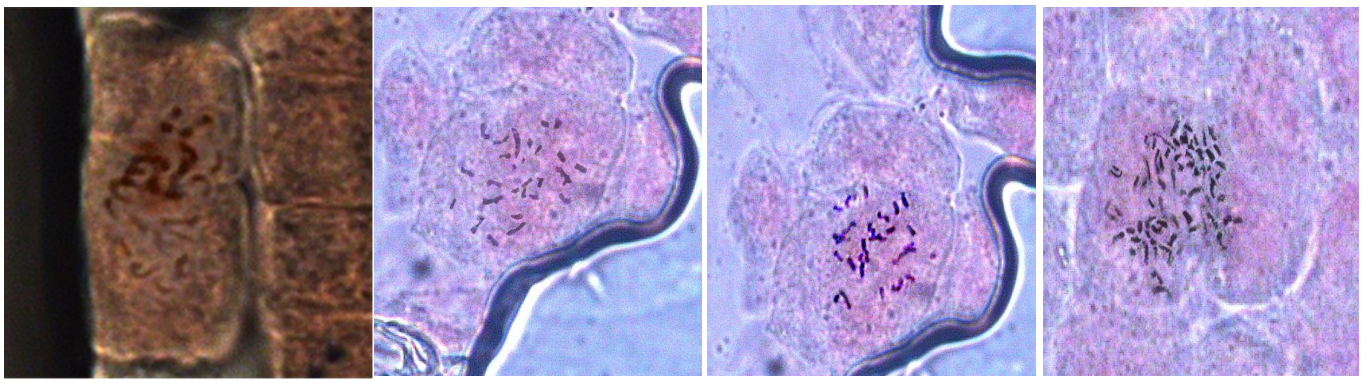


Fig. 9. (A) & (B) Chromosome number 2n = 22 and 2n = 27 recorded in the control T_1 and heteroploid plant of T_1S_5 obtained *in vivo* (C) & (D) Chromosome number 2n = 22 and 2n = 68 recorded in the control T_5 and heteroploid plant of T_5S_1 obtained *in vivo*

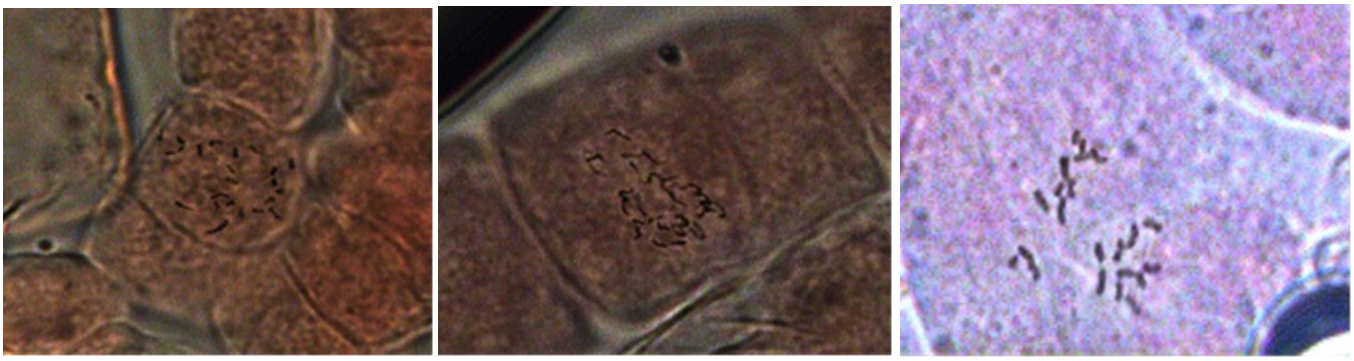


Fig. 10. (A) Chromosome number 2n = 24 in heteroploid T_8S_1 (B) 2n = 30 in heteroploid T_8S_4 produced *in vivo* and (C) 2n = 22 in the control C_8

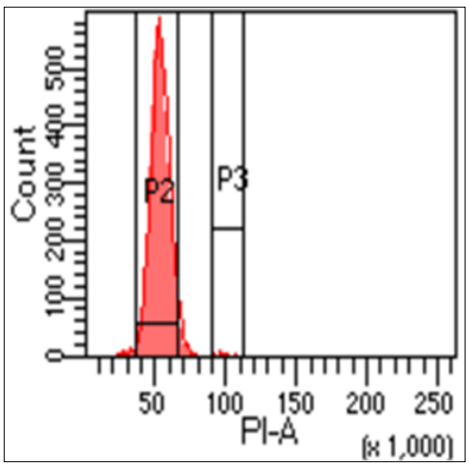
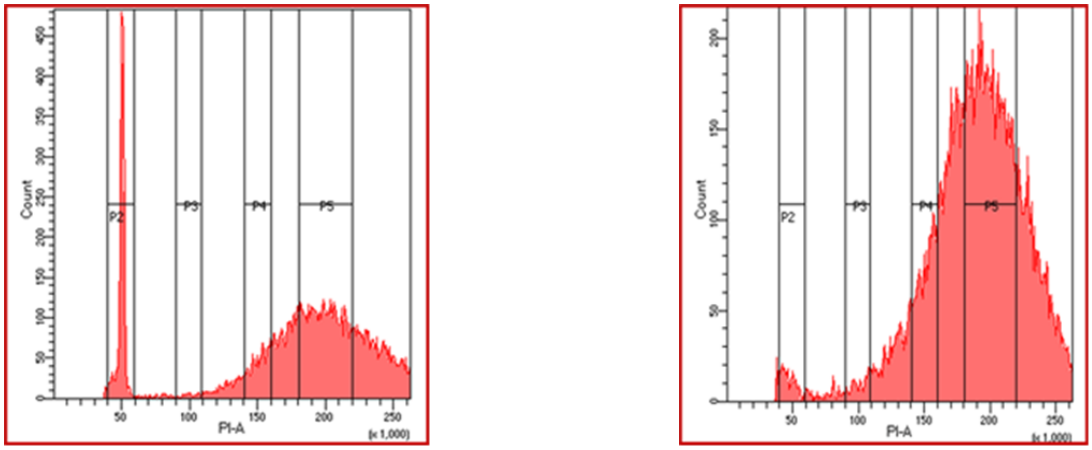


Fig. 11. Flow cytometry histogram of the ginger (standard) which is the diploid control

T_8S_1 and T_8S_4 could be thought of as heteroploid-mixoploids (Figs. 12A, 13A and 13B) having both diploid and tetraploid or triploid or other subsequent ploidy levels as flow cytometry histogram produced peaks at P_2 population (diploid condition) and P_3, P_4 and P_5 populations. But chromosome counting could only confirm chromosome numbers of 2n=27 in T_1S_5, 2n=24 in T_8S_1 and 2n=30 in T_8S_4.

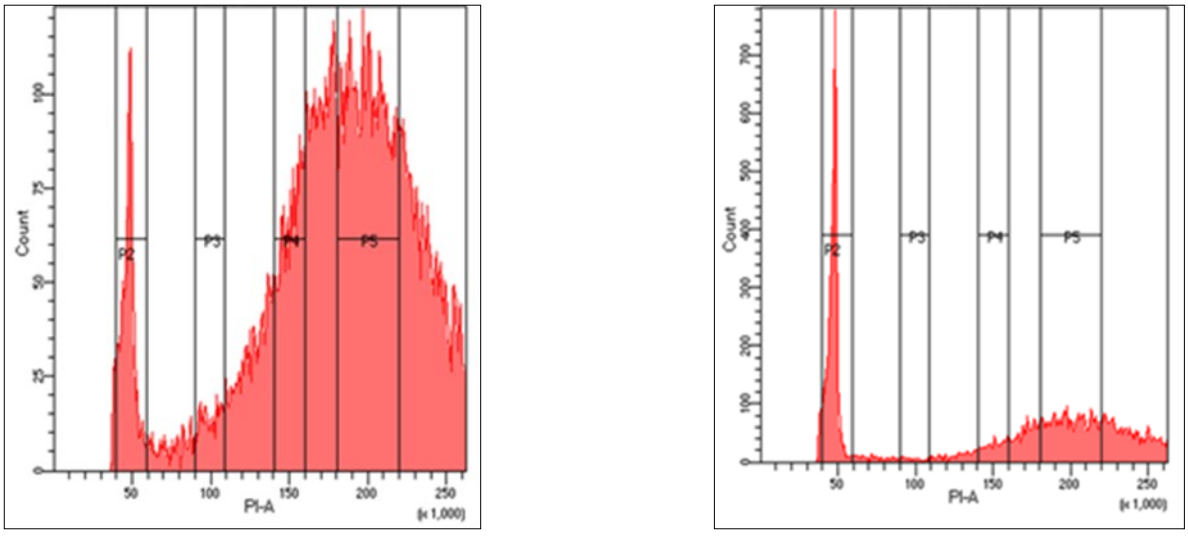
The phenomenon of heteroploidy was frequently observed in our investigation, and FCM was quite efficient in recognizing these cases. It is equally efficient in detecting a huge number of cells in a relatively short span of time. Antimitotic chemicals may not always have the ability to reach all of a plant's meristems, let alone those that are actively dividing, which could lead to the emergence of diploids (33). According to (34), the antimitotic chemical can only penetrate to the deepest meristematic layer after an extended application time during which the cells previously affected might have been killed which can also result in mixoploids. Also, that, the meristematic cells cannot be all at the same time, at the same stage of division. Since all the control plants were diploids, no spontaneous polyploidization occurred but polyploidy was induced in colchicine treated genotypes. (35), obtained four types of polyploids in their study with *Hedychium muluense* (ornamental ginger) plants, which included triploid, tetraploid and mixoploid plants (2x + 4x and 3x + 4x). (30), reported the occurrence of mixoploids having diploid-tetraploid cells in germplasm samples of ginger from China. (36), reported that the progenies of tetraploids consisted of various portions of diploids and tetraploids in chickpea. They observed that this might be either due to the presence of both diploid and tetraploid shoots or due to the reversal of ploidy. They explained that the ploidy can be reversed due to accidental fusion of gametes during meiosis, which results in eight chromosomes generated by uneven chromosome separation at Anaphase I. (37) suggested that the development of diploid cells in tetraploids during pre-meiosis stage could lead to inversion. Another rationale was provided by (38) who stated that the development of multipolar spindles occurs in complement fractionation, which leads to a diploid condition.

Although we obtained heteroploid-mixoploids in the study, it can be considered successful because mixoploid plants can later result in the development of autotetraploids. (39) obtained an entirely autotetraploid population of *T. foenumgraecum* by the culture of seeds stemming from tetraploid branches of mixoploid plant. Tetraploid plants were obtained from mixoploid *Dioscorea*



A B

Fig. 12. Flow cytometry histogram of the heteroploid plants of ginger produced *in vivo* (A) (T_1S_5) having a chromosome number of 2n = 27 (B) (T_5S_1) having a chromosome number of 2n = 68



A B

Fig. 13. Flow cytometry histogram of the heteroploid plants of ginger produced *in vivo* (A) (T_8S_1) having a chromosome number of 2n = 24 (B) (T_8S_4) having a chromosome number of 2n = 30

zingiberensis plants (40). (41) were also able to separate diploid and tetraploid *Lolium perenne* L. from mixoploid ryegrass generated by treating germinating seeds with colchicine.

On the other hand, mixoploid plants are considered unstable as competition occurs between polyploid and original cells resulting in the elimination of the former. Thus, mixoploid status can be reversed to the original ploidy level. In some studies, deliberate efforts are made to eliminate mixoploids as they are often regarded as undesirable by products of polyploidization studies mainly through mechanical isolation of putative polyploids, using nodal segments for regeneration of shoots and by using apical buds for repeated subcultures (42). Another observation with mixoploids is that they do not keep their ploidy level in subsequent generations (43). Stable synthetic tetraploids have been earlier reported in previous studies in *Rhododendron* L. (44). (45) observed that tetraploid plants *Acacia mangium* Willd. were later reclassified as diploids and mixoploids about 16 months later when the plants were transferred under field conditions showing their instability.

The maximum yields at harvest were obtained in control genotype, C_7, treated genotype T_5 and T_8. Similarly, dry rhizome yields were also higher in C_8, T_5 and T_8. Higher dry root yields were obtained in T_5, T_1 and T_8. The greater yield in control genotype, C_7 could be attributed to the larger and bold rhizomes. But induction of polyploidy was difficult in this genotype as the rhizome buds did not survive the lethality of the chemical and subsequently the sprouting percentage was very less. In *Cannabis sativa* (46) polyploid plants were found to be lower and the changes were also hardly visible on comparison with diploids. (36) recorded that although pollen fertility was higher in the tetraploids, pod set was higher in the diploids in chick pea.

Treated genotypes of T_5, T_8, T_4 and T_1 recorded fresh rhizome yields which were on par with control genotype, C_7. Induced tetraploids with larger rhizomes have been previously reported by (8). Higher rhizome yields of up to 0.90 times greater in induced tetraploid ginger than the diploid genotype was obtained by (17). (24) developed an autotetraploid line in ginger plant which yielded larger rhizomes of top-quality confectionary ginger ideal for processing companies. (18) identified two tetraploid lines in ginger with enhanced yield of 320.81 g and 418.65 g compared to diploid (280.33 g). Similarly, dry rhizome yield was higher in the tetraploids (89.91 g). Polyploidy causes the addition of extra gene copies, which results in larger cell size and vigour as reported by (27).

There has also been reports which showed that tetraploids are inferior or sometimes equal to diploids with respect to some characters. Such observations had been made in green gram (47) and black gram (48). They indicated that tetraploids do not express gigantism in all the characters.

The four heteroploid plants that were obtained *in vivo* through colchicine treatment were compared with their respective control to assess which heteroploid performed better among all. Among the five heteroploids developed T_8S_1 was found to be highly promising with respect to plant height, number of leaves plant^{-1}, fresh rhizome yield plant^{-1} and dry rhizome yield per plant. Vigorous nature of polyploids have also been reported in previous studies (49, 50).

Conclusion

Successful *in vivo* polyploidization for induction of heteroploids in ginger using colchicine has been established through the study. The genotypes Athira, Genotype 1 and Genotype 4 proved to be highly efficient for ploidy induction studies among the eight ginger genotypes tried. Two heteroploids could be produced from the Genotype 4. The induced heteroploids although showed poor growth and yield characters in the first generation which might be largely due to the effect of the antimitotic agent, however in the second-generation better characteristics for growth and yield were recorded. Initial screening of putative polyploids by means of morphological characters and stomatal parameters followed by chromosome counting and flow cytometry were found to be the most suitable methods for confirming the ploidy level. Four heteroploids has been identified and the stability has been recorded.

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Authors' contributions

Sreekala G S and Deepa S Nair aided in study conception, manuscript preparation and design of the experiment. Roy Stephen, Swapna Alex, Suhara Beevy S and Beena Thomas contributed towards data collection, analysis and interpretation of the results. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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References

1. Shukla, Y. and Singh, M., 2007. Cancer preventive properties of ginger: a brief review. *Food Chem. Toxicol*. 45: 683-690.
2. Ravindran, P.N., Babu, K.N., and Shiva, K.N., 2005. Botany and crop improvement of ginger. In: Ravindran. P.N., Nirmal Babu, K., (eds). *Ginger-The genus Zingiber.* CRC Press, Boca Raton, FL. pp. 15-85.
3. Jayachandran, B.K. and Vijayagopal, P.D., 1979. Attempts on breaking self-incompatibility in ginger (*Zingiber officinale* Rosc.). *Agri. Res. J. Kerala*. 17(3): 256-57.
4. Ramachandran, K. 1969. Chromosome numbers in Zingiberaceae. *Cytologia* 34: 213-21.
5. Ramachandran, K., 1982. Polyploidy induced in ginger by colchicine treatment. Curr. Sci. 51: 288-9.
6. Jayachandran, B.K. Induced mutations in ginger. Unpublished

Ph.D thesis, Kerala Agricultural University, Kerala, India; 1989.

7. Smith, M.K., Hamill, S.D., Gogel. B.J., and Severn-Ellis, A.A. 2004. Ginger (*Zingiber officinale*) autotetraploid with improved processing quality produced by an *in vitro* colchicine treatment. *Aust. J. Exp. Agric.* 44: 1065-072.
8. Ramachandran, K. and Nair, P.N.C., 1992. Induced tetraploids of ginger *(Zingiber officinale* Rosc). *J. Spices Aromat. Crops* 1: 39-42.
9. Sheeba, P. T. 1996. Induction of autotetraploidy in ginger. M.Sc. (Hort) thesis, Kerala Agricultural University, 73p.
10. Dolezel, J., Greilhuber, J., and Suda, J., 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nature* 2(9): 2233-2244.
11. Gopinath, P.P., Prasad, R., Joseph, B., Adarsh, V.S., 2020. GRAPES: General R shiny based analysis platform empowered by statistics. https://www.kaugrapes.com/home.version 1.0.0.
12. Talebi, S.F., Saharkhiz, M.J., Kermani, M.J., Sharifi, Y., and Fard, R.F., 2017. Effect of different antimitotic agents on polyploid induction of anise hyssop (*Agastache foeniculum* L). *Caryologia* 70(2): 184-93.
13. Nair RR 2016. Variation in pollen fertility and chromosome number among germplasm collections of ginger (*Zingiber officinale* Rosc.) *Int. J. Cytol. Cytosystematics Cytogenet.* 69(1): 73 -81.
14. Sadhu, A., Bhadra, S., and Bandyopadhyay, M. 2016. Novel nuclei isolation buffer for flow cytometric genome size estimation of Zingiberaceae: a comparison with common isolation buffers. *Ann. Bot*. 118: 1057-1070.
15. Ramsey, J. and Schemske, D.W. 1998. Pathways, mechanisms, and rates of polyploidy formation in flowering plants. *Annu. Rev. Ecol. Systemat*. 29: 467-501.
16. Hamill, S. D., Smith, M. K., and Dodd, W. A., 1992. *In vitro* induction of banana autotetraploids by colchicine treatment of micropropagated diploid. *Aust. J. Bot.* 40: 887-896. https://www.publish.csiro.au/bt/bt9920887
17. Hua, K.W., Hua, J.M., Ping, H.H., and Lin, S.G., 2011. Generation of autotetraploid plant of ginger (*Zingiber officinale* Rosc.) and its quality evaluation. *Pharmacognosy Mag*. 7 (27): 200-206. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3173894/
18. Prasath, D., Nair, R.R. and Babu, P.A., 2022. Effect of colchicine induced tetraploids of ginger (*Zingiber officinale* Roscoe) on cytology, rhizome morphology, and essential oil content. *J. Appl. Res. Med. Aromat. Plants* Available: https://doi.org/10.1016/j.jarmap.2022.100422 [2 July 2022].
19. Beck, S.L., Dunlop, R.W., and Fossey, A. 2003. Stomatal length and frequency as a measure of ploidy level in black wattle, *Acacia mearnsii* (De Wild.). *Bot. J. Linn. Soc.* 141: 177-81. https://www.researchgate.net/publication/238420371
20. Omidbaigi, R., Mirzaee, M., Hassani, M.E., and Moghadam, S.M., 2010. Induction and identification of polyploidy in basil (*Ocimum basilicum* L.) medicinal plant by colchicine treatment. *Int. J. Plant Prod.* 4(2): 87-98.
21. Avery, G.S. and Pottorf, L. 1945. Polyploidy, auxin and nitrogen in green plants. *Am. J. Bot.* 32: 669-671. https://www.jstor.org/stable/2437623
22. Nagat, E., Kamla, B., and Hoda, K., 2020. Phenotypic and molecular characterization of polyploid *Vicia faba* induced by colchicine. *GSC Biol. Pharm. Sci.* 11(03): 235-43.
23. Liu, G., Li, Z. and Bao, M., 2007. Colchicine induced chromosome doubling in *Platanus acerifolia* and its effect on plant morphology. *Euphytica* 157: 145-154.
24. Smith, M.K. and Hamill, S.D. 2002. *Zingiber officinale*, ginger, 'Buderim Gold'. *Plant Var. J.* 15: 85.
25. Nasirvand, S., Zakaria, R.A., Zare, N., and Esmaeilpoor, B. 2018. Polyploidy induction in parsley (*Petroselinum crispum* L.) by colchicine treatment. *Cytologia* 83(4): 393-96.
26. Widorento, W. 2016. *In vitro* induction and characterization of tetraploid patchouli (*Pogostemon cablin* Benth) plant. *Plant Cell Tiss. Org. Cult*. 125: 261-267.
27. Sattler, M.C., Carvalho, C.R and Clarindo, W.R., 2016. The polyploidy and its key role in plant breeding. *Planta* 243(2): 281-296.
28. Lindayani., Norzulaani, K., Ibrahim, H and Rahman, N. A. 2010. Effect of colchicine on tissue culture derived plants of *Zingiber officinale* Rosc. and *Zingiber officinale* var. *rubrum* Theilade. *J. Sci. Tech. Trop*. 6: 11-16.
29. Omanakumari, N and Mathew, P.M. 1985. Karyomorphological studies on four species of *Zingiber* Adns. *Cytologia*. 50(3): 445-51.
30. Wang, L., Gao, F., Xu, K and Li, X., 2014. Natural occurrence of mixoploid ginger (*Zingiber officinale* Rosc.) in China and its morphological variations. *Sci. Hortic*., 172(1): 54-60.
31. Dhamayanthi, K.P.M and Zachariah, T.J. 1998. Studies on karyology and essential oil constituents in two cultivars of ginger. *J. Cytol. Genet*. 33(2):195-99.
32. Lee, H. and Lin, T. 2005. Isolation of plant nuclei suitable for flow cytometry from recalcitrant tissue by use of a filtration column. *Plant Mol. Biol. Rpt*. 23:53-58.
33. Carvalho, J.F.R., Carvalho, C.R., and Otoni, W.C. 2005. *In vitro* induction of polyploidy in annatto (*Bixa orellana*). *Plant Cell Tiss. Organ Cult.* 80: 69-75.
34. Beji, M. 1991. Evolutionary relationships in the genus *Hedysarum*. Contribution of experimentally induced tetraploidy. Thesis of Biological Sciences. University of Tunis, Tunis, Tunisia.
35. Sakhanokho, H.F., Rajasekaran, K., Kelley, R.Y., and Islam-Faridi, N. 2009. Induced polyploidy in diploid ornamental ginger (*Hedychium muluense* R. M. Smith) using colchicine and oryzalin. *Hort. Sci.* 44(7): 1809-814.
36. Pundir, N.P.S., Rao, N.K., and Maesen, L.J.G.V.D. 1983. Induced autotetraploidy in chickpea (*Cicer arietinum* L.). *Theor. Appl. Genet*. 65:119-22. https://link.springer.com/article/10.1007/BF00264878
37. Hagberg, A. and Ellerstrom, S. 1959. The competition between diploid, tetraploid and aneuploid rye, theoretical and practical aspects. *Hereditas* 45:369-416.
38. Thompson, M.M. 1962. Cytogenetics of *Rubus*: meiotic instability in some higher polyploids. *Am. J. Bot.* 49:575-87.
39. Marzougui, N., Boubaya, A., Thabti, I., Elfalleh, W., Guasmi, F. and Ferchichi, A. 2009. Polyploidy induction of Tunisian *Trigonella foenumgreaum* L. populations *African J. Biotechnol*. 10(43): 8570-577.
40. Huang, H. P., Gao, S.L., Chen, L.L. and Wei, K.H. 2010. *In vitro* tetraploid induction and generation of tetraploids from mixoploids in *Dioscorea zingiberensis Pharmacognosy Mag*. 6 (21):51-56.
41. Hill, H.D., Myerst, W.M. 1944. Isolation of diploid and tetraploid clones from mixoploid plants of Ryegrass (*Lolium perenne* L.), produced by treatment of germinating seeds with colchicine. *J. Hered*. 35:359-61.
42. Jiang, Y., Liu, S., Hu, J., He, G., Liu, Yet al. 2020. Polyploidization of *Plumbago auriculata* Lam, *in vitro* and its characterization including cold tolerance. *Plant Cell Tiss. Organ Cult.* 140:315-325.
43. Juliao, S.A., Ribeiro, C.V., Lopes, J.M.L., Matos, E.M., et al. 2020. Induction of synthetic polyploids and assessment of genomic stability in *Lippia alba*. *Front. Plant Sci.* 11:292.
44. Vainola A. 2000. Polyploidization and early screening of

Rhododendron hybrids. *Euphytica* 112: 239-44. https://link.springer.com/article/10.1023/A:1003994800440

45. Harbard, J.L., Griffin, A.R., Foster, S., Brooker, C., Kha, L. D and Koutoulis, A. 2012. Production of colchicine induced autotetraploids as a basis for sterility breeding in *Acacia mangium* Willd. *Forestry* 85: 427-36.

46. Parson J. L., Martin, S. L., Golenia, G and James, T. 2019**.** Polyploidization for the genetic improvement of *Cannabis sativa*. *Front. Plant Sci*. 10: 476.

47. Kumar L. S. S. 1945. A comparative study of autotetraploid and diploid types of green gram (*Phaseolus radiatus* L.). *Proc. Indian Acad. Sci.* 21:266-68. https://repository.ias.ac.in/36929/1/36929.pdf

48. Sen, K. N and Chedda, H. R. 1958. Colchicine induced tetraploids of five varieties of black gram. *Indian J. Genet.* 18: 238-48.

49. Zhang, W., Hao, H., Ma, L., Zhao, C and Yu X., 2010. Tetraploid muskmelon alters morphological characteristics and improves fruit quality. *Sci. Hortic.* 125(3):396-400. https://www.researchgate.net/publication/248478129.

50. Tan, F.Q., Zhang, M., Xie, K.D., Fan, Y.J., et al, W.W. 2019. Polyploidy remodels fruit metabolism by modifying carbon source utilization and metabolic flux in Ponkan mandarin. *Plant Sci.* 289: 110276. https://pubmed.ncbi.nlm.nih.gov/31623787/