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Colchicine-induced autotetraploidy and altered plant cytogenetic and morpho-physiological traits in *Catharanthus roseus* (L.) G. Don

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Abstract: Artificially induced polyploidy is often used to alter plant growth pattern and genetic makeup of certain plant species. This experiment was conducted to induce autotetraploidy in *Catharanthus roseus* ('Alba') which contains diploid chromosomes. Application of four levels (0, 100, 200 and 400 mg/l) of colchicine concentrations were utilized at the two true leaf stages of *C. roseus*. It has been observed that 200 mg/l colchicine treatment had the most striking effect on producing polyploid plants. This concentration was able to boost yield performance and survival of tetraploids to 35% and 79% respectively. Increasing of ploidy level was confirmed by flow cytometry and chromosome number. But, plant survival significantly decreased with increased of colchicine concentration. Chromosome number, length and diameter of stomata and chloroplast number in stomata of guard cells increased with increased ploidy level, whereas the numbers of stomata decreased from 390 to 177 mm^2 in tetraploid plants. The overall consequence of colchicines treatment appeared to be a beneficial approach. It elucidated that the chlorophyll content, diameter of the lateral branches, leaf length and width, petal length and width, duration length of flowering, durability of flowering, root diameter, fresh and dry weight of roots, seed length and seed diameter significantly increased in tetraploid as compared to diploid plants.

1. Introduction

Morpho-physiological characters of ornamental and flower plants can be promoted via duplicating their chromosome numbers. Plants with polyploidy chromosomes are usually capable of producing larger organs than diploid chromosomes. It has been proved that the chromosome

numbers in certain plant species can be naturally doubled due to autoploid and alloploid mechanisms that caused to generate plant with polyploidy chromosome (Dhawan and Lavania, 1996). Polyploidy not only occur naturally, it also can be induced artificially using mutation agents. Colchicine is one of these agents (Blakeslee and Avery, 1937). Application of mutation agents on plant is not only able to multiple the chromosomes, but also to induce variations due to changes in chromosome numbers, used to manipulate morphological and biochemical production of plants (Yasuda *et al.,* 2008). A research report indicated that the results have shown colchicine and LAECV treatments to induce different types of mitotic abnormalities including: c-metaphase, vagrant chromosomes, sticky chromosomes, anaphase bridges and increased frequency of micronuclei, along with a reduction in mitotic index in *Allium cepa* root apical meristem cells (Kundu and Ray, 2016). Induction of polyploidy tends to expand nucleus and cell size of organs which causes to have positive impact on enlargement of leaves, branches and flowers of plant. So, a suitable procedure capable of altering chromosomes numbers can become an important approach for improvement of traits such as plant size, flowers size and duration of flowering in horticultural plants (Shao *et al.,* 2003). In overall, polyploidy induction is a beneficial trend for those plant tissues that contain effective compounds in such way that these tissues become enlarge, thus able to sustain more chemical substances than diploid plants (Adaniya and Shirai, 2001).

Catharanthus roseus (L.) G. Don (Apocynaceae) is an ornamental plant that grows up to 30-100 cm in height. It was previously known as *Vinca rosea* (L.) and commonly known as Madagascar periwinkle. Although, it is originated from Madagascar but, widely distributed throughout the world due to survival ability in various habitats and recognized as an ornamental plant (Van Bergen and Snoeijer, 1996). It is a perennial plant with diploid (2n=2x=16) chromosomes (Verma *et al.,* 2011). The Apocynaceae family contains 114 genera and 4650 species. *Catharanthus roseus* is a tropical plant and very sensitive to cold climate. If it grew under a favorite condition, it would produce flowers and its growth development remain over long period of times (Jaleel *et al.,* 2007). It has been reported that polyploidy induction using colchicine and sodium azid on clustered bean plants (*Cyamopsis tetregonoloba*) caused to promote germination, flowering time, plant height, leaf number, cluster numbers of pods, pod length, pod and dry weight (Velu *et al.,* 2008). Similar study of colchicine treatment on lace plant seedling indicated that morphological growth and cytogenetic components significantly changes. These alterations were included: increase in leaf area index, length, thickness and dark leaves, stomata size increases, reduction in the number of stomata and leaf epidermis, increases of chloroplast number in stomata guard cells, increase in the diameter of the flower, pollen diameter, petal length, size of capsules and seeds, and doubling of the chromosomes (Ye *et al.,* 2009; Niu *et al.,* 2016). It has also been observed that application of 0.1% colchicine concentration on *Jatropha curcas* L. plants had the most striking effect on producing polyploidy with yielding improvement of 15% in tetraploids but showed that there was no significant difference between tetraploid and diploid plants in considering plant height. On the other hand, increasing the level of chromosomes in the plant, the stomata numbers and pollen grains became abundant, while stomata density decreased and the leaves became thicker. In general, the tetraploid plants had larger leaves, flowers and seeds as compared to diploid plants (Niu *et al.,* 2016). When the apical meristems and seedlings of hyssop (*Agastache foeniculum* L.) plants received 17.500 µM colchicines and 50 µM trifluralin, a maximum growth of 16% tetraploid induction was obtained. Size of stomata, chloroplast number, morphological traits, leaf length and width, distance between the nodes, leaf area, plant height, fresh and dry weight, and spikes length significantly increased in polyploidy plants (Talebi *et al.,* 2017). An investigation on tissue of Dendrobium plant *in vitro* showed that, when culture medium received 0.075 % colchicines concentration for 14 hours, significant alterations occurred with polyploidy plant. It has revealed that tetraploid plants contained wider and dark leaves, reduce leaf angle and increase the diameter of the roots and stems as compared to diploid plants (Sarathum *et al.,* 2010). The aim of the present investigation is to evaluate the effect of colchicine treatment on *C. roseus* 'Alba' and to study the impact of cytogenetically and morph-physiological alterations in both polyploidy and diploid plants.

2. Materials and Methods

Plant material and autotetraploidy induction

This experiment was conducted as a completed randomized design. *C. roseus* 'Alba' seeds were purchased from Seed-Pakan Company and olchicines

was obtained from Sigma Company. The seeds were planted into culture trays containing cocopeat medium (with EC 0.2 ds/m) and kept in greenhouse with 26±2°C day temperature and 19±2°C night temperature, relative humidity of 70±5% in both dark as well as light (16/8 h photoperiod) conditions, with irrigation round 2 days. The seeds were germinated after 14 days. After seeds germination, 300 apical meristems of the seedlings were treated with different concentrations of colchicine (0, 100, 200 and 400 mg/l with pH=6) at the two true leaf stages using micro spray. Tween 20 (500 µm/l) was also added to colchicine solution in order to increase the surface contact of colchicine with plant's leaves. Colchicine treatments were repeated during seven consecutive days. The treated seedling then kept in greenhouse condition as mentioned above. When the plants reached the sixth-leaf growth stages, the treated plants were transferred into individual pot (22 diameter × 28 cm length) containing sand: clay: rotten manure (1:1:1) and they remained in the pots till the end of the experiment. All the plants within the pots received similar completed Hoagland nutrient solution at flowering stage. The irrigation was applied with intervals of 3 days and same day and night temperatures and photoperiod as mentioned above, except relative humidity of 40±5% in greenhouse condition.

Flow cytometry analysis

Flow cytometry apparatus (Model PA, Partec, Germany D-48161) was used to detect ploidy levels in plant tissues according to Ju *et al.* (2005). The ploidy level of plants was determined at full blooming stage, exactly 15 weeks after transplanting. Chemical agents such as nuclear extraction buffer solution and 4, 6 Diamid-2-phenyl indol (DAPI), under common name of CyStain UV persices, were provided from Partec companies. Parsley (*Petroselinum crispum* Mill.) which has nuclear weight of 4.46 pg was used as a standard plant for calibration of the apparatus. Nuclei suspensions were obtained when approximately 100 mg of fully developed fresh leaf tissue from different parts of the plant was chopped by a sharp razor blade in a specific buffer on ice, according to Gao *et al*. (1996). Nuclear suspensions were filtered through a 50 µm nylon filter and Rnase A (Sigma Aldrich Co.) at a concentration of 2 µg/mL was added to each sample (Gao *et al.,* 1996). Prior to running the experiment all the prepared samples were kept in ice till analyzes via flow cytometry initiated. The internal software of the FCM (BD FAC Station data processing system) was used to analyze histograms for determination of peak position and the relative ploidy index of the samples for each individual plant (Gao *et al.,* 1996). The ploidy level analysis (DNA content) was performed using ratio (peak 1= unknown plants and peak 2= index plant) (Valente *et al.,* 1998). Determination of the ploidy levels of each of the samples were performed in three replicates.

Measurement of stomata

Three matured and developed leaves were cut off from different parts of each tetraploid and diploid plant. Nail varnish technique was used with some modifications to isolate samplings from surface epidermises in order to observe stomata (Smith *et al.,* 1989). The epidermis were mounted on glass slides and a light microscopy "Olympus U-DA" with a digital camera "DPI 12" was used to photograph and measure stomata density. Light microscope was used to assess stomata (numbers/mm^2) and size of stomata (length and diameter) (Fig. 1) with magnification of 100x and 400x respectively (Smith *et al.,* 1989). Since feature of stomata density on the leaf was not uniform at the surrounding nervure, photographs from seven sections of stomata where obtained by rotating each individual sample under the microscope. Then the mean was calculated for each measurement.

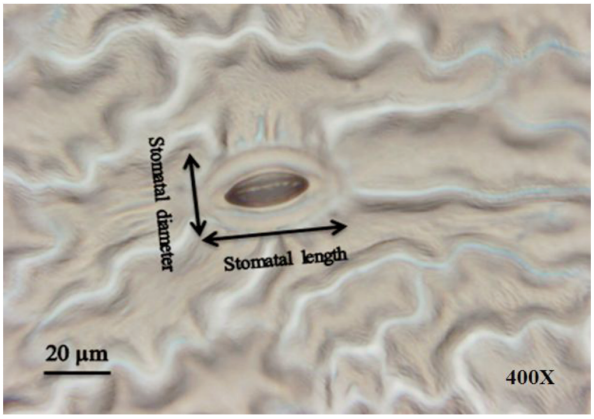

Fig. 1 - Measurement of stomata (length and diameter) with light microscope magnification 400x.

Observation and measure of chloroplast in stomata guard cells

Forceps was used to isolate epidermis from leaf surface in order to observe and count chloroplast in stomata guard cells in the detached leaf. The isolated epidermis was then placed in logol solution (1%) for 5 min. After coloring, each individual sample mounted on glass slides and light microscopy "Olympus U-DA"

with a digital camera "DPI 12" at the 400x magnification was used to count chloroplasts and simultaneously take photograph for the following counting of the number of chloroplasts in the stomata guard cells.

Comparison of morphological traits of diploid and tetraploid plants

After identification of tetraploid plants, morphological and physiological characteristics, as well as growth behavior of both tetraploid and diploid plants were recorded in order to characterize the differences. Number of lateral branches of each sample was counted accurately. A rule (with accuracy 1 mm) and a digital caliper (with accuracy 0.1 mm) were used to measure branch and root diameters. Length of leaf and petal were measured by using the Caliper digital (with accuracy 0.1 mm). As phenological characteristics, it was measured flowering period, which was considered from initial time of flowering to seeds formation, flowers durability on plants was evaluated according to the number of days. Number of seeds per follicle was counted accurately. Fresh root and dry weight was measured using a digital scale (accurately 0.01 g). Oven (48 hours at 70°C) dry weight of root was measured. Seed dimensions including length and diameter were measured using a dial binocular microscope magnification of 40x.

Evaluation of chlorophyll content

Fresh samples of apical leaves were collected from both tetraploid and diploid plants and washed thoroughly with distilled water. Approximately, 0.1 g of leaves was weighed and placed in a mortar then 2 ml of 80% acetone added to the samples and gently crushed the leaves till the mixture was formed in a uniform state. The samples were centrifuged at 6000 rpm for 15 min. A spectrophotometer device with wavelength of 663 and 645 nm was set to read the absorption of chlorophyll. Chlorophyll a and b then were calculated using the following formulas (Arnon, 1949).

1) Chlorophyll a=12.25(A663)-2.55(A645) × V/W
2) Chlorophyll b=20.31(A645)-4.91(A663) ×V/W

where V = volume of extract (ml) and W = weight of tissue (mg)

Observation of chromosome numbers

Study of cytogenetic event was implemented based on counting the set of chromosomes in individual plant cells of diploid and tetraploid plants. The cells of root tip tissue from germinated seeds were used to observe chromosomes numbers. The seeds of each individual plants (diploid and tetraploid) were sterilized separately with Sodium hypocolorid solution (5%) for 5 min (24°C) and rinsed completely with distilled water for 10 min. The seeds were then placed on filter paper in petri dish to germinate at 25±1°C temperature inside the growth chamber. After germination, when the root length reached 5 mm, the roots were separated and washed with distilled water and placed in the pretreatment solution of 8-hydroxyquinoline citrate at temperature of 4°C for two hours. Ethanol and acetic acid in ratio of 1:1 (v:v) were used to stabilize the samples at temperature of 4°C for 20 hours. The samples were washed once with distilled water for 30 min, then with 40% ethanol for 15 min, and kept in 50% ethanol for 15 minutes. After this time the samples were removed and kept in 70% ethanol for 15 min. Ethanol and xylene were used to detect transparency of the samples (Chehrazi *et al.,* 2012).

Hydrolysis of the samples

Hydrolysis of the samples occurred when samples were immersed in 70% ethanol and hydrochloric acid (vol. 2:1) solution for 15 minutes. The samples were then washed with distilled water for 15 minutes and used acetocarmine solution to stain the samples for 5 hours at 25±2°C. Approximately, 2 mm of tip apex of root was removed at the end of the root tip and placed on glass slides. A light microscopy "Olympus U-DA" which capable of magnifying of 400x with a digital camera "DPI 12" was used to get photograph and observe number of chromosome (Chehrazi *et al.,* 2012).

Statistical analysis

SPSS software 16.0 was used to perform statistical analysis of the data. The T-test also was applied for the mean comparison at level of 1% of probability.

3. Results and Discussion

Effect of colchicine on the rates of survival and tetraploid plants

Application of 200 and 400 mg/l colchicine solutions on true two-leaf growth stages of *C. roseus* 'Alba' diploid plants tended to induce tetraploidy. The concentration of 200 mg/l had the highest survival (79%), whereas the highest percentages of tetraploid plants were induced at the 400 mg/l concentration colchicines. The information from results of flow cytometry analysis, cytogenetic and morphological evaluations indicated that concentration of

400 mg/l was not only able to promote autotetraploidy, but caused to generate the highest mortality among treated plants (Table 1). This observation showed the potential of *C. roseus* 'Alba' in responding to colchicines treatments reacted differently, so the certain of plants can't preserve their chromosome sets in balance within the cells, in particular at the high concentration rate of colchicines. Flow cytometry analysis elucidated that the majority of those plants tetraploid with high dose of colchicines developed defective chromosomes; mixed ploidy with abnormal structure and the plants die before reaching the maturity. An investigation implemented to induce polyploidy in *Challistephus chinensis* Nees. (Hanzelka and Kobza, 2001) and *Agastache foeniculum* L. (Talebi *et al.,* 2017) was indicated that the increase of colchicine concentration on the targeted plants decrease the rate of plant survival. Another experiment reported that there is a positive correlation between various concentrations of colchicine applications and mortality in seedlings of Chamomile (*Tanacetum parthenium*) (Saharkhiz, 2007). It has been documented that when high dose of colchicines used as a mutation agent for plant, toxic contamination, phytotoxicity and abnormality became main cause of death in the plants (Han *et al.,* 1999).

Table 1 - Percentage of plant survival and tetraploid plants of *C. roseus* 'Alba' seedlings treated with colchicine

Colchicine concentration (mg/l)	Plant survival (%)	Tetraploid plants (%)
0	98±2 a	0 c
100	96±4 b	0 c
200	79±3 c	35±2 b
400	55±3 d	44±3 a

± Standard error (SE).

Identification of tetraploid plants using flow cytometry

Results on analysis of ploidy levels by flow cytometry are shown in figures 2 and 3. The ploidy level was detected by placing plant tissue from tetraploid and diploid plants into Flow cytometry, and then the DNA content was recorded (Sari *et al.,* 1999). The amount of DNA content was calculated according to formula: which is described by (Bharathan *et al.,* 1994). The rate of DNA contents was equal to 0.35-0.45 and 0.7-0.9 for diploid and tetraploid plants respectively. These findings were similar to the results which were reported by Talebi *et al.* (2017), Niu *et al.* (2016).

Stomata size and density and chloroplast number of stomata guard cells

The results of comparison of stomata at the different locations on the dorsal surface of fully developed leaves have shown that there were significant (1% probability level) differences between diploid and tetraploid plants in relation to density and size of stomata. The results also indicated that density of stomata in diploid and tetraploid plants were 390 and 177 numbers in mm^2 (Table 2 and Fig. 4 b and f). While, the length of stomata and diameter were 17 and 25 µm in diploid and 22.5 and 35.5 µm in tetraploid plants respectively (Table 2 and Fig. 4 a and e). Numbers of chloroplasts of stomata per guard cells were equal to 10 and 20 in diploid and tetraploid plants respectively (Table 2 and Fig. 4 c

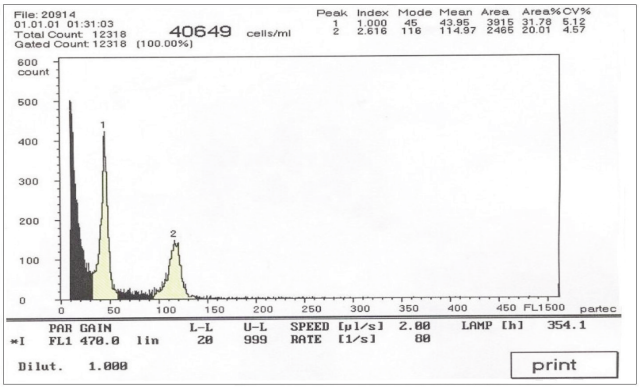

Fig. 2 - Flow cytometry analysis of *C. roseus* 'Alba' cell nuclei in diploid (peak 1) and index plant of parsley in diploid status (peak 2).

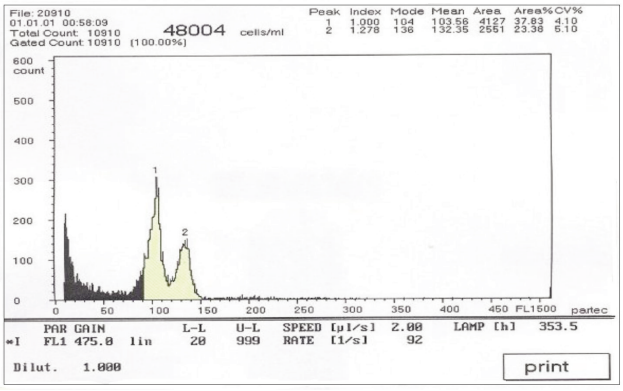

Fig. 3 - Flow cytometry analysis of *C. roseus* 'Alba' cell nuclei in tetraploid (peak 1) and index plant of parsley in diploid status (peak 2).

Table 2 - Mean of stomata size, density and chloroplast numbers in diploid and tetraploid plants of *C. roseus* 'Alba'

Genotype	Stomata length (µm)	Stomata diameter (µm)	Stomata density (n/mm^2)	Chloroplasts number
Diploid	22.5±0.6 b	17±0.3 b	390±2.4 a	10±0.2 b
Tetraploid	35.5±0.4 a	25±0.3 a	177±2.3 b	20±0.3 a

± Standard error (SE).

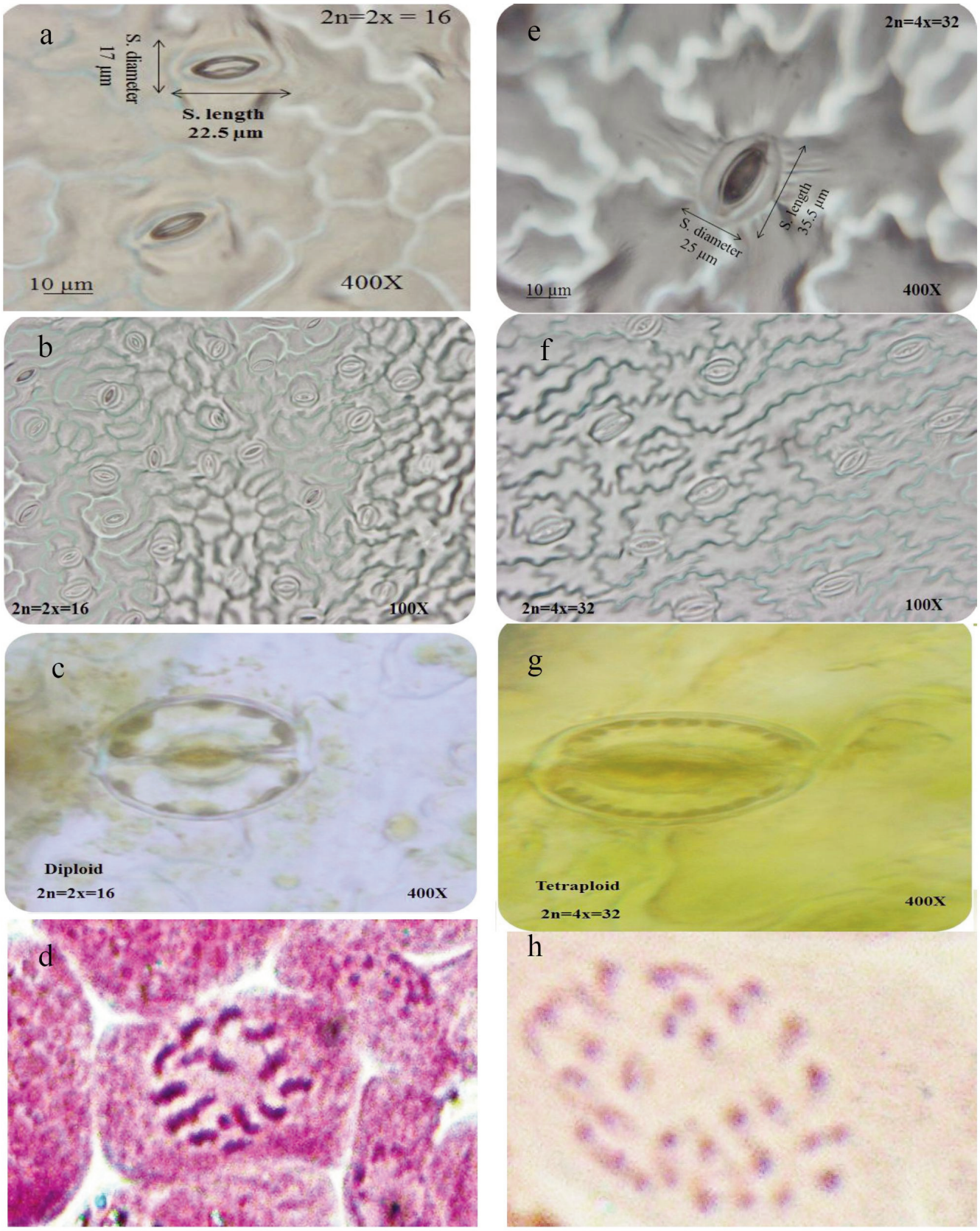

Fig. 4 - Stomata size (a), stomata density (b), chloroplast numbers (c), chromosome numbers (d) in diploid and stomata size (e), stomata density (f), chloroplast numbers (g) and chromosome numbers in tetraploid (h) of *C. roseus* 'Alba' plants.

and g). Early research reports on the size of stomata in the guard cells indicated that these cells are more dependent on genetic than environmental factors as compared to other cells in plant (Yasuda *et al.,* 2008). Another study on *Jatropha curcas* L. indicated that with increase ploidy, the stomata and pollen grains became larger, but stomata density decreased (Niu *et al*., 2016). A study on the increase polyploidy level of Japanese persimmon *(Diospyrus kaki* L.) has shown that with increasing in ploidy level, plant's stomata guard cells have become enlarge but with less density (Tamura *et al.,* 1996). In a report by Roy *et al.* (2001) it has been shown that the length and diameter of the stomata were set to be standard parameters for identification of tetraploid plants. Similar work has been done to identify autotetraploid using colchicines treatments in *Humulus lupulus* plant (Roy *et al.,* 2001). Several other studies showed that application of colchicines treatments tend to increase of ploidy level in plant, the length and diameter of stomata and chloroplast numbers increased (Gu *et al.,* 2005; Talebi *et al.,* 2017).

Observation and chromosomes counting

In order to determine the ploidy level in the treated plants, the cells of root tip were isolated from both diploid and tetraploid plants after the series of preparation, stained and then light microscope used to count chromosomes sets. Chromosome numbers of root tip cells of diploid and tetraploid were 16 and 32 respectively (Fig. 3). Application of colchicine tends to prevent the activity of the subunits join of microtubule (tubulin protein) and/or keep them apart that impedes the formation of the spindle fibers during the cell division and stops chromosomes movement in metaphase stage (Kundu and Ray, 2016). Thus, cell division occurs without cell wall formation which leads to double the number of chromosomes in plant cells. Colchicine treatment could induce different types of mitotic abnormalities including c-metaphase, vagrant chromosomes, sticky chromosomes, anaphase bridges and increased frequency of micronuclei (Gupta, 2002; Kundu and Ray, 2016).

Phenotypic variation between diploid and tetraploid plants

The t-test at the 1% probability level showed significant differences between diploid and tetraploid plants. As chromosome numbers duplicated, stem number, stem diameter, leaf area, leaf number, flower diameter, diameter of flower ovary, total chlorophyll content, fresh and dry weight of roots, duration of flowering length, durability of flowering, root diameter, length and diameter of seeds were significantly increased in the tetraploid as compared to diploid plants while. On the other hand, increases ploidy level from diploid to tetraploid decreased length of lateral branches and root and number of seed in follicle (Table 3). In the present experiment, promotion of ploidy level caused to reduce lateral branch length, but tended to increase diameter and number of branches (Table 3). In fact, the formation of polyploidy in plant is simply due to reduction of the frequency of cell division during initiation of growth and development which caused to lowering growth rate in tetraploid when compared to diploid plants. In an experimental study that was conducted to induce polyploidy in Cumin plant it has been reported that autotetraploid plants have lower growth at the early growth stages and shorter time to flowering stage than diploid plants (Dijkestra and Speckmann, 1980). While, at the same time, the diameter of lateral branches and its numbers significantly enhanced in tetraploid plants (Table 3). Rubuluza *et al.* (2007) in their research proved that colchinice treatments are capable of producing similar out puts on seedling of *Colophospermum mopane* L. plants.

Table 3 - Comparison of morphological and physiological traits in diploid and tetraploid plants of *C. roseus* 'Alba'

Characteristics	Diploid	Tetraploid
Length of lateral branches (cm)	24.5±0.5 a*	13.3±0.6 b*
Diameter of lateral branches (mm)	3.2±0.2 b	5.3±0.2 a
Lateral branch number	3.3±0.2 b	8.4±0.3 a
Leaf length (mm)	60±1 b	97±3 a
Leaf width (mm)	20±0.04 b	47±0.2 a
Petal length (mm)	22±0.25 b	27±0.4 a
Petal width (mm)	15±0.05 b	25.3±0.03 a
A chlorophyll (mg/g)	0.6±0.015 b	0.9±0.01 a
B chlorophyll (mg/g)	0.21±0.01 b	0.37±0.01 a
Duration length of flowering (day)	148±2 b	181±2 a
Durability of flowering (day)	3.6±0.24 b	7±0.15 a
Root length (cm)	30.8±1.8 a	17.5±1.7 b
Root diameter (mm)	3.5±0.2 b	6±0.3 a
Fresh weight of roots (g)	8.5±0.2 b	14.2±0.3 a
Dry weight of root(g)	1.4±0.03 b	2.3±0.05 a
Number of seeds in follicle	17.5±0.4 a	7.7±0.4 b
Seed length (mm)	2±0.04 b	3±0.06 a
Seed diameter (mm)	1.1±0.02 b	1.5±0.04 a

± Standard error (SE).

* In each row, means with similar and dissimilar letters are no significant and significant respectively according to t-test.

The length and width of leaves were larger in tetraploid than diploid plants (Table 3 and Fig. 5). So, a plant with such characteristic is being able to sustain more chemical substances in vegetative organs. Artificially Induction of polyploidy in many plant species caused to increase cell size and consequently enhance flower size, inflorescence, leaves, vegetative and generative organs (Watrous and Wimber, 1988; Omidbaigi, 2009; Niu *et al.,* 2016; Talebi *et al.,* 2017). Other researchers reported the beneficial effect of artificially inducing polyploidy in the *Melaleuca alternifolia* (Zhang, 2000), *Tanacetum parthenium* L. (Saharkhiz, 2007), *Jatropha curcas* (Niu *et al.,* 2016) *and Agastache foeniculum* L. plants (Talebi *et al.,* 2017) which caused to enlarge size of leaves and branches. The amount of chlorophyll a and b were significantly increased in the leaves of tetraploid (Table 3). The accumulation of high chlorophyll contents in the leaves of tetraploid plants may be relevant to increase number of chloroplasts in the stomata guard cells (Fig. 4 c and g). As shown above, the number of chloroplasts in stomata guard cells of tetraploid plants was two folds greater than those in diploid plants. In a similar experiment conducted on Acacia (*Acacia mearnsii*), Mathura *et al.* (2006) have shown that chlorophyll content was significantly higher in tetraploid than diploid plants. Polyploidy tended to have positive effect on size of reproductive organs. The growth of length and width of petals developed significantly higher in tetraploid than diploid plants (Table 3 and Fig. 5). A considerable growth increases was occurred in petal and flower size of tetraploid when carnation and *Jatropha curcas* plants received leaf foliar application of colchicines (Roy Chowdhury and Tah, 2011; Niu *et al.,* 2016).

In the present study, colchicine treatments caused to make the length of flower duration and durability of flowers longer in tetraploid than diploid plants (Table 3). Generally, in tetraploid plants, the beginning of flowering is delayed and the flowering period is longer than the diploid plants (Blakeslee and Avery, 1937; Lavania and Srivastava., 1991). The roots length in tetraploid (17.5 cm) is less than diploid plants (30.8 cm) (Table 3). This reduction may be due to reduction of cell division in the longitudinal direction, but it has been reported that with reduction of root length, root diameter increased in these plants (Sarathum *et al.,* 2010). It has been found that when ploidy level was increased in salvia plant, root diameter significantly increased as compared to control plants (Gao *et al*., 1996). Increase in fresh and dry weight of root in tetraploid can be due to production of lateral roots and enhance of root diameter (Table 3). A series of experimental studies which were carried on *Hyoscyamus niger* L. and *Agastache foeniculum* L. plants, elucidated that doubling level of chromosome from diploid to tetraploid caused to increase fresh and dry weight of root and shoot (Lavania and Srivastava, 1991; Talebi *et al.,* 2017).

In the present experiment, colchicines treated plants were able to produce seeds with a bigger size than untreated plants. Lengths of seeds in diploid and tetraploid plants were 2 and 3 mm, respectively. And seed diameter measurement indicated that tetraploid had thicker seed in diameter than diploid, but the number of seeds per follicle has decreased from 17.7 to 7.7 numbers in tetraploid plants (Table 3). The implication behind the reduction of the seed number per follicle may be attributed to increasing the seed size in the plant. It has been reported that artificially induced polyploidy plant usually caused to produce seeds with bigger size as compared to diploid plants, whereas the seed numbers simply reduced. Niu *et al.* (2016) have shown when chromosomes of *Jatropha curcas* plant duplicated due to colchicines treatment the seeds of the tetraploid plants grown bigger than those of the diploid. Study the application of colchicines on other plants such as Chickpea, Crape myrtle and Dendrobium could promote the seeds with bigger size and weight in autotetraploidy plant (Pundir *et al.,* 1983; Ye *et al.,* 2009; Sarathum *et al.,* 2010). Niu *et al.* (2016), Dijkestra and Speckmann (1980) reported that low seed formation in autotetraploid may be relevant to meiotic abnormalities during cell division in which caused to produce the bigger size of seed in the plant.

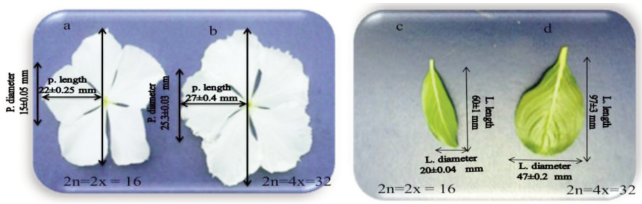

Fig. 5 - Size of diploid petal (a), size of tetraploid petal (b), size of diploid leaf (c) and size of tetraploid leaf (d) in diploid and tetraploid genotypes of *C. roseus* 'Alba'.

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