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Comparative Studies on Diploid and Tetraploid Levels of *Nicotiana alata*

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Abstract: The present investigation was planned to induce polyploid plants of *Nicotiana alata* by treating seeds in an aqueous solution of colchicine and identify the ploidy level by counting the chromosome number. Compare between diploid and tetraploid plants for some phenotype characters and the mineral content of nitrogen (N) and phosphorus (P) in the different parts of the plant was carried out. Results indicated that colchicine treatments of *Nicotiana alata* seeds were very effective for producing a large number of tetraploid plants. Chromosome number of tetraploid plants was determined and was confirmed by microscopic observations in both mitotic and meiotic divisions. Morphologically, tetraploid plants showed an increase in the vegetative and flowering characters than the diploid plants with new ornamental characteristics. The increase in the rate of N and P nutrients in both diploid and tetraploid plants of *Nicotiana alata* caused an obvious increase in their content in different parts of the plant. In addition, the mineral content in root, stem and leaves recorded higher values in the tetraploid plants than in the diploid ones. It was concluded that morphological characters and mineral contents (N and P) in colchitetraploid plants were very obviously changes than in diploid plants.

Key words: *Nicotiana alata* · Colchicine · Diploid · Tetraploid · Mineral content

INTRODUCTION

The Family Solanaceae is composed of 98 genera and about 2300 species belonging to 14 tribes grouped in three sub families [1, 2]. The genus *Nicotiana* consists of 64 recognized species, some species are cultivated as drug or ornamental plants [3].

The ploidy manipulation is considered as a valuable tool in genetic improvement of many plants including the *Solanum* spp. [4], citrus [5],pomegranate [6], *Allium* spp. [7] and azaleas [8]. In addition; the polyploids also provide a wider germplasm base for breeding studies [9].

Since the discovery of colchicine as an agent for doubling chromosomes in the plants [10], ploidy may be induced with colchicine treatment. Colchicine ($C_{22}H_{25}O_6N$) is a poisonous compound extracted from seeds and bulbs of *Colchicum autumnal* L. The optimum dose of colchicine and incubation time depends on the species and environmental conditions [11].

Colchicine acts by binding to the tubulin dimmers, preventing the formation of microtubules and consequently, spindle fibers during cell division [12, 13]. So, the chromosome has been duplicated, but mitosis has not yet taken place and restriction of cell wall formation at this stage results in the polyploid cells. These cells were larger than diploid counterparts with greater cell volume frequently developed into thicker tissues, thus resulting in large size plant organs [14].

Morphological comparison between diploid and tetraploid plants showed some differences such as an earlier induction of enhanced growth of lateral branches and multiple shoots [15]. Also, colchicine induces mutagenic changes on gross and micro-morphological features which may be of agronomic utility [16]. Polyploidy has been used in horticulture as a breeding tool to enhance ornamental characteristics such as plant size, leaf thickness and increased width to length ratio of leaves and flower size [6].

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The tetraploid plants have higher nitrogen (N), potassium (K), calcium (Ca) and magnesium (Mg) concentrations and lower sodium (Na) and boron (B) concentrations than the diploid plants [17] they have higher uptake of water, N and K with a consequent increase in leaf area and the amount of photosynthate [18]. The increase in nitrogen concentration in leaves of maize plant with increasing the levels of nitrogen might be attributed to the supplement amounts of nitrogen applied to plants [19].

The present investigation aimed to compare between diploid and tetraploid levels in *Nicotiana alata* plants for the changes of the morphological characters and some mineral content of (N and P) in the different parts of the plants.

MATERIALS AND METHODS

Seed Treatment: Seeds of the *Nicotiana alata* were immersed for 24 hours in distilled water, then soaked in aerated colchicine solution with different concentrations 0.10, 0.25 and 0.50 % (w/v) for 12, 24 and 48 hours before transfer to the pots and after growing, seeds of tetraploid plants were obtained from the plants treated with the highest concentration of colchicine.

Cytological Analysis

Determination of Ploidy Level: Ploidy level of *Nicotiana alata* plants was determined by chromosome counting to confirm the tetraploid level (4n) using mitotic and meiotic examinations.

For mitotic examination, seeds of diploid and tetraploid plants of *Nicotiana alata* were germinated, the root tips were collected and pre-treated by cooling then fixed in carnoy's solution. The root tips were stored until microscopic observations according to Darlington and La cour [20]. For meiotic study at the flowering stage, six plants were selected from the effective concentration of colchicine (0.5 %) to collect flower buds and fixed for 24 hours in Carnoy's solution and stored in ethanol at 4°C until cytological analysis according to Belling [21].

Morphological Characteristic: The morphological features of diploid and tetraploid plants were visually examined and some changes were recorded due to colchiploidization in aspects of the plant growth habit. These morphological features were vegetative and flower characteristics. The vegetative characters were the stem length, stem diameter, number of branches and number of leaves on plant at flowering initiation, leaf length, leaf width, mean internodes number and mean internodes length, whereas the recorded flowering characters were the number of flowers per plant, mean pedicel flowers length, style length, corolla tube length, sepals length, stamen length, stigma diameter and capsule diameter.

Fertilizing NPK Nutrients: The germinated seedlings of either diploid or tetraploid plants were treated by NPK. Nitrogen was added as Urea [CO $(NH_2)_2$ 46% N] at rates of 0.0, 50, 100 and 150 kg/Fed. While, phosphorus was added as ordinary Super phosphate (15.5 % P_2O_5) at rates of 0.0, 50, 100 and 150 kg/fed. Potassium was added to each rates of nitrogen and phosphate as potassium sulphate (K_2SO_4 48% K_2O) at recommended rate (80 kg/Fed).

Different rates NPK nutrients were used in the present study; to throw some light on their effect on Nitrogen as urea [CO $(NH_2)_2$ 46% N], Phosphate was added as ordinary super phosphate (15.5% P_2O_5) and Potassium was added as K_2SO_4 (48 % K_2O).

Design and Treatments: The field experiment was carried out at the farm of Faculty of Agriculture Al-Azhar University. The experimental design was laid out in a complete randomized plot in four replicates including control. Each plot area was 6 m^2 (2 × 3). At the end of the experiment the plants were harvested and separated to leaves, stems and roots for chemical analysis.

Plant Analysis: The harvested plant samples (leaves, stems and roots) were air dried,over dried at 70 °C, ground and stored for determined dry matter yield of different organs. For wet ashing; a 0.2 g of ground sample was digested using a mixture of sulphuric – perchloric acid (2:1) for N and P determination. Nitrogen was determined by Kjeldahel technique according to Jackson [22], where Phosphorus was determined colormitrically by using Spectrophotometer (Spectronic 20) as described by Jackson [22].

Statistical Analysis: The obtained data for the morphological changes of diploid and tetraploid plants were statistically analyzed using t-test where, the mineral Content results were exposed to analysis of variance (ANOVA) according to Snedecor and Cochran, [23] using MSTAT program and least significant ranges (LSR) were used to compare between means of treatments according to Duncan [24] at probability of 5 %.

RESULTS

Induction of Tetraploid Plants: The results indicated that colchicines could be efficiently utilized for the induction of tetraploid plants of *Nicotiana alata*. The study indicated that the effective dose of colchicine was 0.5% after 48 hours of treatment.

Cytological analysis was carried out on root tips and flower buds of tetraploid plants of *Nicotiana alata* to determine the true effects of chromosome doubling. Mitotic analysis of apical meristems of the seedlings revealed the occurrence of tetraploid level, where the diploid cells (2n) had 18 chromosomes whereas those in tetraploid cells(4n) were 36 chromosomes as shown in Fig. 1. The meiotic studies confirmed the tetraploids by counting the chromosome number in the pollen mother cells of healthy growing buds of tetraploid plants. Fig. 2 shows the tetraploid cells (4n) had 18 bivalents and the diploid (2n) possessed 9 bivalents.

Morphological Characters: The induction of polyploidy is associated with changes in plant morphology; several morphological variations were recognized in tetraploid plants induced by colchicine treatment.

The morphological differences between the diploid and tetraploid plants are shown in Table 1 and 2. Table 1 represents the vegetative characteristics of diploid and tetraploid plants of *Nicotiana alata*.The morphological changes of tetraploid plants were significant ($P<0.01$) than the diploid plants in stem length, number of leaves on plant at flower initiation, leaf breadth, leaf length, mean internodes number and mean internodes length while they were not significant for stem diameter and number of branch/plant. Although, the leaves of tetraploid plants were typically of green color darker than the leaves of diploid plants.

In comparison with the control, the tetraploid plants showed other morphological changes in the flowering characteristics as shown in table 2, the tetraploid plants

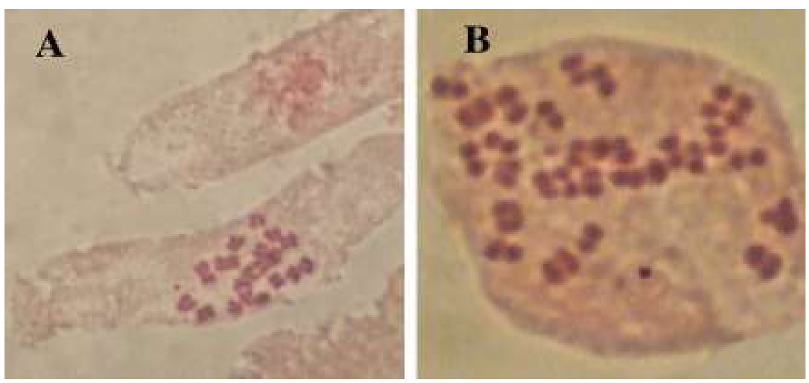

Fig. 1: Chromosome number in mitotic division of *Nicotiana alata* for diploid cell (A) 2n = 18 chromosome and for tetraploid cell (B) 4n = 36 Chromosome

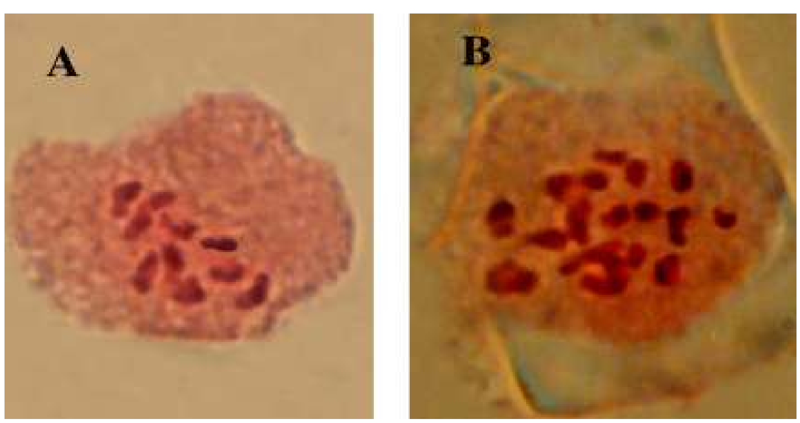

Fig. 2: Metaphase I configurations in PMCs of *Nicotiana alata* A = 9 bivalents for diploid cell and B = 18 bivalents for tetraploid cell

Table 1: Vegetative characteristics diploid and tetraploid plants of *Nicotiana alata*. (Mean ±SE)

Ploidy Level	meanof stem length/cm	Mean of stem diameter /cm	Mean of no. branch/plant	Mean of No. of leave on plant at flower initiation	Mean of leaf breadth/cm	Mean of leaf length/cm	Mean of internodes	Numbers Mean Internodes Length (cm)
Diploid	116.67± 0.66	1.62± 0.11	2.7 ± 0.33	24.17± 0.60	14.62± 0.28	21.88 ± 0.48	14.5 ± 0.43	6.12 ± 0.11
Tetraploid	136.83**±0.60	1.75± 0.23	3.83 ± 0.48	37** ± 0.97	18.25**±0.38	26.33** ± 0.57	18.67**±0.49	6.8** ± 0.73

* P< 0.05 ** P< 0.01

Table 2: Flowering characteristics diploid and tetraploid plants of *Nicotiana alata*(Mean ±SE)

Ploidy Level	Mean No of days for Flower initiation	Mean of flower /plant	Mean pedical flower length/mm	Mean of sepals length/cm	Mean of style length/cm	Mean ±SE of stamen length /cm	Mean ±SE of stigma Diameter /mm	Mean ± SE of capsule Diameter /mm
Diploid	166.5±0.76	82.5±0.76	15.75±0.38	1.62±0.6	3.68±0.37	2.55±0.63	1.77±0.82	8.4 ± 0.33
Tetraploid	152±0.76	123.33**±0.88	18.13** ±0.32	1.83±0.4	4.2±0.61	3.55±0.63	2.1** ± 0.77	9.4** ± 0.17

* P< 0.05 ** P< 0.01

produced more flowers, longer pedicel flower length, larger stigma and capsule diameter significantly (P<0.01) than diploids. However produced longer sepals length, style length and stamen length less significantly than diploids.

The Mineral Content: The content of N and P nutrients in different parts of either the diploid or tetraploid plants of *Nicotiana alata* were presented in Tables 3 and 4. Generally in both diploid and tetraploid plants, the increasing the rate of N and P nutrients caused a significant increase (P<0.05) in their content in different parts of plant under investigation.

Nitrogen Content: The N contents in the leaves, stems and roots in both diploid (2n) and tetraploid (4n) plants were represented in Table 3.

It is seen that the N content in different parts (leaves, stems and roots) were significantly (P<0.05) increased in both diploid and tetraploid plants as the rate of N (kg/fed) increased. The value of N content in the tetraploid is higher than in diploid plant. This was true for different parts of plants. The values of N. content in leaves in tetraploid were ranged from 1.27 to 2.87 % while the values of diploid were ranged from 1.09 to 2.01 %.

In stem the values for diploids were in the range of 1.02 to 1.82 % while the values of tetraploids were in the range of 1.16 to 2.61 %.

Approximately the obtained results of roots were similar to that obtained in the case of either leaves or stems.The values of N content were ranged from o.76 to 1.60 % in diploid plants, while the values of nitrogen content in tetraploid plant were ranged from 0.91 to 2.19%.

Based on the aforementioned results, it was noticed that the N content in leaves was higher than in both stems and roots. But, the N content in the stem still higher than

Table 3: Nitrogen content (%) in different parts of Nicotiana alata plants as affected by nitrogen rate in diploid (2n) and tetraploid (4n)

	Leaves	
Rate of N Kg/Fed	2n	4n
0	1.09 F	1.27 E
50	1.55 D	1.20 C
100	1.85 C	2.64 B
150	2.01 C	2.87 A
Stem		
Rate of N Kg/Fed	2n	4n
0	1.02 F	1.16 E
50	1.37 D	1.83 C
100	1.50 D	2.18 B
150	1.82 C	2.61 A
Root		
Rate of N Kg/Fed	2n	4n
0	0.76 G	0.91 F
50	1.28 E	1.44 D
100	1.43 D	1.84 B
150	1.60 C	2.19 A

Table 4: Phosphorus content (%) in different parts of Nicotiana alata plants affected by phosphate rate in Diploid (2n) and Tetraploid (4n)

	Leaves	
Rate of N Kg/Fed	2n	4n
0	0.03 D	0.03 D
50	0.10 C	0.11 C
100	0.15 B	0.15 B
150	0.19 A	0.20 A
Stem		
Rate of N Kg/Fed	2n	4n
0	0.06 E	0.08 E
50	0.15 D	0.15 D
100	0.16 C	0.24 B
150	0.23 B	0.27 A
Root		
Rate of N Kg/Fed	2n	4n
0	0.04 G	0.10 F
50	0.12 D	0.13 D
100	0.17 C	0.18 C
150	0.19 B	0.21 A

Mean in each column with the similar letters are not significantly different at 5 % level. Diploid (2n) Tetraploid (4n) that in roots. The distribution of N content in all plant parts indicated that the mobility of N in plant was high. This was true for either diploid or tetraploid plants.

Phosphorous Content: The results of Table 4 indicate the content of P in leaves, stems and roots in both diploid (2n) and tetraploid (4n) plants. It was clearly seen that the P content in diploid and tetraploid plants was significantly ($P<0.05$) increased as the rate of phosphorus increased. This was true for different parts of plant (leaves, stems and roots). For leaves the P content is corresponding to different rates of P (kg/Fed). At 0 kg/fed, the P content is equal in both diploid and tetraploid plants (0.03), but at 50, 100 and 150 kg/fed the P content in tetraploid plant were higher than in diploid plant. Exception was found at control treatment, where the obtained values in either diploid or tetraploid were about equal. The P content in leaves of either ploidy levels were in the range of 0.11 to 0.20 and 0.10 to 0.19 % for the tetraploids and diploids respectively.

In stem the P content in tetraploid was higher than in diploid plants. The values in case of tetraploid plant were in the range of 0.08 to 0.27%, while the values in case of diploid plant were in the range of 0.06 to 0.23%.

In root, also the P content in tetraploid (0.10 to 0.21 %) was higher than in diploid plants (0.04 to 0.19 %).

Moreover, the increase in P content was more pronounced in the stem than leaves and roots. Also, the P content was higher in leaves than roots. This distribution was differed from that in nitrogen content.

DISCUSSION

The use of chemicals to induce direct changes in the original genome of the plants has been particularly useful in the study of genetics and plant improvement. The induction of tetraploid plants in *Nicotiana alata* indicated that colchicine is an anti-mitotic agent which inhibit the formation of spindle fibers and effectively arrest mitosis at the metaphase stage leading to polyploidy [25] by binding to the tubulin dimmers, preventing the formation of microtubules and consequently, spindle fibers during cell division [13]. So, the chromosome have been duplicated and obtained tetraploid plants of *Nicotiana alata*. Similar results indicated various responses to colchicine in different species or varieties as wheat [13] Pomegranate, [6] sweet beet [9] and cotton [11].

The tetraploid level was confirmed by counting the chromosome number in both mitotic and meiotic division, several studies evaluating ploidy level based on the chromosome number which is the only reliable method to confirm the ploidy levels [26].

The tetraploid cells were larger than diploid counterparts with greater cell volume frequently developed into thicker tissues, thus resulting in large size plant organs [14, 27].

Morphological comparison between diploid and tetraploid plants showed some differences in both vegetative and flower characteristics. The morphological changes of tetraploid plants were highly significant than the diploid plants in stem length, number of leaves on plant at flower initiation, leaf breadth, leaf length, leaf internodes number and leaf internodes length. Other morphological changes in the flowering characteristics showed that the tetraploid plants produced more flowers, longer pedicel flower length, larger stigma and capsule diameter significantly than diploids. The obtained results were in agreement with the results reported previously [28] whereas, it was found that typical polyploid characters like gigantism, bigger leaves, flowers and pods were exhibited by the induced colchitetraploids in *Vicia faba*. Finally, the induction of polyploidy as tetraploids has been used in horticulture as a breeding tool to enhance ornamental characteristics such as plant size, leaf thickness and increased width to length ratio of leaves and flower size [6, 29].

Plants depend on nutrients from the soil for their metabolic reactions, while, continuous use of the soil after several plant harvesting, the nutrients dwindle and this results in a reduction in the quality and yield of plants. So fertilizers are substances that added to the soil to provide one or more of the chemical elements essential for plant nutrition. The most important plant nutrients provided by fertilizers are nitrogen (N), phosphorus (P) and potassium (k).

In the present investigation, the N content in different parts (leaves, stems and roots) significantly increased in both diploid and tetraploid plants as the rate of N (kg/fed) increased. The value of N content in the tetraploid is higher than in diploid plants. This was true for different parts of plants. This result of N content was in agreement with other previous reports [19,30,31]. Where they reported that the increase in the rate of N fertilizer increased the N content. Moreover, Jones *et al.* [17] obtained the same results, where the tetraploid rohodes grass plants had higher N than the other diploid ones.

The P content in diploid and tetraploid plants significantly increased as the rate of P increased. This was true for different parts of plant (leaves, stems and roots).

For leaves, the P content is corresponding to different rates of P (kg/Fed), whereas the P content in tetraploid plant were higher than in diploid plant. Exception was found at control treatment, where the obtained values in either diploid or tetraploid were about equal. Also, in stem and root the P content in tetraploid was higher than in diploid plants. The stem had the highest amount of phosphorus content. Also, the P content was more pronounced in the leaves than roots, as previously demonstrated in among different parts of *Pueraria phaseoloides*, stem tissues contained more P followed by leaf and root [32]. However, this distribution was differed from that in N content. This means that the mobility of P in all plant parts is lower than the mobility of N in the same plant parts, as mentioned and supported in previous research [33].

In conclusion, the morphological changes and the increase in mineral content of N and P in colchitetraploid plants were very obvious than diploid plants. So, the induction of ploidy levels could be efficiently used in horticulture to enhance new ornamental characters.

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