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## Research Article

# Detection and Evaluation the Tetraploid Plants of *Celosia argentea* Induced by Colchicines

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## Abstract

**Background:** Chromosome doubling using colchicine has been used in plant breeding programs to produce the polyploidy plants that have superior agronomic traits over their diploid counter part. They have an increase in cell size, leading to larger reproductive and vegetative organs, increasing the production of important medicinal compounds and a higher tolerance to environmental stress. **Methodology:** Seeds of *Celosia argentea* were treated by 0, 0.01, 0.02, 0.05, 0.1 and 0.2% colchicine for 48 h to produce polyploidy plants. Stomata size and density, pollen grain size, number of chloroplast per guard cell and DNA content as well as morphological traits were used to detect the polyploidy plants. **Results:** Putative tetraploid plants were increased significantly in all studied traits either vegetative, flowering growth or phytochemical compound compared to diploid ones. Pollen viability and seed germination percentage were decreased in the tetraploid plants compared to the diploid ones. The DNA content in the tetraploid plant was two times more than the diploids. Other traits used to screen the tetraploid plants were found to be effective. **Conclusion:** Finally, colchicine is an effective method in the breeding programme of *Celosia argentea*.

**Key words:** Colchicine, polyploidy, DNA content, stomata size, number of chloroplast, pollen grain

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Celosia argentea* is a cultivated annual plant belongs to the family Amaranthaceae. It is mainly used for flowerbeds in gardens. *Celosia argentea* is used as vegetable and medicinal plants. This plant is one of the main sources of pigments, proteins, calories, vitamins and minerals such as calcium, phosphorus and iron. It is also used for treating dysenter, diarrhea, acute abdominal pain, inflamed stomach and skin eruption. It contains different compositions which occur as secondary metabolites such as alkaloid, glycosides, flavonoids, saponins, tannins and carbohydrate<sup>1-4</sup>.

Chromosome doubling using colchicine has long been used in plant breeding programs and progress in genetic study. Polyploidy plants may be found in agriculture and horticulture as they often-superior agronomic traits over their diploid counter part. For example, polyploids may have larger leaves and flowers, thicker stems and roots, darker green leaves and an increased width to length ratio of the leaves. They have an increase in cell size, leading to larger reproductive and vegetative organs, increasing the production of important medicinal compounds and a higher tolerance to environmental stress<sup>5-7</sup>.

Indirect methods have been developed for ploidy determination, cytological characteristics such as size of stomata cells, stomata density, pollen grain diameter and number of chloroplasts in guard cells can be used as indirect ways for determining polyploids. Total DNA content can also be a good indicator of ploidy level as it increases by chromosome doubling that happens in polyploidy. Therefore, optical density of extracted DNA, measured by spectrophotometry is quick and simple way to determine the ploidy change<sup>8</sup>.

This study aimed to produce tetraploid plants in *Celosia argentea* using colchicine treatments and evaluated their performance in vegetative and flowering growth and their content from phytochemical composition. Also, determine the ploidy induction using stomata size and density, pollen grain size, number of chloroplast per guard cell and DNA content.

## MATERIALS AND METHODS

This study was carried out at the Nursery of Ornamental Plants, Faculty of Agriculture, South Valley University, Qena, Egypt.

Seeds of *Celosia argentea* var. *spicata* were treated by colchicine solutions at 0, 0.01, 0.02, 0.05, 0.1 and 0.2% for 48 h on 15 February, 2015. One hundred and twenty seeds were

used for each treatment. The seeds were sown in three replications; each one contained four plastic pots. Each pot contained a mixture of clay, sand (1:1 v/v). After 2 months, the seedlings were transplanted into plastic pots containing the soil mixture of clay and sand (3:1 v/v).

All plants of the different treatments, which possess a superior growth were examined for tetraploid state.

**Morphological observations:** After eight month from sowing, plant height, numbers of branches and leaves per plant, leaf area, stem diameter, length of the inflorescence in the main stem and number of inflorescences per plant were determined. Open pollinated seeds from diploid and confirmed tetraploid plants were collected and seed germination percentage was examined in petri dishes.

**Phytochemical determination:** Chlorophyll content (SPAD unit) was determined in the leaves with SPAD apparatus as described by Yadawa<sup>9</sup>. Anthocyanin determination was conducted in the inflorescences<sup>10</sup>.

Alkaloids were determined in the leaves<sup>11</sup>. Five grams of the powdered sample was extracted with 10 mL of petroleum ether. Then, petroleum ether were removed using aspirator. One gram of the extract was suspended in 10 mL of double distilled water and the pH was adjusted to 7.6. After shaking for 1 h the suspension was centrifuged. One milliliter of the supernatant was diluted to 50 mL with phosphate buffer. The absorbance was measured spectrophotometrically at 580 nm.

Total phenols were determined with the folin-ciocalteu assay methods<sup>12,13</sup>. One milliliter of ethanolic extract was added to 9 mL of distilled deionized water. One milliliter of folin-ciocalteu reagent was added to the mixture and shaken. After 5 min, 10 mL of 7% Na<sub>2</sub>CO<sub>3</sub> solution was added to the mixture. After incubation for 90 min at room temperature, the absorbance was determined at 750 nm with a UV spectrophotometer. The data was expressed as milligrams of Gallic Acid Equivalent (GAE) per gram extract.

**Ploidy determination:** Putative tetraploid plants were selected based on morphological traits from different treatments for further estimation to confirm the ploidy induction.

Size and density of stomata and chloroplast number measurements: Epidermal cells of two leaves from nine diploid plants and nine putative tetraploid plants were used. Epidermal cells of plant were obtained by applying thin layer of clear nail polish on the lower leaf surface and removing it after drying the polish (a basal side). Imprints were placed

on a microscope slide and observed through the light microscope at 1000x magnification equipped with digital camera. Number of stomata, number of chloroplast, length and width of stomata were measured using a line scale implanted in the micrometer ocular<sup>8</sup>.

**Pollen fertility and diameter:** Fertility of the pollen (PF) was determined using acetocarmine technique<sup>4</sup>. Poorly stained and shrunken pollens were considered as sterile.

$$PF (\%) = \frac{\text{No. of fertile pollen grains}}{\text{Total No. of pollen grains}} \times 100$$

Pollen diameter was also estimated in micrometer using a line scale implanted in the micrometer ocular.

**DNA content determination:** Genomic DNA was extracted from young leaves using extraction kit according to manufacture's instruction (Promega, USA). The DNA content was quantified by measuring absorbance at 260 nm wave length using UV spectrophotometer and calculated<sup>14,15</sup>.

**Statistical analysis:** Data of the diploid and confirmed tetraploid plants were statistically analyzed using t-test after calculation the variance homogeneity<sup>16</sup>.

## RESULTS AND DISCUSSION

Table 1 presents the mean values and variances of morphological traits for both diploid and tetraploid plants. The results of t-test indicated statistically highly significant differences between diploid and tetraploid plants for all studied traits.

Gigantism growth was found in polyploidy plants compared to diploids as shown in Fig. 1. Plant height was 45.6 and 98.0 cm for diploid and tetraploid plants, respectively as shown in Table 1. Numbers of branches were 9 and 91 for diploid and tetraploid plants, respectively. Large leaf number

and area were found in tetraploid (227.5 and 132, respectively) compared to (44.0 and 63.6) for diploid plants. Also, stem diameter was increased in tetraploid plant (1.37 cm) compared to diploid (0.63 cm). Number of inflorescences and length of inflorescence were increased significantly in tetraploid 17.0 and 15.0, respectively compared to diploid (5.3 and 6.0).

Colchicine inhibits the formation of spindle fibers and temporarily arrest mitosis at the anaphase stage. At this point, the chromosomes are replicated but cell division do not occur resulting in polyploidy cells. Increasing ploidy often results in increased cell size that in turn results in thicker, broader leaves and larger flowers<sup>7</sup>.

Seeds of both diploid and putative tetraploid were collected and germinated on petri dishes. Seed germination percentage was significantly decreased from 69.53-16.35% when the ploidy level turned from diploid to tetraploid as shown in Table 1. This may be due to their thicker seed coat and seedling emergence strength<sup>17</sup> or may be due to acute chromosomal damage, delay in the onset of mitosis, chromosomal aberrations induced enzyme activity such as catalase and lipase and hormonal activity resulted in reduced germination<sup>18</sup>.

Darker leaves and higher chlorophyll content in tetraploid were detected (41.6) compared to diploids (34.1). The dark green color is the result of bigger cells and more chlorophyll content<sup>19</sup>.

For medicinal plants, polyploids are usually more valuable because they exhibit increased biomass and content of effective compound<sup>19,20</sup>. Alkaloid content was 4.89-15.37 mg g<sup>-1</sup> for diploid and tetraploid plants respectively as shown in Table 2. Phenols also was increased significantly in tetraploid plants (15.0 mg g<sup>-1</sup>) compared to 8.1 mg g<sup>-1</sup> in diploid. Anthocyanin content as an antioxidant substance was also increased in the tetraploid plants (22.2 mg g<sup>-1</sup>) compared to diploids (10.6 mg g<sup>-1</sup>).

Figure 2 shows that stomata size and density. Length and width of the stomata in putative morphologically tetraploid plants were measured as shown in Table 3. The obtained results showed that, stomata size was highly significant

Table 1: Comparison between diploid and tetraploid plants of *Celosia argentea* concerning to plant height, number of branches and leaves, leaf area, stem diameter, length of inflorescence, number of inflorescences per plant and seed germination percentage

Treatments	Plant height (cm)		No. of branches		No. of leaves		Leaf are (cm <sup>2</sup> )		Stem diameter (cm)		Length of inflorescence		No. of inflorescences per plant		Seed germination (%)	
	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>
Diploid	45.6 <sup>b</sup>	292.6	9 <sup>b</sup>	18	44.0 <sup>b</sup>	216.0	63.6 <sup>b</sup>	537.9	0.63 <sup>b</sup>	0.006	6.0 <sup>b</sup>	9.5	5.3 <sup>b</sup>	0.66	69.53 <sup>a</sup>	6.6
Tetraploid	98.0 <sup>a</sup>	32.0	91 <sup>a</sup>	72	227.5 <sup>a</sup>	220.5	132.0 <sup>a</sup>	800.0	1.37 <sup>a</sup>	0.000	15.0 <sup>a</sup>	18.0	17.0 <sup>a</sup>	18.00	16.35 <sup>b</sup>	9.0
t-calculated	9.8**		29.1**		29.6**		6.3**		24.6**		5.8**		8.3**		33.2**	

\*\*Highly significant at p = 0.01,  $\bar{X}$ : Mean values and S<sup>2</sup>: Variance



Fig. 1(a-b): Photograph showing, (a) Tetraploid plant of *Celosia argentea* produced by colchicine treatments compared to (b) Diploid plants

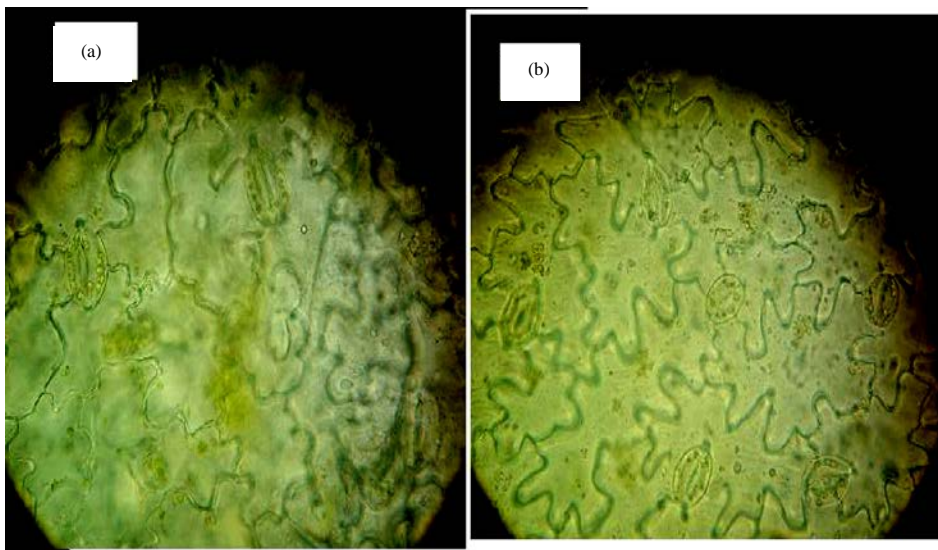


Fig. 2(a-b): Photograph showing stomata size and density in the (a) Tetraploid plant of *Celosia argentea* produced by colchicine treatments compared to (b) Control (diploid) plants

Table 2: Comparison between diploid and tetraploid plants of *Celosia argentea* concerning to chlorophyll content (SPAD unit), anthocyanin content, alkaloid and phenols content

Treatments	Leaf chlorophyll content (SPAD unit) (mg g <sup>-1</sup> )		Anthocyanin in inflorescence fresh weight		Alkaloid concentration (mg g <sup>-1</sup> dry weight)		Phenols concentration (mg g <sup>-1</sup> dry weight)	
	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>
Diploid	34.1 <sup>a</sup>	152.0	10.6 <sup>b</sup>	18.00	4.85 <sup>b</sup>	2.6	8.1 <sup>b</sup>	0.53
Tetraploid	41.6 <sup>a</sup>	99.9	22.2 <sup>a</sup>	27.38	15.37 <sup>a</sup>	2.5	15.0 <sup>a</sup>	0.98
t-calculated	1.5 <sup>NS</sup>		4.2 <sup>**</sup>		11.4 <sup>**</sup>		14.0 <sup>**</sup>	

NS: Not significant at p = 0.01, \*\*Significant at p = 0.01,  $\bar{X}$ : Mean values and S<sup>2</sup>: Variance

increased compared to diploids. Mean values were 100.0 and 147.5  $\mu\text{m}$  for diploid and tetraploid respectively with respect

to stomata length and 55 and 96.6  $\mu\text{m}$  for stomata width. Stomata size and number can be an indicator of polyploidy

Table 3: Comparison between diploid and tetraploid plants of *Celosia argentea* concerning to number of stomata/microscopic field, number of chloroplast per guard cell, length and width of stomata ( $\mu\text{m}$ ), pollen viability percentage, pollen diameter ( $\mu\text{m}$ ) and total DNA content ( $\mu\text{g mL}^{-1}$ )

Treatments	No. of stomata/ microscopic field		No. of chloroplast per guard cell		Length of stomata ( $\mu\text{m}$ )		Width of stomata ( $\mu\text{m}$ )		Pollen viability (%)		Pollen diameter ( $\mu\text{m}$ )		Total DNA content ( $\mu\text{g mL}^{-1}$ )	
	X	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>	$\bar{X}$	S <sup>2</sup>
Diploid	24.0 <sup>a</sup>	32.0	7.6 <sup>b</sup>	0.66	100.0 <sup>b</sup>	0.0	55.0 <sup>b</sup>	50.0	94.3 <sup>a</sup>	193.0	131.6 <sup>b</sup>	16.6	14.0 <sup>b</sup>	5.8
Tetraploid	13.3 <sup>b</sup>	0.6	12.8 <sup>a</sup>	2.80	147.5 <sup>a</sup>	75.0	96.6 <sup>a</sup>	66.6	88.0 <sup>a</sup>	40.7	286.0 <sup>a</sup>	1720	30.0 <sup>a</sup>	2.0
t-calculated	5.6**		3.8**		16.9**		13.0**		1.9 <sup>NS</sup>		11.1**		12.3**	

\*\*Highly significant at  $p = 0.01$ ,  $\bar{X}$ : Mean values and S<sup>2</sup>: Variance

levels and it has been used in different plant types for determining ploidy levels<sup>7,8</sup>. The counted stomata number in the microscope field was 24 for diploids and 13.3 in the polyploidy plants as shown in Table 3.

Larger stomata cells and decrease in stomata number were found in tetraploids in many studies research<sup>19</sup>. They stated that, stomata size and counting were identified as the most effective criteria of polyploidy, due to easier and economical application. When polyploidy level increased, plant cells became larger, while the number of stomata per area decreased.

Stomata measurement was used with morphological changes as pre selection method after colchicine treatment<sup>21</sup>. Number of chloroplast per guard cell was highly significantly greater in the tetraploid plant (12.8) than diploid plants (7.6). No significant decrease were found in the tetraploid plants with respect to pollen viability (88%) compared to diploid plant (94.3%) as shown in Table 3. The chromosomal aberrations, some genetic and physiological changes might have caused pollen sterility<sup>18</sup>. Pollen diameter in the putative tetraploid was significantly increased (286  $\mu\text{m}$ ) compared to diploid ones (131.66  $\mu\text{m}$ ). Greater number of chloroplasts per guard cell and pollen diameter were found in the polyploidy plant rather than diploid in the other studies<sup>7,22</sup>.

Total DNA content was increased significantly in the tetraploid plants (30  $\mu\text{g mL}^{-1}$ ) compared to (14  $\mu\text{g mL}^{-1}$ ) in the diploid ones as shown in Table 3. Many colchicine studies proved by treatment that the tetraploid cells contained DNA almost two times more than diploid ones<sup>15</sup>. Total DNA content of plants increases as a result of chromosome doubling that happens in polyploidization. This can be used as an indicator for ploidy determination<sup>8</sup>.

Colchicine has been widely used for chromosome doubling in a variety of plant species leading to the production of novel germplasm that can be used as bridging materials or as direct commercial cultivars with improving characteristics<sup>15</sup>.

## CONCLUSION

Tetraploid *Celosia argentea* plants with bigger vegetative and flowering growth and with highly phytochemical

compound content were obtained using colchicine treatments. Putative polyploidy was detected through examination of stomata size, stomata number, pollen grain size, number of chloroplast per guard cells and confirmed with DNA content.

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