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STUDIES ON INDUCTION OF POLYPLOIDY AND MOROPHOLOGICAL CHARACTERS OF SNAPDRAGON (*Antirrhinum majus*) AND LARKSPUR (*Delphinium ajacis*) PLANTS BY USING COLCHICINE

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ABSTRACT

In an effort to increase gigantism in diploid snapdragon 2n=16 and larkspur plants 2n= 16, the present study was undertaken to induce polyploidy by colchinization. Colchicine was applied as soaking seeds or to the emerging apical buds of transplants at 0.05, 0.1, 0.2 and 0.4 percent aqueous concentration employing cotton plugs for 24, 48, 72 and 96 hrs. The investigated parameters were emergence rate, stem length, stem thickness, leaf characteristics and flower characteristics. Results revealed that soaking seeds in 1% colchicine and 24-hour operating time induced the highest numbers of tetraploid plants in *Antirrhinum majus*, and 0.5 % for 48 hour gave the same result in *Delphinium ajacis*. Expression of gigantism in morphological characters such as plant height, size of leaves and flowers were observed in tetraploid plants.

Studies on morphological characters showed that the colchicine exhibited delayed reduction in number of leaves per plant. There was reduction in the number of flowers per plant and floral variants observed including bigger sized flowers.

Keywords: polyploidy, tetraploid, snapdragon (*Antirrhinum majus*), larkspur (*Delphinium ajacis*), colchicine.

INTRODUCTION

Polyploidy has been defined as the possession of four or more complete sets of chromosomes and has been an important feature of chromosome evolution in many floriculture and ornamental plants. There are various modes for the induction of polyploids, these include mechanisms of somatic doubling during mitosis by using colchicine (Kaarthik, 2004)

Polyploidy has played an important role in improving many plant species and hybrids. The field of floriculture and ornamental plants has probably benefited the most because polyploidy can increase genetic variability and tends to increase the size, genetic modifications, then the improvement of vegetative and flowering characters, so the economic importance of the material will have to be considered when planning breeding programmes. (Andersen, 2004).

Generally, polyploids sometimes seem to be more tolerant to stresses including drought, cold (Pašakinskienė, 2000) and poor soils (Buggs and Pannell, 2007) than their diploid progenitors.

The ease by which chromosome number in plants may be doubled by using colchicine appears likely to greatly stimulate interest in flower breeding. As a rule, tetraploid have larger flowers and may be bloom later and thus extend the flowering season (Gu. *et al.*, 2005).

In Egypt and Mediterranean countries, snapdragon (*Antirrhinum majus*) and larkspur *Delphinium ajacis* are two of the important winter annuals that can be used as cut flowers for exportation or for local markets, or for landscaping. The genus *Antirrhinum* have 2n=16, is a member of the Family *Plantaginaceae. Delphinium* is a plant genus belongs to the Family *Ranunculaceae* and has 2n=16 (Dalgaard, 1986).

Colchicine is used for inducing polyploidy in plant cells during cellular division by inhibiting chromosome segregation during mitosis. It is not only usually well tolerated, but in fact frequently results in plants which are larger, hardier, faster growing, and in general more desirable than the normally diploid parents; for this reason, this type of genetic manipulation is frequently used in breeding plants commercially. Treatment of diploid plants to obtain tetraploid can be done through (Kerr, 2001):

- Seed treatment: the most effective and safest way.
- Apical or adventitious meristem (shoot meristem treatment) of seedlings or intact plants, and
- *In-vitro* for cells, callus or portions grown on either solid or liquid medium.

The entire plant growing from a colchicine-treated seed could be polyploidy according to the concentration and time of soaking. Colchicine is nearly always lethal to seeds, and in the treatment there is a very fine line between polyploidy and death. So, the concentration and time for application of colchicine solutions must be define for every plant, and this is our main objective.

MATERIALS AND METHODS

The experiments were carried out at the Experimental Farm of the Faculty of Agriculture, Suez Canal University, during two successive seasons of 2004/2005 and 2005/2006. The strain Rose Bicolor of *Antirrhinum majus* and the strain Cliveden Beauty of *Delphinium ajacis* were examined for their chromosome number and used in the present study.

In the first experiment seeds of both genera were soaked in colchicine aqueous solutions. The concentrations of colchicine were 0.00, 0.10, 0.25, 0.50 and 1.00% (Ajalin *et al.,* 2003) for four periods as 24, 48, 72 and 96 hours (Gu *et al*., 2005 and Thao *et al*., 2003) on a shaker at room temperature. Seeds imbibed in distilled water served as a control. Each variant of colchicine application included three replicates, each replicate contained 50 seeds. They were kept for 24 hour on wet filter paper before colchicine application flush of treated seeds for taking out the rest of colchicine from tissues. After the period of application, the treated seeds were washed with distilled water and then planted in pots until seeds were germinated. After the true leaves appearing, seedlings were transplanted in the field.

In the second experiment, four-day seedlings of both snapdragon and larkspur were treated with colchicine solutions of 0.1, 0.25, 0.50, and 1.0% between cotyledon leaves using small pieces of cotton. Shoots covered with aluminum foil and operating time were 3, 5 and 7 days (Koksal *et al.,*

2002), applications were performed every day in the early morning. Direct sun light was avoided and humidity was carefully controlled to prevent the evaporation of colchicine solution. The cotton swab was kept wet by adding drops of colchicine using means of a dropper when required during daytime. The shoot apices were then washed to be free of colchicine. When the seedlings in the two methods of treatments reached four stage leaves, they were planted in prepared beds, and treated normally in respect of irrigation, fertilizer applications, and other horticulture practices.

Data were recorded for emergence rate or survival rate (percentage), vegetative characters (plant height and stem diameter), leaf characteristics (average leaf length, average leaf width and average leaf area (for snapdragon), flower characteristics (date to anthesis, florets length and number of florets in inflorescence).

Statistical analysis

The factorial experimental was layed and in randomized complete block design according to Gomez and Gomez (1984). The differences between means were separated by t- paired test and by Duncan's Multiple Range test. The differences were examined by t-paired test and Duncan's Multiple Range test to compare means of treatments.

RESULTS AND DISCUSSION

The introduction of new varieties or strains of larger flowers, better color, more satisfactory keeping quality, as well as types resistant to pests, diseases, drought and salinity, has further possibilities in greater development in the flower industry. In the breeding for new varieties or strains of flowers, the plant breeder needs to be aware of the requirement of superior plants. The development of polyploidy varieties or those with more sets of chromosome than the two sets found in diploid plants is one of the methods used for improvement of some flower crops which offer a certain amount of possibility to the plant breeder. (Naiki and Nagamasu 2004).

Conclusion driven from observation of DNA content, stomata number per microscopic field, stomata length and stomata width would indicate that there were different lines or strains. Accordingly, the following specific treatments were selected and subjected in subsequent studies (Silva *et al.,* 2000).

According to Bird (2007) the epigenetic changes play a role in the process of cellular differentiation, allowing cells to stably maintain different characteristics despite containing the same genomic material. Epigenetic refer to chromatin and DNA modifications that are stable over rounds of cell division but do not involve changes in the underlying DNA sequence of the organism. The modern usage of the word "epigenetic" is referring to heritable traits that do not involve changes to the underlying DNA sequence (**Akimoto** *et. al.* 2007). The genome sequence is static (with some notable exceptions), but cells differentiate in many different types, which perform different functions, and respond differently to the environment and intercellular

signaling. Thus, as individuals develop, morphogens activate or silence genes in an epigenetically heritable fashion, giving cells of new strains of either snapdragon or larkspur plants in this study.

Antirrhinum majus			
Soaking seeds		**Immersion of apical meristem**	
Line number	**Treatment**	**Line number**	**Treatment**
Control	Control	Control.	Control.
A-1	0.25% colchicine for 48 hours	A-6	0.25% colchicine for 5 days
A-2	0.25% colchicine for 96 hours	A-7	0.50% colchicine for 7 days
A-3	0.50% colchicine for 48 hours	A-8	1.00% colchicine for 5 days
A-4	0.50% colchicine for 72 hours	A-9	1.00% colchicine for 7 days
A-5	1.00% colchicine for 48 hours		
Delphinium ajacis			
Soaking seeds		**Immersion of apical meristem**	
Line number	**Treatment**	**Line number**	**Treatment**
Control	Control	Control.	Control.
D-1	0.10% colchicine for 48 hours	D -6	0.25% colchicine for 7 days
D -2	0.10 % colchicine for 72 hours	D -7	0.50% colchicine for 5 days
D -3	0.25% colchicine for 72 hours	D -8	0.50% colchicine for 7 days
D -4	0.50% colchicine for 48 hours	D -9	1.00% colchicine for 5 days
D -5	1.00% colchicine for 48 hours		

1-Percentage of emergence rate

First experiment: (Soaking seeds in different colchicine concentrations)

The emergence rate was evaluated in variants with swell seeds soaked in colchicine solution and in the distilled water. The percentage of emerged seedling from number of sown seeds was calculated. Data were transformed with angles corresponding to percentage.

As shown in Table (1) there was a continuous decrease in the survival percentage from the twenty-four hours treatment to the ninety sex hours of treatment with colchicine solution, regardless the colchicine concentration. Besides, the colchicine concentration of 0.1% showed a significant increase in the survival rate more than the other concentrations.

The results in the same Table (1) indicated that soaking seeds in any colchicine solution, in the two seasons, had lower emergence rate than the control.

Ajalin *et al.,* (2002), reported similar results on *Viola x wittrockiana*. They found that increasing soaking period and colchicine concentration lead to higher mortality rate. Also Blakesley *et al.,* (2002) concluded that the germination percentage of *Acacia dealbata* seeds tended to decline with increasing levels of colchicine, and increasing length of exposure. The findings of Rubuluza *et al.,* (2007) on *Colophospermum mopane* were in harmony with the obtained results.

Second experiment: Immersion of apical meristem.

Data concerning survival rate percentage of *A. majus* seedlings under investigation as affected by colchicine application, in both seasons, are given in Table (1). The survival rate is illustrated as a comparison between

emerged seedlings number after colchicine treatment and survived plants, which were planted in the field.

Number of survivals in all treatments was significantly lower than that of the control. Survival rate percentage was gradually decreased with increasing dose application during the two seasons. It is concluded from the above mentioned discussion that the application of the same concentrations of colchicine has less injurious effects when applied to the seedlings of *Antirrhinum majus* than when applied directly to the seeds.

Data of survival rate (percentage) of *D. ajacis* seedlings under investigation as affected by colchicine application, in both seasons, are given in Table (1). Under the effect of colchicine, the data revealed that survival (percentage) was reduced under all treatments compared with control, in both seasons.

Large differences were found between the two methods of colchicine application. The soaking of swell seeds brings a considerable suppress of an emerged rate and a high seedling dieback. In the application of the colchicine solution on the apex between cotyledons leaves, the survival rate was relatively high. The highest dieback was found in the 7th day's variant of a colchicine application on the apex.

In this respect Kahane *et al.,* (1997) found that survival rates of *in vitro* culture *Allium cepa* L. depended on plant line and colchicine concentration. These results were in agreement with the finding of Rubuluza *et al.,* (2007) who stated that increasing colchicine concentrations and exposure time were severely detrimental to growth and survival of *Colophospermum mopane*. In addition, similar results for colchicine applications were obtained from *in vitro* cultivated onion plants (Grzebelus and Adamus 2004). The survival rate was significantly fallen down with higher colchicine concentration.

Vegetative characteristics:

First experiment: (Soaking seeds in different colchicine concentrations)

Data in Table (2) show that the average length of main stem of *A. majus* cv. Rose Bicolor was 55.17±0.73 cm in diploid plants and ranged from 42.00±1.15 to 68.33±1.45 cm in tetraploid lines, in the first season, and ranged from 39.59±0.76 to 70.67±1.20 cm, in the second season. Data of the t-paired analysis showed a highly significant difference in stem length between diploid and tetraploid lines.

For *D. ajacis* cv. Cliveden Beauty data presented in Table (2) indicate that the average length of the main stem was 90.92±0.30 cm in diploid, and ranged from 93.95±0.33 to 100.17±1.01 cm in tetraploid, in the first season and from 93.08±0.55 to 99.00±0.29 cm, in the second season.

Data in Table (2) show the effect of interaction between colchicine concentrations and soaking time on plant height and stem diameter for the selected lines during both seasons. It is evident that the heights of *A. majus* and *D. ajacis* were decreased with the higher concentrations of colchicine.

Table (1) Effect of different colchicine concentrations and soaking time on emergence rate of *Antirrhinum majus* cv. Rose Bicolor and *Delphinium ajacis* cv. Cliveden Beauty, during both seasons.

Colchicine concentration (%)	Soaking time (h)	*Antirrhinum majus* cv. Rose Bicolor		*Delphinium ajacis* cv. Cliveden Beauty	
		1st season 2004/2005	2nd season 2005/2006	1st season 2004/2005	2nd season 2005/2006
Soaking seeds					
0.00	24	94.67 a	91.00 a	81.33 a	83.33 a
	48	92.67 a	92.00 a	82.00 a	82.00 a
	72	89.33 b	89.00 a	72.00 b	71.33 b
	96	86.67 b	85.00 b	65.33 c	66.00 c
0.10	24	86.00 b	84.67 b	71.33 d	70.00 d
	48	80.67 c	81.33 c	62.00 e	63.33 e
	72	75.33 d	74.67 d	47.33 e	46.67 e
	96	61.33 e	63.00 e	38.67 g	40.00 g
0.25	24	73.33 d	72.67 d	48.67 e	49.33 e
	48	66.00 e	62.00 e	37.33 e	38.00 e
	72	54.00 f	54.00 f	28.00 f	30.00 f
	96	29.33 g	27.33 h	20.00 h	21.00 h
0.50	24	64.67 e	61.33 e	39.33 f	38.00 f
	48	50.67 f	49.33 g	28.67 g	30.67 g
	72	27.33 g	26.00 h	16.67 i	20.67 i
	96	20.67 h	19.33 i	00.00 k	00.00 k
1.00	24	22.67 h	20.67 i	34.00 h	35.33 h
	48	15.33 l	10.00 j	13.33 j	12.67 j
	72	00.00 j	00.00 k	00.00 k	00.00 k
	96	00.00 j	00.00 k	00.00 k	00.00 k
Immersion of apical meristem					
0.00	3	100.00 a	100.00 a	100.00 a	100.00 a
	5	100.00 a	100.00 a	100.00 a	100.00 a
	7	100.00 a	100.00 a	100.00 a	100.00 a
0.10	3	98.00 b	96.67 b	84.67 b	86.00 b
	5	93.33 c	94.00 c	81.33 cd	78.67 cd
	7	80.67 f	82.00 f	72.67 e	72.67 e
0.25	3	93.33 c	92.00 d	86.00 b	84.67 b
	5	89.33 d	91.33 d	79.33 d	77.33 d
	7	74.67 g	73.33 g	65.33 b	65.67 b
0.50	3	94..00 c	91.33 d	81.33 c	82.00 c
	5	88.00 d	86.67 e	74.67 e	75.33 e
	7	79.00 f	81.33 f	64.67 f	64.00 f
1.00	3	94.00 c	90.67 d	73.33 e	75.33 e
	5	84.00 e	82.00 f	74.00 e	76.00 e
	7	70.67 h	72.67 g	60.67 g	62.67 g

Means with the same letters within a column are not significantly different according to Duncan's Multiple Range test within a column at 5% level.

These results were in agreement with those obtained by Kermani *et al.,* (2003) who reported that the internodes of *Rosa* were longer in tetraploids

than diploid plants. Besides the results of Oliveira *et al.,* (2004) on *Stevia rebaudiana*; Naiki and Nagamasu (2004) on *Damnacanthus indicus*; Eeckhaut *et al*., (2004) on *Spathiphyllum* cultivars and Escandón *et al*., (2005) on *Scoparia montevidiensis*, found that tetraploid plants show significant enhanced growth vigor and larger than diploid plants. Generally, Otto and Whitton (2000) stated that polyploidy is sometimes, but not always, associated with larger overall plant size. It is clear from Table (2) that stem diameter was gradually augmented as the colchicine concentrations increased, in both experimental seasons.

Data showed statistical differences in stem diameter between diploid and tetraploid lines. The average stem diameter of diploid plants was 0.71±0.03 cm and ranged from 1.42±0.06 to 1.75±0.03 cm for tetraploid lines, in the first season, and from 1.43±0.04 to 1.67±0.04 cm, in the second one, for *A. majus.* In addition, in *D. ajacis* the difference in stem diameter between diploid and individual tetraploid lines with t-paired test was highly significant. The average stem diameter of diploid plants was 0.51±0.01 cm and from 0.86±0.01 to 0.92±0.02 cm in tetraploid lines, in the first season, and from 0.86±0.03 to 0.93±0.01 cm, in the second season.

Van Tuyl *et a*l.,(1992) on *Lilium* and *Nerine*; Rupa *et al*., (1996) on *Catharanthus roseus* and Jacob *et al*., (1997) on *Anemone coranaria,* reported similar results. They found that the tetraploid level increase size of the most vegetative and reproductive plant organs among them: cotyledon size, stem size, diameter and rigidity, flower diameter and plant height.

Second experiment: Immersion of apical meristem.

Data presented in Table (2) show the effect of interaction between colchicine concentrations and soaking time on plant height for the selected lines and control plants, during both seasons for *A. majus* and *D. ajacis.*

On *A. majus,* when apical bud immersed in different concentrations of colchicine, it was noticed that the apical bud of some plants continued its growth, and in some others bud ceased to grow and the branches, developed from a lateral bud.

Data of the t-test analysis showed highly significant differences in stem length between diploid and tetraploid lines for either *A. majus* or *D. ajacis*. These data indicated that the tetraploid plants were taller than diploid plants.

Results showed that tetraploid plants had longer stem than diploid plants. This is in agreement with the findings of Jacob *et al.,* (1997) on *Anemone coranaria*; Silva *et al.,* (2000) on *Cattleya intermedia* and Zeldin and McCown (2003) on cranberry.

Sugiyama (2005) stated that cell division and cell expansion are fundamental processes for growth and development of plant organs. It is well known that polyploidy leads to an increase in organ size, which may be caused by changes in activities of cell division and expansion, resulting from duplication of gene loci and the increase in nuclear DNA content. This was confirmed with the findings of Rauf *et al.,* (2006) on *Gossypium arboreum*. They stated that the increase of both dimension and area were probably due to the fact that cells with a larger complement of chromosomes grow larger to maintain a constant ratio of cytoplasmic to nuclear volume, and express more proteins with the presence of more genes.

This increase in size may be translated to an increase in plant and its organ morphological characteristics in new ploidy levels.

Data of stem diameter for *A. majus* and *D. ajacis as* affected by colchicine application are given in Table (2). Data show statistical differences in stem diameter between diploid and tetraploid plants. In *A. majus* the average stem diameter of diploid plants was 0.73±0.01 cm and from 1.41±0.03 to 1.48±0.03cm for tetraploid plants, in the first season, and from 1.40±0.03 to 1.47±0.08 cm, in the second one. It is clear that stem diameter was gradually augmented as the colchicine concentration was going upward until the concentration of 1.00% for 7 days. While in the *D. ajacis* plants the average stem diameter of diploid plants was 0.54±0.01 cm, ranged from 0.88±0.01 to 0.92±0.01 cm in tetraploid lines, in the first season, and gave 0.52±0.02 cm in diploid plants while ranged from 0.85±0.00 to 0.91±0.00 cm in tetraploid lines, in the second season.

These results are in agreement with the findings of Eeckhaut *et al.,* (2004) on *Spathiphyllum wallisii*. They reported that polyploidization usually causes thicker stem.

Table (2) Plant height and stem diameter in the parental diploid and different tetraploid lines of *Antirrhinum majus* cv. Rose Bicolor and *Delphinium ajacis* cv. Cliveden Beauty during both seasons.

		Soaking seeds			Immersion of apical meristem		
		Line number	Plant height (cm)	Stem diameter (cm)	Line number	Plant height (cm)	Stem diameter(cm)
Antirrhinum majus	First season 2004/2005	Control	55.17±0.73	0.71±0.03	Control	56.17±1.09	0.73±0.01
		A-1	65.33±0.88**	1.47±0.02**	A-6	66.50±0.50**	1.44±0.02**
		A-2	68.33±1.45**	1.46±0.04**	A-7	63.67±1.20**	1.43±0.02**
		A-3	65.00±0.33**	1.42±0.06**	A-8	63.00±2.08**	1.41±0.03**
		A-4	64.67±0.58**	1.43±0.04**	A-9	58.00±1.15*	1.48±0.03**
		A-5	42.00±1.15**	1.75±0.03**			
	Second season	Control	52.67±1.33	0.74±0.02	Control	54.67±0.33	0.79±0.02
		A-1	69.33±0.67**	1.43±0.04**	A-6	73.50±0.58**	1.40±0.03**
		A-2	70.67±1.20**	1.44±0.03**	A-7	67.85±0.33**	1.41±0.10**
		A-3	63.33±0.67**	1.45±0.03**	A-8	62.33±1.01**	1.47±0.04**
		A-4	60.17±1.30**	1.49±0.02**	A-9	60.16±2.03*	1.47±0.08**
		A-5	39.59±0.76**	1.67±0.04**			
Delphinium ajacis	First season 2004/2005	Control	90.92±0.30	0.51±0.01	Control	91.08±0.85	0.54±0.01
		D-1	97.33±1.01**	0.86±0.01**	D-6	98.17±0.44**	0.91±0.01**
		D-2	100.17±1.01**	0.89±0.01**	D-7	96.33±0.88**	0.92±0.01**
		D-3	95.58±0.58**	0.87±0.02**	D-8	93.50±1.04**	0.88±0.01**
		D-4	96.50±0.58**	0.88±0.01**	D-9	89.75±0.14 ns	0.89±0.02**
		D-5	93.95±0.33**	0.92±0.02**			
	Second season	Control	90.08±1.40	0.54±0.01	Control	89.92±1.45	0.52±0.02
		D-1	96.83±0.83**	0.88±0.01**	D-6	96.50±0.76**	0.90±0.01**
		D-2	97.67±0.51**	0.89±0.02**	D-7	97.00±0.58**	0.85±0.00**
		D-3	98.08±0.55**	0.91±0.02**	D-8	94.17±0.73**	0.91±0.00**
		D-4	99.00±0.29**	0.86±0.03**	D-9	88.42±0.68 ns	0.86±0.01**
		D-5	93.08±0.55*	0.93±0.01**			

Results are given as mean values ± standard error * * Significant differences P < 0.01

Leaf characteristics

First experiment: (Soaking seeds in different colchicine concentrations).

The width, length and leaf area of the fifth leaf of *A. majus* were measured in diploid and tetraploid lines, but only the length and width of fifth leaf were measured in diploid and tetraploid plants of *D. ajacis*. Studying these characteristics would give an indication of the occurrence of polyploidy (Table 3).

The statistical analysis, using the t- paired test between every line and control indicated that the average length, width and area were highly significantly increased with polyploidy, or in other words, with the increase in number of genomes. It is noticed that colchicine caused a great increase in the length of the leaves of tetraploid lines as compared with diploid plants. It is clear that the differences in leaf length were highly significant between tetraploid lines and diploid. Generally, all tetraploid lines had larger leaves than control plants in *A. majus* and *D. ajacis.*

Imery and Cequea (2001) obtained similar results and Wang *et al*., (2001) found that expression of gigantism in morphological characters such as length, width and thickness were recorded for leaves of succulent leaves of *Aloe vera.* In addition, Chen and Gao (2007) stated that the leaves of tetraploid *Astragalus membranaceus* were larger than those of diploids.

Data on the width of fifth leaf are presented in Tables (3 and 4). A comparison of leaf width of diploid plants and tetraploid lines as represented by the fifth leaf blade of each showed that there was a highly significant increase in leaf width of tetraploid plants over the diploid ones.

As for leaf area of *A. majus,* data in Table (3) show that a comparison of leaf area between every line and control plants, as represented by the fifth leaf blade of each plant, using the t- paired test showed that there was a highly significant difference in leaf area .The average leaf area of *A. majus* was 11.90±0.7 cm^2 for diploid plants and ranged from 17.12±0.15 to 17.68±0.20 cm^2 in tetraploid lines, in the first season, and ranged from 16.63±0.35 to 17.22±0.47 $cm,^2$ in the second season

Sugiyama (2005) concluded that leaf size increased with the induction of cell ploidy, but the mechanism of this effect is poorly understood. Moreover tetraploid cultivars had faster leaf elongation rates than did diploid cultivars of *Lolium perenne* and *L. multiflorum*, resulting in longer leaves, mainly due to their longer mature cells, which lead to increase in cell length of the tetraploid cultivars caused by a faster cell elongation rate, not by a longer period of cell elongation and that polyploidy increases leaf size mainly by increasing the cell elongation rate, and thus increase final cell size.

Second experiment: Immersion of apical meristem

Data presented in Tables (3 and 4) show the response of leaf characteristics of *A. majus* cv. Rose Bicolor and *D. ajacis* cv. Cliveden Beauty to colchicine application.

It can be clearly noticed that leaf length was increased in tetraploid lines compared with control plants in both season. The statistical analysis using t-paired test between every line and control plants showed that colchicine caused a great increase in the length of the leaves of tetraploid plants as compared with diploid plants.

Increased leaf length is agreed with those found by Imery and Cequea (2001) on *Aloe vera;* Gao *et al.,* (2002) on *Scutellaria baicalensis;* Thao *et al.,* (2003) on *Alocasia* and Sakhanokho *et al.,* (2004) on daylilies. They stated that leaves of tetraploid plants were larger than those of diploid plants.

Data related to the leaf width of *A. majus* cv. Rose Bicolor and *D. ajacis* cv. Cliveden Beauty as affected by colchicine solution applications are given in Table (3and 4).

The statistical analysis with t-paired test for the average leaf width indicated that highly significant differences were found between diploid and all tetraploid lines, in both seasons of experiment.

It can be clearly noticed that leaf width increased in tetraploid lines compared with the control. Increased mean values of leaf width are in agreement with those reported by Lu and Bridgen (1997) on *Alstroemeria aurea*; Imery and Cequea (2001) on *Aloe vera* and Rauf *et al.,* (2006) on *Gossypium arboreum*. They stated that the expression of gigantism on morphological characteristics such as leaf width was noticed in tetraploid plants. For *A. majus,* results pertaining to average leaf area as affected by colchicine solutions application are shown in Table (3).

The average leaf area of tetraploid plants ranged from 17.96±0.42 to 19.50±0.13 cm, ^{2} in the first season and from 18.40±0.12 to 19.48±0.46 cm,^{2} in the second one. While, it was 11.83±0.48 cm^2 for diploid plants, in the first season. It can be noticed that, a comparison of leaf area of diploid plants and tetraploid as represented by fifth leaf blade area showed that there was a highly significant difference in leaf area, between diploid plants and tetraploid lines, during both seasons of the experiment.

Table (4) Leaf characteristics in different tetraploid lines as compared to diploid plants of *Delphinium ajacis* cv. Cliveden Beauty in two seasons.

***Delphinium ajacis* cv. Cliveden Beauty**					
Line number	**Leaf length (cm)**	**Leaf width (cm)**	**Line number**	**Leaf length (cm)**	**Leaf width (cm)**
Soaking seeds			**Immersion of apical meristem**		
First season, 2004/2005					
(Control)	6.10±0.06	8.80±0.06	**(Control)**	6.22±0.03	8.47±0.07
D-1	7.80±0.15**	10.47±0.09**	**D-6**	8.13±0.07**	10.87±0.03**
D-2	7.60±0.26**	10.43±0.12**	**D-7**	8.27±0.09**	11.07±0.13**
D-3	7.66±0.12**	10.47±0.09**	**D-8**	7.97±0.03**	11.17±0.13**
D-4	7.98±0.02**	10.20±0.12**	**D-9**	8.12±0.14**	11.40±0.06**
D-5	7.83±0.17**	10.70±0.06**			
Second season 2005/ 2006					
(Control)	6.22±0.03	8.47±0.07	**(Control)**	6.50±0.06	8.67±0.03
D-1	7.85±0.17**	10.50±0.00**	**D-6**	8.03±0.03**	11.13±0.07**
D-2	7.87±0.03**	10.53±0.07**	**D-7**	8.13±0.03**	11.21±0.05**
D-3	7.75±0.08**	10.53±0.07**	**D-8**	8.10±0.06**	11.03±0.12**
D-4	7.94±0.03**	10.43±0.03**	**D-9**	8.07±0.07**	11.20±0.15**
D-5	7.90±0.05**	10.77±0.03**			

Results are given as mean values ± standard error ** Significant differences at P < 0.01

Flower characteristics

First experiment: (Soaking seeds in different colchicine concentrations).

Tables (5 and 6) demonstrate the effect of colchicine treatments on number of days to anthesis, florets length, and number of florets per plant as compared with the control plants of *A. majus* and *D. ajacis.* It was interest to investigate whether or not the colchicine would affect the number of days required for anthesis (the first inflorescence on main stem). Data for average of days to flowering are presented in Tables (13 and 14). The data showed that colchicine treatments delayed date to anthesis. Results of 2004/2005 season showed that the average number of days required from planting to opening the first flower was 140.33±3.23 days, whereas it ranged from 161.78±1.22 to 168.44±0.44 days in tetraploid lines. In 2005/2006, it was 141.67± 4.19 in the diploid cultivar compared with 162.00±2.60 to 168.67±0.67 days in tetraploid lines for *A. majus.* In addition, in *D. ajacis* plants, the average number of days required from planting to opening of the first inflorescence was 137.67±1.45 days and ranged from 146.67±0.88 to 151.00±1.53 days for diploid and tetraploid lines, respectively, in the first season. The same trend was found in the second season.

However, polyploidy delayed flowering in *A. majus* and *D. ajacis* as indicated by number of days to anthesis. Richa and Srivastava (2004) stated similar results on *Helianthus annus*. They found that the tetraploid sunflower plants were delayed in flowering as compared with the diploids. They added that the possible delay in flowering in polyploidy has been attributed to the lower permeability accompanied by lower respiration resulting in the upset of translocation of food material from the site of production to the growing point.

As for florets length, it is noticed that, there were highly significant differences in floret length between diploid plants and tetraploid lines. Generally, colchicine treatments increased average florets length in *A. majus* and *D. ajacis* (Fig 1 &2). This increase was found to be statistically significant using the t- paired test, between any line and control plants, during both seasons for two plants. The larger size of florets in tetraploid lines were also reported by several investigators as Griesbach (1985) on *Phalaenopsis* orchids; Jacob *et al.,*(1997) on *Anemone coranaria*; Hancock (1997); Eeckhaut *et al*., (2004) on *Spathiphyllum* cultivars ,Gu *et al*., (2005) on *Zizyphus jujube* and Rauf *et al.,* (2006) on *Gossypium arboreum*. They concluded that polyploids had greatest values that can have much larger and heavily textured flowers (which might also last longer). They added that flowering might be delayed and/or prolonged.

The total number of florets produced by every plant used to determine the effect of polyploidy on this characteristic. Most of the workers stated that polyploidy decreases the number of florets produced per plant, and in this research, polyploidy plants showed a reduction in number of florets per plant (fewer florets per inflorescence).

Data presented in Tables (13 and 14) indicated that number of florets per plant was decreased due to polyploidy. The average numbers of florets per plant were 101.67±4.41 florets in diploid plants and ranged from 76.00±2.31 to 92.44±1.55 florets in tetraploid lines, in the first season, for *A.*

majus .On *D. ajacis* the average numbers of florets per plant were 79.67±2.19 in diploid plants and ranged from 101.33±4.48 to 111.67±1.33 in tetraploid lines in the first season. The same trend was found in the second season Significant differences were noticed in number of florets between control plants and the other colchicine treatments used during both seasons.

The results of number of florets per plant agree with the results of Boora *et al.,* (2003) on *Polianthes tuberosa* who stated that the number of florets increased with ploidy incidence. Although Zaffar *et al*., (2004) on saffron, they stated that there was a reduction in the number of flowers per plant in tetraploid plants compared with diploid plants.

Second experiment: Immersion of apical meristem

Data presented in Table (5 and 6) represent the response of *A.majus* and *D. ajacis* lines flowering to colchicine solution treatments, in both seasons.

It can be clearly noticed that number of days to anthesis was increased in tetraploid lines compared with the diploid plants, in both seasons, gradually with increasing colchicine concentration. Results showed highly significant differences between diploid and tetraploid lines in their date of flowering.

Increased mean values of days to anthesis is in agreement with those reported by Eeckhaut *et al*., (2004) on *Spathiphyllum wallisii;* Richa and Srivastava (2004) on *Helianthus annus* and Gu *et al., (2005)* on *Zizyphus jujube*. As for florets length, it is noticed that there were highly significant differences in floret length between any line and control plants, during both seasons. Generally, colchicine treatments increased average floret length. (Tables 5 and 6).

Several investigators also stated the larger size of florets in tetraploid plants as Griesbach (1985) on *Phalaenopsis* orchids; Hancock (1997) and Jacob *et al*., (1997) on *Anemone coranaria*. Comai (2000) stated that polyploid plants often show immediate phenotypic effects such as increasing cell size and organ size. These results are in agreement with the findings of Kermani *et al.,* (2003) on Rosa; *Eeckhaut et al.,* (2004) in *Spathiphyllum wallisii;* Gu *et al.,* (2005) on *Zizyphus jujube* and Escandón *et al* (2006) in *Bacopa monnieri.*

Data presented in Table (5) indicated that number of florets decreased due to polyploidy. The statistical analysis with t- paired test showed that significant differences were noticed in number of florets between diploid and tetraploid lines, during both seasons, of *A. majus.* The decrease in number of florets agreed with Griesbach (1990) on *Anigozanthos humilis*. In addition, Zaffar et al., (2004), obtained similar results on saffron. They reported that there was a reduction in number of flowers in tetraploid plants compared with diploid ones.

Meanwhile, in *D. ajacis,* it is noticed that highly significant differences were found in number of florets per plant between any line and control plants, during both seasons. Generally, colchicine treatments increased average number of florets per plant (Table 6)

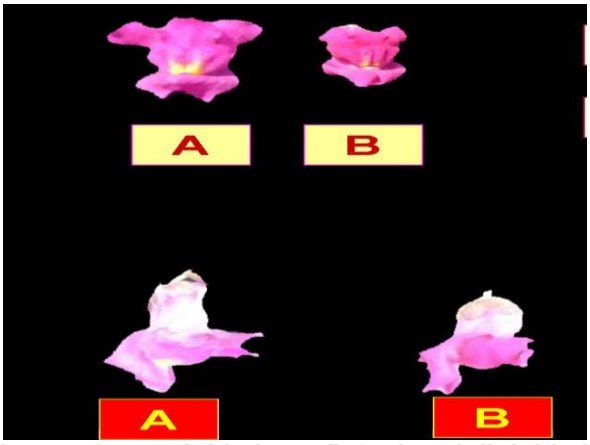

Fig (1) Flower of tetraploid plants (left A) and diploid plants (right B) of *Antirrhinum majus*

Fig (2) Flower of tetraploid and diploid plants of *Delphinium ajacis*

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دراسات علي إحداث التضاعف والصفات المورفولوجية لنباتي حنك السبع والعايق باستخدام الكولشيسين

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أجريت هذه الدراسة بمزرعة كلية الزراعة – جامعة قناة السويس لدراسة كفاءة الكولشيسين فى إحداث التضاعف فى كل من نباتى حنك السبع صنف روز بايكلر ونبات العايق صنف كليفيدين بيوتى باستخدام طرق وتركيزات وفترات زمنية مختلفة للمعاملة بالكولشيسين. تمت الدراسة على كل نبات على حدة وشملت الدراسة الآتى :

- نقع البذور فى محلول الكولشيسين بتركيزات: صفر ، 0.10 ،0.25، 0.50، 1 % ولفترات زمنية 24، 48، 72، 96 ساعة.
- معاملة القمة النامية للبادرات بالكولشيسين بنفس التركيزات السابقة ولفترات زمنية 3 ، 5 ، 7 أيام متتالية .

الصفات التى تم دراستها كانت كالتالى: نسبة الإنبات، طول وسمك الساق وصفات الورقة وصفات الزهرة لكل نبات من النباتات تحت الدراسة.

وكانت أهم النتائج المتحصل عليها هى :

- أعطت معاملة نقع البذور فى محلول الكولشيسين بتركيز 1 % ولمدة 24 ساعة أعلى نسبة من النباتات المتضاعفة لنبات حنك السبع ، أما فى نبات العايق فتم الحصول على نفس النتيجة عند نقع البذور فى محلول تركيزه 0.5% لمدة 48 ساعة .
- عند معاملة القمة النامية للنباتات كانت أفضل المعاملات هى المعاملة بتركيز 1 % لمدة 7 أيام متتالية فى نبات حنك السبع ولكن فى نبات العايق كانت أعلى نسبة تضاعف عند المعاملة بتركيز 0.25% لمدة 7 أيام.
- تأثرت نسبة الإنبات بدرجة كبيرة بتركيز الكولشيسين وكذلك بفترة النقع حيث أدت المعاملة بالتركيز 1 % لمدة 72 و 96 ساعة الى عدم إنبات البذور المعاملة فى نبات حنك السبع ، أما فى نبات العايق فأن المعاملة بكل من تركيز 0.50 % لمدة 96 ساعة و كذلك المعاملة بالتركيز 1 % لمدة 72 و 96 ساعة أدت الى عدم إنبات البذور المعاملة .
- توقفت نسبة النباتات الحية عند معاملة القمة النامية للنباتات على تركيز الكولشيسين وعلى مدة المعاملة فزيادة أي منهما تؤدى الى خفض نسبة النباتات الحية فى كلا النباتين.
- تم ملاحظة العملقة فى الصفات المورفولوجية مثل طول النبات ، حجم الأوراق والأزهار فى النباتات الرباعية.
- كذلك لوحظ تأخر موعد الأزهار مع نقص عدد الأزهار وكبر حجمها عند المعاملة بالكولشيسين ، أما الأوراق فكانت اكبر وأدكن لونا وذلك فى نباتى حنك السبع والعايق.

Table (3): Average length, width and area of fifth leaf in the diploid plants and the induced tetraploid lines during 2004/2005 and 2005/2006 seasons.

***Antirrhinum majus* cv. Rose Bicolor**							
Line number	**Leaf length (cm)**	**Leaf width (cm)**	**Leaf area (cm^2)**	**Line number**	**Leaf length (cm)**	**Leaf width (cm)**	**Leaf area (cm^2)**
Soaking seeds				**Immersion of apical meristem**			
First season, 2004/2005							
Control	7.53±0.15	2.30±0.06	11.90±0.7	**Control**	7.71±0.10	2.35±0.07	11.83±0.48
A-1	8.63±0.09**	2.93±0.05**	17.12±0.15**	**A-6**	9.67±0.16**	3.78±0.22**	17.96±0.42**
A-2	8.80±0.12**	3.19±0.16**	17.47±0.12**	**A-7**	10.08±0.06**	4.01±0.06**	19.04±0.19**
A-3	8.73±0.24**	3.24±0.70**	17..61±0.3**	**A-8**	9.47±0.24**	3.87±0.12**	19.50±0.13**
A-4	8.97±0.15**	3.10±0.09**	17.68±0.20**	**A-9**	9.78±0.35**	3.92±0.09**	18.97±0.23**
A-5	8.90±0.06**	3.01±0.09**	17.33±0.3**				
Second season 2005/ 2006							
Control	7.40±0.10	2.17±0.09	11.27±0.23	**Control**	7.45±0.23	2.31±0.15	11.95±0.25
A-1	8.43±0.07**	3.01±0.07**	16.94±0.33**	**A-6**	9.40±0.30**	3.85±0.14**	19.06±0.34**
A-2	8.40±0.06**	2.87±0.09**	16.87±0.31**	**A-7**	9.94±0.24**	3.50±0.17**	18.75±0.48**
A-3	8.23±0.15**	2.99±0.08**	16.63±0.35**	**A-8**	9.80±0.12**	3.72±0.11**	18.40±0.12**
A-4	8.46±0.03**	2.91±0.07**	17.22±0.47**	**A-9**	9.73±0.13**	3.89±0.13**	19.48±0.46**
A-5	8.23±0.12**	2.89±0.03**	16.73±0.31**				

Table (5) Effect of colchicine concentrations and duration on flower characteristics of diploid and polyploidy plants of *Antirrhinum majus*, in the two studied seasons

Antirrhinum majus cv. Rose Bicolor							
Line number	**No. of days to anthesis**	**Floret length (cm)**	**No. of florets per plant**	**Line number**	**No. of days to anthesis**	**Floret length (cm)**	**No. of florets per plant**
Soaking seeds				**Immersion of apical meristem**			
First season, 2004/2005							
Control	140.33±3.23	2.23±0.15	101.67±4.41	**Control**	140.00±2.89	2.30±0.08	96.33±3.41
A-1	161.78±1.22**	3.53±0.04**	92.44±1.55*	**A-6**	161.33±1.76**	3.53±0.04**	85.00±1.53*
A-2	163.67±1.86**	3.59±0.07**	91.11±2.06*	**A-7**	168.00±1.00**	3.54±0.06**	83.00±0.58*
A-3	164.67±2.14**	3.65±0.03**	81.67±3.18*	**A-8**	173.33±2.40**	3.47±0.04**	81.01±1.01*
A-4	166.00±2.00**	3.54±0.07**	85.00±2.89*	**A-9**	172.67±3.71**	3.49±0.05**	84.00±1.15*
A-5	168.44±0.44**	3.52±0.02**	76.00±2.31*				
Second season 2005/ 2006							
Control	141.67±4.19	2.40±0.05	109.33±4.19	**Control**	142.33±2.33	2.20±0.10	98.00±4.36
A-1	162.00±2.60**	3.54±0.02**	85.00±1.00*	**A-6**	165.67±1.86**	3.57±0.03**	88.33±1.67*
A-2	163.33±1.67**	3.67±0.07**	88.00±1.15*	**A-7**	173.33±1.76**	3.57±0.07**	84.33±1.20*
A-3	166.67±1.76**	3.53±0.06**	90.33±0.33*	**A-8**	174.67±1.45**	3.43±0.03**	87.33±1.33*
A-4	166.67±1.33**	3.6±0.05**	88.33±1.67*	**A-9**	175.33±1.86**	3.53±0.12**	86.67±1.67*
A-5	168.67±0.67**	3.48±0.02**	81.33±1.33*				

Results are given as mean values ± standard error

*** and ** significant differences at P < 0.05 and P < 0.01 respectively**

Table (6) Effect of colchicine concentrations and duration on flower characteristics of diploid and polyploidy plants of *Delphinium ajacis*, in the two studied seasons

Delphinium ajacis cv. Cliveden Beauty							
Line number	**No. of days to anthesis**	**Floret length (cm)**	**No. of florets per plant**	**Line number**	**No. of days to anthesis**	**Floret length (cm)**	**No. of florets per plant**
Soaking seeds				**Immersion of apical meristem**			
First season, 2004/2005							
Control	137.67±1.45	2.60±0.12	79.67±2.19**	**Control**	142.33±1.45	2.53±0.09	82.67±2.67
D-1	148.00±1.15**	3.52±0.04**	106.33±2.03**	**D-6**	155.33±0.33**	3.33±0.03**	103.00±1.73**
D-2	146.67±0.88**	3.51±0.05**	101.33±4.48**	**D-7**	157.33±0.33**	3.47±0.07**	102.67±2.33**
D-3	151.00±1.53**	3.52±0.05**	104.33±5.36**	**D-8**	159.67±0.33**	3.53±0.03**	109.00±3.79**
D-4	150.00±1.53**	3.63±0.02**	111.67±1.33**	**D-9**	163.00±1.00**	3.40±0.06**	97.67±1.45**
D-5	150.00±1.15**	3.58±0.03**	105.67±4.10**				
Second season 2005/ 2006							
Control	140.00±1.15	2.53±0.09	84.00±0.58	**Control**	143.67±1.20	2.67±0.07	85.33±2.40
D-1	150.33±1.33**	3.42±0.02**	105.00±3.46**	**D-6**	157.00±0.58**	3.48±0.04**	102.67±3.71**
D-2	152.33±0.33**	3.60±0.00**	97.67±0.33**	**D-7**	155.67±0.33**	3.36±0.04**	111.00±2.08**
D-3	155.67±0.33**	3.43±0.03**	109.00±2.08**	**D-8**	157.33±0.88**	3.58±0.09**	101.67±1.67**
D-4	154.00±1.00**	3.57±0.03**	98.67±1.20**	**D-9**	161.33±1.20**	3.62±0.03**	110.00±5.00**
D-5	157.00±1.15**	3.53±0.03**	107.67±1.45**				

Results are given as mean values ± standard error

*** and ** significant differences at P < 0.05 and P < 0.01 respectively**