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MUTAGENIC EFFECTS OF GAMMA IRRADIATION ON *Centaurea cyanus*, L.

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ABSTRACT

The experiments were carried out to study the effect of different doses of gamma-rays on some morphological traits in the M1 and M2 - generations of *Centaurea cyanus*. Seeds were irradiated with different doses of gamma- rays (0,5,10,15,20 and 25 kr.). Observations on germination percentage, plant height, number of branches, leaf chlorophyll content, flowering date, number of inflorescences, inflorescence diameter, morphological changes and mutation aberrations were recorded.

In the M1 and M2 -generations all doses of gamma radiation decreased the seed germination percentage. The plant height was reduced in all gamma radiation treatments in the M1 -generation of both seasons. The higher the dose the higher growth reduction obtained. In the M2 –generation the differences in plant height were not significant in both seasons. The number of branches was not affected by gamma-radiation treatments in all generations for both seasons. The effect of gamma-rays on the leaves chlorophyll content was not significant in both seasons. The flowering date was significantly affected by the different doses of gamma-rays in all generations in both seasons. There was an increase in the number of days to flowering with an increase in the gamma-radiation doses as compared with the control. The dose of 5Kr. produced the largest average number of inflorescences in the M1-generation of both seasons, however, in the M2-generation of both season, gamma-rays did not significantly affect the number of inflorescences. As for the inflorescence diameter, gamma-rays did not significantly affect the inflorescence diameter in all generations in both seasons. In the M2-generation, the results showed that there were slight change in the flower colour at the treatments of 15 and 25 Kr. in the first season and at 5 and 25 kr in the second one. The colour was lighter than normal in 4 plants. The doses of 25 and 20 kr in the first and second seasons respectively caused some changes in the shape of the floral organs in the M2 -generation in two plants.

INTRODUCTION

Centaurea cyanus is a winter annual plant that belongs to the family Compositae, growing to 40-90 cm tall, with grey-green branched stems. The leaves are lanceolate, 1-4 cm long. The flowers are most commonly an intense blue colour, produced in flowerheads (capitula) 1.5-3 cm diameter, with a ring of a few large, spreading ray florets surrounding a central cluster of disc florets. It is grown as an ornamental plant in gardens, as border plants and for cut flowers. It is also occasionally used as a culinary ornament, and as an ingredient in tea. Other names sometimes used in cultivation include "bachelor's button" or "basket flower" or "boutonniere flower".

Genetic variation is the starting point of any breeding programme. Genetic variation may already be present in nature, may be obtained after several years of selection, or may be produced through hybridization (for seed propagated crops). Spontaneous somatic mutations have played an essential role in the speciation and domestication of ornamental plants. Unfortunately, the rate of occurrence of spontaneous mutations is too low to satisfy practical breeding needs. Mutagenic agents such as radiation and certain chemicals can be used to induce mutations at a higher frequency and generate genetic variation from which desired mutants may be selected (Van Harten. 1998). Gamma irradiation is the main physical mutagen used to induce genetic variation (Novák, 1990). Induced mutations using ionizing radiation have produced a large number of new varieties by bringing about genetic changes in different ornamental plants which have already been commercialized such as Petunia (Kashikar and Khalatkar, 1981), Chrysanthemum, Bougainvillea, Hibiscus, Portulaca, Rose and Tuberose (Datta, 1991) and Gladiolus (Cantor *et al*., 2002) .

Centaurea cyanus is one of the plants that has narrow spectrum of natural morphological variation, which can be enhanced by using chemical or physical mutagens.

The main objective of the present study was to study the effect of different doses of gamma-radiation on some morphological traits of the M1- and M2-generations of *Centaurea cyanus*.

MATERIALS AND METHODS

Two experiments were conducted from 2003 to 2006 in the Floriculture and Ornamental Horticulture Research Garden, at El-Shatby. The first experiment dealt with the M1-generation while the second one dealt with the M2-generation.

M1-generation

Seeds of *Centaurea cyanus* L. “Double Bleuet” were used in these experiments. These seeds were obtained from Truffaut company, France. Gamma-rays used in this study were generated from the cobalt-60 source, in Gamma-Cell installed in Irradiation Laboratory at Middle East Regional Radio-isotope Center for the Arab Countries at El-Dokky , Cairo, Egypt.

The layout of the experiments was designed to provide complete randomized blocks experiment containing three replicates (Steel and Torrie, 1980). One hundred seeds were used for each treatment in every replicate.

On Oct. 7, 2003 and Oct. 12, 2004 in the first and second seasons respectively, dry seeds were exposed to different doses of gamma-rays. The used doses were 0, 5, 10, 15, 20 and 25 Kr. (at exposure rate 82 and 84r/sec. in the first and second seasons respectively).

The germination ratios were recorded in the laboratory, using Petri dishes containing wet filter papers. Fifty seeds were sown in each dish on Oct. 8, 2003 and Oct. 13, 2004 in the first and second seasons respectively. Three replicates were used for each treatment.

On Oct. 8, 2003 and Oct. 13, 2004 in the first and second seasons respectively. the seeds were sown in 50 cm. diameter clay pots containing 1 sand :1 clay soil : 1 peat moss (by volume). The layout of the experiments was designed to provide complete randomized blocks experiment containing three replicates (Steel and Torrie, 1980). One hundred seeds was used for each treatment in every replicate. Six weeks later, the plants were transplanted to 30 cm pots one plant per pot using the same experimental design. Every replicate contained 6 treatments. Twenty plants were used as an experimental unit for each treatment within every replicate. The M1-experiments were terminated on May 7, 2004 and May 10, 2005 in the first and second seasons respectively.

Observations were recorded for the M1-generation in the two successive experimental seasons based on seeds germination percentage, plant height (cm), chlorophyll content of the leaves (mg/100g fresh weight of leaves according to Wellburn (1994), flowering date (the number of days between sowing and the appearance of the first inflorescence on the plant), number of inflorescence , inflorescence diameter (cm) and any changes in plant growth and flowering.

Data were statistically analyzed and the mean comparisons were made according to least significant difference (LSD) at the 5% level of probability. For germination percentage, angular transformation was settled and the statistical analysis was carried out using values resulting from transformation.

M2-generation

The collected selfed seeds from M1-generations for each treatment were sown on Oct. 19, 2004 and Oct. 21, 2005 for the first and second seasons respectively, in 50 cm. diameter clay pots containing 1 sand :1 clay soil : 1 peat moss (by volume). Six weeks later, the plants were transplanted to 30 cm pots one plant per pot containing the same soil mixture used before and 3 replicates were used in the first and second seasons respectively. Each replicate contained 6 treatments for the first and second seasons respectively. Twenty plants were used as experimental unit. All characters of M2-generation were measured in the same manners mentioned in the M1-generations. Variations in the M2-generation included inflorescence colour and ray florets number were recorded.

RESULTS AND DISCUSSION

Seed Germination

In the M1 and M2 -generations all doses of gamma radiation decreased the seed germination percentage. The highest dose (25 kr) had the lowest germination percentage (Table 1). This reduction may be due to the effect of gamma radiation doses which inhibit the synthesis of enzymes, or may be due to the role of physical mutagen doses in awakening the meristemic cell division in the seeds.

These results are in agreement with those obtained by Abdel-Maksoud (1992) on *Solanum pseudo-capsicum*, Zaharia *et al.* (1991) on *Tagetes erecta*, Boncheol and Maluszynski (1997) on barley, Kumari and Singh (1997) on *Pisum sativum* and Cheema and Atta (2003) on basmati rice.

Table 1- Mean values of germination percentage of *Centurea cyanus* as affected by gamma radiation in the M_1 and M_2 generations of the first and second seasons.

Gamma -rays Dose kr	Germination Percentage (%)			
	First season		Second season	
	M_1	M_2	M_1	M_2
0	87.10	88.65	88.30	84.44
5	86.59	86.01	86.54	83.68
10	84.54	82.87	84.53	81.68
15	79.41	79.96	81.39	77.91
20	77.52	77.06	79.33	74.07
25	75.70	76.76	73.01	72.91
L.S.D. $_{0.05}$	2.76	2.41	3.12	3.09

Plant Height

The results presented in Table 2 show that the plant height was reduced in all gamma radiation treatments in the M1 -generation of both seasons. The higher the dose the higher growth reduction obtained. In the M2 –generation the differences in plant height were not significant in both seasons. This reduction in plant height might be due to the effect of gamma rays on the inhibition of DNA or enzymes synthesis which affect the cell division and elongation (Bidwell, 1979).

Similar results were reported by Sarawgi and Soni (1993) on *Oryza sativa,* Sareen and Koul (1994) on *Plantago ovata* and Badr *et al*. (2004) on *Gomphrena globosa*.

Table 2- Mean values of plant height of *Centurea cyanus* as affected by gamma radiation in the M_1 and M_2 generations of the first and second seasons.

Gamma -rays Dose kr	Plant height (cm)			
	First season		Second season	
	M_1	M2	M_1	M2
0	87.4	67.15	80.7	75.06
5	85.2	65.23	79.5	72.18
10	91.4	61.85	76.1	69.34
15	81.5	54.73	75.3	66.05
20	82.9	48.10	72.0	59.06
25	75.7	59.41	62.5	56.54
L.S.D. $_{0.05}$	5.60	N.S	4.66	N.S

N.S = not significant

Number of branches

Data reported in Table3 show that the number of branches was not affected by gamma-radiation treatments in all generations for both seasons. These results were in agreement with those reported by Venkatachalam and Jayabalan (1991) on *Zinnia elegans* Nasare and Choudhary (2003) on *Ocimum sanctum* and Badr *et al*. (2004) on *Gomphrena globosa.*

Table 3: Mean values of number of branches of *Centurea cyanus* as affected by gamma radiation in the M_1 and M_2 generations of the first and second seasons.

Gamma -rays Dose kr	Number of branches			
	First season		Second season	
	M_1	M_2	M_1	M_2
0	6.21	5.79	6.00	5.77
5	7.07	6.31	6.33	6.20
10	7.06	6.82	7.00	6.61
15	6.80	7.07	6.58	7.22
20	6.79	6.91	5.96	6.12
25	6.78	6.31	6.37	6.19
L.S.D. $_{0.05}$	N.S	N.S	N.S	N.S

N.S = not significant

Leaves chlorophyll content

The leaves chlorophyll content was studied in the M1 only. The differences among treatments were not significant in both seasons (Table 4).

It is known that the changes in chlorophyll content is associated with the changes in the chloroplasts. The important factors that control chloroplast differentiation area are: (1) Genetic information present in plastids which contain the chloroplast DNA, (2) Cytokinins have been shown to control chloroplast differentiation independently of their action on cell division and (3) inorganic salts (iron, magnesium, copper, potassium and ammonium salts) play important roles in the synthesis or metabolism of chlorophyll in plants (Konzak *et al.*,1972).

The effect of gamma-rays which resulted in chlorophyll mutant can be attributed to enhancement in chloroplast differentiation or any other reason from the previous ones.

Similar findings were reported by Misiha and Hussein (1992) on *Althea rosae* Badr *et al.* (2000) on *Tagetes erecta* and Youssef *et al*. (2000) on *Pelargonium graveolens.*

Table 4: Mean values chlorophyll content of *Centurea cyanus* as affected by gamma radiation in the M_2 generations of the first and second seasons.

Gamma -rays Dose kr	Chlorophyll content (mg/100 g) leaves	
	First season	Second season
	M_2	M_2
0	61.23	60.19
5	61.11	58.01
10	58.06	57.06
15	53.91	56.11
20	57.84	51.39
25	53.43	50.55
L.S.D. $_{0.05}$	N.S	N.S

N.S = not significant

Flowering Date

Data reported in Table 5 show that the flowering date was significantly affected by the different doses of gamma-rays in all generations in both seasons. There was an increase in the number of days to flowering with an increase in the gamma-radiation doses as compared with the control. The delayed flowering may be explained as a result of delaying or inhibiting the synthesis of florigens. The mechanism of floral initiation is a dramatic event involving a total change over the character and developmental pattern of the meristem. There are many discussions about florigens, or flower induction substances that act in the doses which enhanced the beginning of flowering. In this study, it may be explained as a result of delaying or inhibiting the synthesis of florigens which resulted in an increase by the number of days to flowering under the external environmental conditions because the induction of flowering can be affected by many factors and varied and may be either internal or external (Bidwell,1979).

These results were in agreement with those reported by Badr *et al.* (2000) on *Tagetes erecta,* Singh (2000) on *Lablab purpureus* and Khan (2004) on *Crocus sativus.*

Table 5: Mean values of the number of days to flowering of *Centurea cyanus* as affected by gamma radiation in the M_1 and M_2 generations of the first and second seasons.

Gamma -rays Dose kr	Number of days to flowering			
	First season		Second season	
	M_1	M_2	M_1	M_2
0	123.4	114.5	124.8	115.3
5	124.9	117.6	126.0	116.2
10	126.6	121.1	127.7	116.9
15	127.1	122.3	130.6	122.7
20	130.4	126.7	134.1	120.1
25	134.2	129.9	132.6	121.8
L.S.D. $_{0.05}$	1.13	1.39	2.09	1.76

Number of inflorescences

Data presented in Table 6 revealed that the dose of 5Kr. produced the largest average number of inflorescences in the M1-generation of both seasons, which significantly differed from the other treatments including the control. On the contrary, in the M2-generation of both season, gamma-rays did not significantly affect the number of inflorescences.

Table 6 Mean values of the number of inflorescences of *Centurea cyanus* as affected by gamma radiation in the M_1 and M_2 generations of the first and second seasons.

Gamma –rays Dose kr	Number of inflorescences per plant			
	First season		Second season	
	M_1	M_2	M_1	M_2
0	11.08	11.98	12.68	12.31
5	15.98	13.73	14.08	13.29
10	13.82	12.78	13.32	13.14
15	12.87	12.77	12.59	12.22
20	15.21	11.31	11.89	10.51
25	13.59	11.09	11.69	11.9 7
L.S.D. $_{0.05}$	1.09	N.S	1.17	N.S

N.S = not significant

Such different effects of the mutagen were also reported by Stepanenko and Regir (1983) on *Calendula officinalis*,L., Arnold *et al.* (1998) on roses and Badr *et al*. (2004) on *Gomphrena globosa.*

Bidwell (1979) mentioned that all steps in the flowering process are preprogrammed in the totipotent cells of the meristem. All that is needed as trigger or a release that sets these cells on the way in the program for flowering. The capacity to flower is inherent, like the capacity to form leaves.

Inflorescence diameter

Gamma-rays did not significantly affect the flower diameter in all generations in both seasons (Table7). However, the doses from 5 and 10 kr increased flower diameter slightly compared with the control. These results may be due to the effect of gamma-radiation doses on cell growth during flower initiation which affected cell number and/ or size. These results support the findings of Chauhan and Patra (1993) on Opium poppy, Badr *et al*. (2000) on *Tagetes erecta and* Khan (2004) on *Crocus sativus.*

Table 7: Mean values of inflorescences diameter of *Centurea cyanus* as affected by gamma radiation in the M_1 and M_2 generations of the first and second seasons.

Gamma -rays Dose kr	Inflorescence diameter(cm)			
	First season		Second season	
	M_1	M_2	M_1	M_2
0	6.53	6.98	6.50	6.77
5	7.99	6.31	5.98	6.58
10	8.01	7.82	5.31	6.25
15	7.67	7.79	6.82	6.17
20	6.60	6.31	6.77	6.32
25	5.98	5.62	6.19	6.11
L.S.D. $_{0.05}$	N.S	N.S	N.S	N. S

N.S = not significant

Induction of variability

Inflorescence colour

In the M2-generation, the results showed that there were slight change in the flower colour at the treatments of 15 and 25 Kr. in the first season and at 5 and 25 kr in the second one.The colour was lighter than normal in 4 plants (Figure1). This change in the flower colour can be attributed to the effect of the mutagen treatments together with temperature and light on the development of pigments (Bidwell,1979). Similar results were reported by Stepanenko and Regir (1983) on *Callendula officinalis*, Venkatachalam and Jayabalan (1994) on *Zinnia elegans,* Badr *et al. (2000)* on *Tagetes erecta* and Dhankhar and Dhankhar (2003) on okra.

F1

Inflorescence form

The doses of 25 and 20 kr in the first and second seasons respectively caused some changes in the shape of the floral organs in the M2 -generation in two plants (Figure 2). This may be due to that gamma-rays doses caused some changes in the flower bud during the time of its initiation**.** When a plant is damaged by radiation two basic things can happen. The radiation can go directly damage the cell's vital points, such as the cytoplasm (Chandorkar and Dengler, 1987). The second outcome is that the radiation will damage other things inside of the cell, the biggest thing to worry about is water in the cell. The water, when irradiated, makes free radicals that defuses and damages different parts of the cell. Commonly radiation doses affect the cell wall causing it to break down and shrink in size.

Similar findings were reported by Rani and Jayabalan (1992) on *Tagetes patula* , Geetha and Vaidyanathan (1998) on *Glycine max,* Badr *et al. (2000)* on *Tagetes erecta,* Devi *et al.* (2002) on rice and Korthica and Subba Lakshmi (2006) on soybean.

Figure 2: Types of inflorescence form abnormalities in the M2 of the first (left) and second(right) seasons.

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التأثيرات المطفرة لأشعه جاما على نبات عنبر سنتوريا

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الهدف من هذا البحث هو دراسة تأثير الجرعات المختلفة من أشعه جاما علي احداث بعض الاختلافات فى الصفات الظاهرية فى نبات عنبر سنتوريا صنف Double Bleuet و قد أجريت التجارب خلال السنوات 2003-2006 بحدائق ابحاث الزهور ونباتات الزينة بالارشاد التابعة لكلية الزراعة بالشاطبى –جامعة الإسكندرية. و تم زراعة موسمين متتاليين كالاتي M_1, M_2 في عامي ٢٠٠٣ , ٢٠٠٤كموسم أول , M1 , M2 في عامي ٢٠٠٤, ٢٠٠٥كموسم ثاني وعوملت البذور باشعه جاما بالجرعات صفر, ٥, ١٠, ١٥, ٢٠و ٢٥ كيلو راد. ويمكن تلخيص النتائج كما يلي:-

فى الجيل الطفورى الأول و الثانى وجد أنه كلما زادت الجرعات المستعملة من أشعة جاما كلما انخفضت نسبة الإنبات و قل ارتفاع النبات و ذلك خلال الموسم الأول والثانى.

لم توجد أي فروق معنوية بالنسبة لعدد الافرع و محتوى الأوراق من الكلوروفيل.

أخرت كل الجرعات المستخدمة من أشعة جاما تاريخ الإزهار فى الجيل الطفورى الأول و الثانى و لكلا الموسمين ولم تؤثر المعاملات على قطر النورة. لوحظ أنة عند الجرعة١٥ كيلو راد من أشعة جاما زاد عدد النورات مقارنة بالكنترول و كل الجرعات الاخرى المستخدمة من أشعة جاما .

تم الحصول علي أشكال متعددة من الاختلافات مثل ظهور اختلافات فى لون النورات (أفتح لونا) و ذلك فى الجيل الطفورى الثانى عند ١٥ و٢٥ كيلو راد فى الموسم الاول و٥ و٢٥ كيلو راد فى الموسم الثانى و كذلك شكل الأزهار الشعاعية فى النورة لنباتين عند ٢٥ كيلو راد فى الموسم الاول و عند ٢٠ كيلو راد فى الموسم الثانى. وقد تم جمع بذور هذه النورات وزراعتها لانتاج الجيل الطفورى الثا لث والرابع للتأكد من ثبات الصفة.