



MORPHOLOGICAL AND FLOWERING VARIABILITY OF *Lathyrus odoratus* BY USING SOME PHYSICAL AND CHEMICAL MUTAGENS

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ABSTRACT

An investigation to study the influence of gamma rays as seeds exposing (0, 1, 5, 10, 15 and 20 kr) and N – Nitroso – N – Methyl Urea (NMU) as seed soaking in concentrations (0.0, 30, 60, 90, 120 and 150 ppm) on *Lathyrus odoratus*, it was conducted at the Experimental Farm of Fruit and Ornamental Plants Breeding Research Department, Horticulture Research Institute, ARC, Egypt during the two consecutive seasons of 2012/2013 and 2013/2014. The two experiments were arranged in randomized complete block design (RCBD) with three replicates. In M₁-generation, the results revealed lower in seed germination percentage, plant height, leaf chlorophyll content and number of flowers with increasing doses and concentrations of physical and chemical mutagens. However, the effect of mutagens on leaf length and flower anthocyanin content was insignificant at M₁ and M₂-generations. The higher doses of gamma-rays and concentrations of NMU led to delay the flowering date in M₁ and M₂-generations, decrease the seed germination percentage in M₂-generation, while lower doses and concentrations caused an increase in plant height, number of branches/plant, number of leaves/plant, number of flowers/plant and leaf chlorophyll content in M₂-generation. The variations means squares indicated insignificant difference between gamma-rays and NMU in M₁ and M₂-generations, in seed germination percentage, number of branches per plant, number of leaves per plant, flowering date and number of flowers per plant. However, higher variation was observed between gamma-rays and NMU in M₁ and M₂-generations, for plant height, leaf length and leaf chlorophyll content. The obtained results clearly indicated that different doses and concentrations of mutants can be effectively utilized to create variability for plant height (dwarfness plants and compact) fasciata, chlorophyll mutation, leaf shape and flowers cluster length of sweet pea plants.

Key words: *Lathyrus odoratus*, gamma rays, Nitroso Methyl Urea, fasciata, mutation

INTRODUCTION

The species of the genus *Lathyrus* (Leguminosae-Fabaceae) are distributed mainly in temperate zones of the Northern hemisphere, Africa and South America (Goyder, 1986). The genus consists of about 160 annual and perennial species (Allkin *et al.*, 1986; Plitman *et al.*, 1995). The species are separated into 13 sections based on morphological traits (Kupicha, 1983). Some species such as *Lathyrus odoratus*, *L. sativus*, *L. cicera* and others were of agricultural importance as forage, fodder or

ornamental plants and had a long history as cultivated plants.

Gamma rays (are a part of electromagnetic spectrum) belong to ionizing radiation can be energetically charged particles, such as electrons or high-energy photons. The biological effect of gamma rays based on the interaction with atoms or molecules in the cell, particularly with water to produce free radicals in cells (Wi *et al.*, 2005). The effects of gamma irradiation on different parts of plant; *i.e.*, bulb, tuber, stem cutting, fruits and seeds were investigated. Irradiated seeds with gamma rays induced

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biochemical contents; *i.e.*, enzyme, protein and phytohormones. They were severely changed by exposing seeds to gamma rays. It is worthy to notice that gamma irradiation induced either stimulation or inhibition of growth and endogenous hormones. Lower dosage of gamma rays could stimulate growth.

The number of chemical mutagens are very great and in continually increasing. However, for practical purposes of mutation in cultivate plants, so far only a few are really useful, most of these belong to the special class of alkylating agents and may be listed as follows: Nitroso Methyl Urea (NMU), Nitroso Ethyl Urea (NEU), Ethyl Methane Sulphonate (EMS), Methyl Methane Sulphonate (MMS), Diethyl Sulphate (DES), Ethyleneimine (EI). Azides are also effective mutagens, also cholicine is important for inducing chromosomal doubling (Badr *et al.*, 1990).

This investigation aimed to study the effect of different doses of physical mutagen (gamma radiation) and concentrations of chemical mutagen (N-Nitroso-N-Methyl Urea) on *Lathyrus odoratus* to induction some variations and mutations in vegetative growth and flowering.

MATERIALS AND METHODS

The present work was carried out on *Lathyrus odoratus* at the Experimental Farm of Breeding Research Department for Fruit trees, Ornamental Plant and Woody Plants, Horticulture Research Institute, Agricultural Research Center, Egypt. Seeds of a local variety (Apollo) of *Lathyrus odoratus* were taken from The Orman Botanical Garden Farm, during the two successive seasons of 2012/2013 and 2013/2014. The physical and chemical properties of the experimental farm soil are shown in Table 1.

The present experimental work involved studying the following two main treatments:

Effect of Gamma Rays

This experiment included six doses of gamma rays; *viz.*, 0, 1, 5, 10, 15 and 20 krad. Gamma rays used were generated from Cobalt – 60 source in gamma cell installed in Irradiation Laboratory at Middle East Regional Radio – Isotope Center for Arab Country at Cairo,

Egypt. The cobalt source emitting radio energy of 86 rad / second (it's called chronic in dilation. Healthy and dry seeds were irradiated by gamma rays at different doses.

Effect of N-Nitroso-N- Methyl Urea (NMU)

This experiment included six concentrations of NMU *viz.*, 0.0, 30, 60, 90, 120 and 150 ppm. The used NMU in this study was obtained from mark W. Germany. Seeds were soaked in freshly prepared solutions from NUM for 12 hours and thoroughly rinsed in tap water. Then, it immediately washed in running water to remove excess solution from the surface of seeds.

The plot area of two main experiments was 6 m². Seeds were sown in the field in hills 25cm apart. It was sown on 15th October in the first and second seasons of 2012/2013 and 2013/2014. Seedlings were thin to one plant per hill after three weeks from sowing. The treatments were arranged in a randomized complete block design (RCBD) with three replicates for the two experiments. All the plants received normal agricultural practices whenever they were needed.

Data Recorded

Growth parameters

After 21 days from sowing, seeds germination percentage as well as after 90 days of sowing, plant height (cm), branche number per plant, number of leaves per plant and leaf dimension (as leaf length) were determined.

Flowering parameters

After 105 days from sowing, days to flowering and flower number per plant were calculated.

Chemical analysis

Leaf chlorophyll content was estimated in leaves by using SPAD-502 meter as described by (Yadava, 1986). A sample of dry petals was randomly taken from each treatment for chemical analysis. Furthermore, samples of sweet pea petals were air-dried until a constant weight was obtained. The anthocyanin content (mg/100 g) in dried petals was colorimetrically determined according to the method described by Fuleki and Francis (1968) and adopted by Francis (2000) for *Lathyrus odoratus*.

Table 1. Physical and chemical properties of experimental farm soil

Character	Clay (%)	Silt (%)	Sand (%)	Texture	pH	Organic matter	Available nutrients (ppm)		
							N	P	K
Value	48.78	28.46	22.76	Clay	7.85	1.75	16.72	10.90	80.8

Mutation frequency

Percentage and number of survival plants of *Lathyrus odoratus* were estimated.

Statistical Analysis

Analysis of variations, (F- test and mean comparison by LSD) for each mutagen, in each generation was run according to completely randomized block design (Snedecor and Cochran, 1980). Comparing variation observed and test the significance; larger mean square/smaller mean square was done according to Snedecor and Cochran (1980). A test of equality of two variances was computed.

RESULTS AND DISCUSSION

Results of physical (gamma-rays) and chemical (N-Nitroso-N-Methyl Urea) mutagens in first (M_1) and second (M_2) mutated generations of *Lathyrus odoratus* plants, will be presented below. It is known that, the effect of mutagens in the M_1 -generation was mostly on the plant physiology, but in the M_2 -generation was the mutated.

Effect of gamma Irradiation and N-Nitroso-N-Methyl Urea (NMU) on Growth Parameters

Seed germination percentage

In the M_1 -generation, seed germination percentage was decreased with increasing the doses of gamma rays and concentrations of NMU, the dose of 20 kr, was more effective in reducing seed germination percentage (less than 50% germination) (Table 2), in M_2 -generation, the results recorded highly significant and significant reduction in seed germination percentage when subjected to the doses of gamma rays and NMU concentration, respectively in *Lathyrus odoratus* plants, compared with control.

When comparing the variations mean squares (Table 3), as a result of the effect of both gamma rays and NMU mutagens, it was insignificant. That means that no differences of the effect on seed germination of all the cases compared could be recorded.

The obtained results are in harmony with those reported by Abo El-Kheir (2004) on *Brassica oleraceae*, Ilbas *et al.* (2005) on barley, Khalaf (2008) on *Amaranthus caudatus*, Mostafa (2011) on *Helianthus annuus*, Ariraman *et al.* (2014) on pigeon pea and Gaswanto *et al.* (2016) on chilli plant.

Plant height

Effect of gamma-rays and NMU on plant height (Table 4) indicated that the values recorded were reduced with the increasing of the doses and concentrations, while 5 and 1 krd in the M_1 and M_2 generations and 30 ppm in the M_2 - generation increased the plant height. The reduction was highly significant than the control in the M_1 and M_2 generations.

Comparing the variations means squares (Table 5) results indicate significant difference between gamma-rays in M_1 and M_2 generations, but the variation were insignificant between NMU in the M_1 and M_2 - generation significant variation was observed between NMU and gamma-rays in M_2 and M_1 generation, respectively.

These results are in a accordance with those found by El-Ashry *et al.* (1992) on *Lathyrus odoratus*, Mahmoud (2002) on delphinium and mathiola, khalaf (2008) on *Amaranthus caudatus*, Quecini *et al.* (2008) on *Petunia hybrid*, Ariraman *et al.* (2014) on Pigeon pea and Mostafa (2015) on *Khaya senegalensis*.

Table 2. Effect of physical (gamma rays) and chemical (NMU) mutagens on seed germination percentage of *Lathyrus odoratus* in the M₁ and M₂ generations during (2012/2013 and 2013/2014) seasons

Gamma rays (kr)	Seed germination percentage				
	M ₁	M ₂	Nitroso methyl urea (NMU) (ppm)	M ₁	M ₂
0 kr	94.443	91.11	0.00 ppm	95.55	91.11
1 kr	93.33	87.776	30 ppm	87.773	83.33
5 kr	91.106	84.44	60 ppm	81.106	75.553
10 kr	86.663	75.553	90 ppm	77.773	71.106
15 kr	68.886	58.886	120 ppm	75.553	69.996
20 kr	48.886	38.886	150 ppm	72.22	64.44
F. test	**	**	F. test	**	**
LSD 5%	11.125	9.128	LSD 5%	8.06	6.611
LSD 1%	15.913	13.065	LSD 1%	11.534	9.461

** = Highly significant at 0.01.

Table 3. Comparison of variations resulted from (gamma rays) and (NMU) mutagens on seed germination percentage of *Lathyrus odoratus* in the M₁ and M₂ generations during (2012/2013 and 2013/2014) seasons

Mutagen				Comparison			
Gamma		Chemical		Gamma		Chemical	
M ₂ .L.g	NS	M ₂ .L.c	NS	M ₁ .L.g	NS	M ₁ .L.g	NS
————	1.244	————	1.278	————	4.410	————	3.454
M ₁ .L.g		M ₁ .L.c		M ₁ .L.c		M ₂ .L.c	
				M ₂ .L.c	NS	M ₂ .L.c	NS
				————	1.009	————	1.161
				M ₁ .L.g		M ₂ .L.g	

M₁; First mutated generation

C; Chemical mutagen

M₂; Second mutated generation

NS; Not significant

L; *Lathyrus odoratus*

G; Gamma ray

Table 4. Effect of physical (gamma rays) and chemical (NMU) mutagens on plant height (cm) of *Lathyrus odoratus* treated seeds in the M₁ and M₂ generations during (2012-2013 and 2013-2014) seasons

Gamma rays (kr)	Plant height (cm)				
	M ₁	M ₂	Nitroso methyl urea (NMU) (ppm)	M ₁	M ₂
0 kr	134.213	139.65	0.00 ppm	135.076	141.203
1 kr	135.226	154.35	30 ppm	130.966	151.033
5 kr	135.583	139.756	60 ppm	128.553	147.513
10 kr	133.886	134.606	90 ppm	127.32	137.38
15 kr	130.946	130.89	120 ppm	126.163	131.973
20 kr	127.43	129.366	150 ppm	122.503	127.416
F. test	**	**	F. test	*	**
LSD 5%	2.369	4.222	LSD 5%	6.609	5.726
LSD 1%	3.389	6.043	LSD 1%	9.458	8.195

** = Highly significant at 0.01.

* = Significant at 0.05

Table 5. Comparison of variations resulted from (gamma rays) and (NMU) mutagens on plant height of *Lathyrus odoratus* in the M₁ and M₂ generations during (2012/2013 and 2013/2014) seasons

Mutagen				Comparison			
Gamma		Chemical		Gamma		Chemical	
M ₂ .L.g	*	M ₂ .L.c	NS	M ₁ .L.c	NS	M ₂ .L.c	*
————	8.354	————	1.278	————	————	————	7.832
M ₁ .L.g		M ₁ .L.c		M ₁ .L.g	1.878	M ₁ .L.g	
				M ₂ .L.c	NS	M ₂ .L.g	NS
				————	4.447	————	1.066
				M ₁ .L.g		M ₂ .L.c	

M₁; First mutated generation C; Chemical mutagen M₂; Second mutated generation
 NS; Not significant L; *Lathyrus odoratus* *; Significant at 0.05 G; Gamma - ray

Number of branches per plant

In the M₁ and M₂ –generations (Table 6) 1 and 5 krd of gamma–rays showed significant stimulation effect on branch number of *Lathyrus odoratus*, but in the M₁–generation, it was reduced with the increase of the concentration of NMU, whereas, in the M₂-generation 30 ppm of NMU had significant stimulation effect on branch number of *Lathyrus odoratus*.

Recorded data in M₁ and M₂ –generations (Table 7) showed highly significant reduction in branch number with gamma rays and NMU. However, testing the variation at this growth stage (Table 7) between gamma rays and NMU treatments, in the M₁ and M₂ generation were found to be insignificant, and at this growth stage the response was similarly, and no difference was recorded between M₁ and M₂ –generations.

These results are in harmony with those reported by El-Tony (1999) on *Tagetes erecta*, Badr *et al.* (2004) on *Gomphrena globosa*, Hussein (2005) on *Anethum graveolens*, Karthika and Lakshmi (2006) on soybean and Mostafa (2015) on *Khaya senegalensis*.

Number of Leaves / plant

Results in Table 8 show that the mutagenic treatments of gamma rays and NMU caused highly significant effect on leaf number per plant. Moreover, gamma – rays and NMU treatments had significant stimulation effect on number of leaves per plant at 1 kr and 30 ppm on both M₁ and M₂ –generations, respectively.

Comparing the variation mean squares (Table 9) results indicate insignificant difference between gamma rays and NMU in M₁ and M₂-generations. These results are in agreement with those reported by El-Ashry *et al.* (1992) on *Lathyrus odoratus*, Badr *et al.* (2004) on *Gomphrena globosa*, Khalaf (2008) on *Amaranthus caudatus*, Ibrahim *et al.* (2009) on *Lathyrus odoratus*, Mostafa (2011) on sunflower plant and Mostafa (2015) on *Khaya senegalensis*.

Leaf length

The effect of gamma rays and NMU treatments on leaf length of M₁ and M₂ –generations are listed in Table 10. In M₁ and M₂ –generations it had insignificant effect when compared with control in each, respectively. So that, at this growth stage the response was similar, and there were no differences between M₁ and M₂ –generations.

Comparing variations (Table 11) showed higher significant mean square from gamma rays when compared mean square in M₁ and M₂ –generations. Such variation detected that NMU produced higher variation in M₁ and M₂ generations. However, it was observed that the variations between gamma rays and NMU were higher in M₂ and M₁ –generations, respectively on leaf length in *Lathyrus odoratus*.

The results are in accordance with those found by Dila *et al.* (2003) on chrysanthemum, Encheva *et al.* (2003) on sunflower, Ibrahim *et al.* (2009) on *Lathyrus odoratus*, Naik and Murthy (2009) on *Guizotia abyssinica* and Li *et al.* (2010) on *Stenotaphrum secundatum*.

Table 6. Effect of physical (gamma rays) and chemical (NMU) mutagen on number of branches per plant of *Lathyrus odoratus* in the M₁ and M₂ generations during (2012/2013 and 2013/2014) seasons

Gamma rays (kr)	Number of branches per plant				
	M ₁	M ₂	Nitroso methyl urea (NMU) (ppm)	M ₁	M ₂
0 kr	9.48	8.076	0.00 ppm	9.72	10.336
1 kr	11.603	10.283	30 ppm	9.316	13.72
5 kr	11.633	11.35	60 ppm	8.306	12.886
10 kr	10.376	9.91	90 ppm	9.423	9.813
15 kr	10.016	8.456	120 ppm	8.65	8.523
20 kr	8.583	7.96	150 ppm	8.206	7.796
F. test	**	**	F. test	N.s	**
LSD 5%	1.33	0.631	LSD 5%	—	0.803
LSD 1%	1.903	0.902	LSD 1%	—	1.148

** = Highly significant at 0.01.

NS = Not significant at 0.05

Table 7. Comparison of variations resulted from (gamma rays) and (NMU) mutagens on number of branches per plant of *Lathyrus odoratus* in the M₁ and M₂ generations during (2012/2013 and 2013/2014) seasons

Mutagen				Comparison			
Gamma		Chemical		Gamma		Chemical	
M ₂ .L.g	NS	M ₂ .L.c	NS	M ₁ .L.c	NS	M ₂ .L.c	NS
—————	1.612	—————	4.169	—————	3.578	—————	3.868
M ₁ .L.g		M ₁ .L.c		M ₁ .L.g		M ₁ .L.g	
				M ₂ .L.g	NS	M ₂ .L.c	NS
				—————	5.769	—————	2.399
				M ₁ .L.c		M ₂ .L.g	

M₁; First mutated generation
L; *Lathyrus odoratus*

M₂; Second mutated generation
NS; Not significant

C; Chemical mutagen
G; Gamma ray

Table 8. Effect of physical (gamma rays) and chemical (NMU) mutagen on number of leaves per plant of *Lathyrus odoratus* in the M₁ and M₂ generations during (2012/2013 and 2013/2014) seasons

Gamma rays (kr)	Number of leaves per plant				
	M ₁	M ₂	Nitroso methyl urea (NMU) (ppm)	M ₁	M ₂
0 kr	259.633	254.533	0.00 ppm	258.62	255.983
1 kr	272.556	266.99	30 ppm	276.66	284.393
5 kr	266.12	266.52	60 ppm	270.30	280.010
10 kr	256.40	259.633	90 ppm	263.58	271.106
15 kr	256.47	257.79	120 ppm	255.61	255.656
20 kr	257.49	242.78	150 ppm	250.356	249.060
F. test	**	**	F. test	**	**
LSD 5%	4.068	4.891	LSD 5%	4.804	8.595
LSD 1%	5.823	6.999	LSD 1%	6.904	12.291

**; Highly significant at 0.01.

Table 9. Comparison of variations resulted from (gamma rays) and (NMU) mutagens on number of leaves of *Lathyrus odoratus* in the M₁ and M₂ generations during (2012/2013 and 2013/2014) seasons

Mutagen				Comparison			
Gamma		Chemical		Gamma		Chemical	
M ₂ .L.g	NS	M ₂ L.c	NS	M ₁ .L.c	NS	M ₁ .L.g	NS
—————	1.864	—————	2.232	—————	1.81	—————	2.203
M ₁ .L.g		M ₁ .L.c		M ₂ .L.g		M ₁ .L.c	
				M ₂ .L.c	NS	M ₂ L.c	NS
				—————	2.638	—————	4.919
				M ₂ L.g		M ₁ .L.g	

M₁; First mutated generation C; Chemical mutagen M₂; Second mutated generation
 NS; Not significant L; *Lathyrus odoratus* G; Gamma - ray

Table 10. Effect of physical (gamma rays) and chemical (NMU) mutagen on leaf length of *Lathyrus odoratus* treated seeds in the M₁ and M₂ generations during (2012/2013 and 2013/2014) seasons

Gamma rays (kr)	Leaf length				
	M ₁	M ₂	Nitroso methyl urea (NMU) (ppm)	M ₁	M ₂
0 kr	4.443	4.223	0.00 ppm	4.41	4.25
1 kr	4.613	5.13	30 ppm	4.543	5.483
5 kr	4.31	4.816	60 ppm	4.486	4.516
10 kr	4.39	4.336	90 ppm	4.56	4.383
15 kr	4.426	4.093	120 ppm	4.45	4.273
20 kr	4.376	4.353	150 ppm	4.533	4.35
F. test	N.S	N.S	F. test	NS	NS
LSD 5%	—	—	LSD 5%	—	—
LSD 1%	—	—	LSD 1%	—	—

NS = Not significant

Table 11. Comparison of variations resulted from (gamma rays) and (NMU) mutagens on leaf length of *Lathyrus odoratus* treated seeds in the M₁ and M₂ generations during (2012/2013 and 2013/2014) seasons

Mutagen				Comparison			
Gamma		Chemical		Gamma		Chemical	
M ₂ .L.g	*	M ₂ L.c	*	M ₂ .L.g	*	M ₁ .L.g	NS
—————	15.225	—————	63.750	—————	45.38	—————	2.980
M ₁ .L.g		M ₁ .L.c		M ₁ .L.c		M ₁ .L.c	
				M ₂ .L.c	NS	M ₂ L.c	*
				—————	1.404	—————	21.387
				M ₂ L.g		M ₁ .L.g	

M₁; First mutated generation C; Chemical mutagen M₂; Second mutated generation
 NS; Not significant L; *Lathyrus odoratus* *; Significant at 0.05 G; Gamma - ray

Effect of Gamma Irradiation and N-Nitroso-N-Methyl Urea on Flowering Parameters

Days to Flowering

Results in Table 12 explain that the mutagenic treatments (gamma rays and NMU) in M_1 and M_2 -generations recorded highly significant effect on flowering data of *Lathyrus odoratus*. However, the earliest day to flowering (117.826 days) and (120.586 days) was recorded by the treatments of 5 kr gamma rays and 30 ppm NMU in M_2 -generation. A significant delay of flowering was found at the treatments of 20 kr gamma rays and 150 ppm NMU in M_2 -generation.

Comparing the variability by those treatments produced in M_1 and M_2 -generation with gamma rays and NMU (Table 13) were insignificant on flowering date on *Lathyrus odoratus*.

The previous results are in line with those found by El-Ashry *et al.* (1992) on *Lathyrus odoratus*, Khan *et al.* (2006) on *Vigna radiate*, Khalaf (2008) on *Amaranthus*, Naik and Murthy (2009) on *Guizotia abyssinta*, Dhakshanamoorthy *et al.* (2010) on *Jatropha curcas* L and Mostafa (2011) on sunflower plant as for the effect of physical and chemical mutagens on flowering date.

Number of flowers / plant

Data of Table 14, reveal in M_1 -generation that gamma rays treatments had insignificant effect on the number of flowers/plant, but it was highly significant in the M_2 -generation gamma-rays and M_1 and M_2 -generation of NMU treatments on the number of flowers / plant.

Comparing the variability by those treatments (Table 15) produced by gamma-rays and NMU was insignificant in all generations.

The results are in agreement with those found by, Khalaf (2008) on *Amaranthus caudous*, El-Tony (1999) on *Tagetes erecta* and Ibrahim *et al.* (2009) on *Lathyrus odoratus*.

Effect of Gamma Irradiation and N-Nitroso-N-Methyl Urea on Some Chemical Constituents

Leaf chlorophyll content (SPAD-502)

In the M_1 and M_2 -generations (Table 16), gamma-rays treatments had significant and highly significant effect on leaf chlorophyll

content in *Lathyrus odoratus*, but in the M_1 and M_2 -generation, no significant effect on leaf chlorophyll content was observed with NMU in *Lathyrus odoratus*.

Comparing variation (Table 17) significant mean squares resulted from gamma rays and NMU in M_1 -generation, However, higher significant between M_1 generation gamma rays and M_1 generation NMU such variation, it was observed for gamma rays and NMU in M_2 -generation on leaf chlorophyll content.

This result agreed with those obtained by Rybinski (2003) on *Lathyrus sativus*, Karthika and Lakshmi (2006) on soybean, Abo El-Kheir (2004) on *Brassica oleracea* as well as Hussein (2005) on *Anethum graveolens*.

Flowers anthocyanin content

Results in Figs. 1 and 2 show that the mutagenic treatments effect gamma-rays and NMU in M_2 -generation which had insignificant effect on flowers anthocyanin content (mg/100g) in *Lathyrus odoratus* petals.

When comparing the variations mean square as result of this effect, it was insignificant. That means no differences of the effect on flowers anthocyanin content of all the cases compared. These results agreed with these reported by, Odeigah *et al.* (1998) on *Vigna unguiculata* L.

Effect of Gamma Irradiation and N-Nitroso-N-Methyl Urea on Mutation Characteristics in the M_2 -Generation

Plant height (dwarfness)

The treatment of 20-kr. gamma rays caused plant dwarfism (8.57 %) in Table 18 and Figs. 3 and 4. In addition, the treatment of 30 ppm NMU caused plant dwarfism (mutation rate 2.66% in Table 18 and Figs. 3 and 5).

Growth habit (compact plant)

The treatment of 1 kr gamma rays caused compact plant (2.26% in Table 18 and Figs. 6 and 7). While, The treatment of 60 ppm NMU caused compact plant (2.94% in Table 18 and Figs. 6 and 8).

These results agreed with those reported by Combacedes *et al.* (1992). They found that a range of gamma rays from 10 to 60 Gy was applied

Table 12. Effect of physical (gamma rays) and chemical (NMU) mutagen on days to flowering of *Lathyrus odoratus* in the M₁ and M₂ generations during (2012/2013 and 2013/2014) seasons

Gamma ray (kr)	Days to flowering				
	M ₁	M ₂	Nitroso methyl urea (NMU) (ppm)	M ₁	M ₂
0 kr	124.653	125.806	0.00 ppm	124.21	126.63
1 kr	122.72	117.826	30 ppm	120.203	120.586
5 kr	127.036	126.636	60 ppm	128.353	125.516
10 kr	129.646	133.79	90 ppm	136.323	137.276
15 kr	133.26	135.133	120 ppm	137.493	137.333
20 kr	138.526	139.723	150 ppm	139.016	138.983
F. test	**	**	F. test	**	**
LSD 5%	3.322	3.092	LSD 5%	3.898	5.17
LSD 1%	4.754	4.423	LSD 1%	5.462	7.398

** = Highly significant at 0.01.

Table 13. Comparison of variations resulted from (gamma rays) and (NMU) mutagens on flowering date of *Lathyrus odoratus* in the M₁ and M₂ generations during (2012/2013 and 2013/2014) seasons

Mutagen				Comparison			
Gamma		Chemical		Gamma		Chemical	
M ₂ .L.g	NS	M ₁ .L.c	NS	M ₂ .L.g	NS	M ₁ .L.c	NS
	1.826		1.011		1.025		1.780
M ₁ .L.g		M ₂ .L.c		M ₁ .L.c		M ₁ .L.g	
				M ₂ .L.g	NS	M ₂ .L.c	NS
					1.037		1.759
				M ₂ .L.c		M ₁ .L.g	

M₁; First mutated generation
NS; Not significantC; Chemical mutagen
L; *Lathyrus odoratus*M₂; Second mutated generation
G; Gamma ray**Table 14. Effect of Physical (gamma rays) and chemical (NMU) mutagen on number of flower per plant of *Lathyrus odoratus* in the M₁ and M₂ generations during (2012/2013 and 2013/2014) seasons**

Gamma ray (kr)	Number of flowers per plant				
	M ₁	M ₂	Nitroso methyl urea (NMU) (ppm)	M ₁	M ₂
0 kr	99.306	98.02	0.00 ppm	100.906	99.65
1 kr	107.466	99.633	30 ppm	99.026	104.563
5 kr	105.323	99.616	60 ppm	92.63	103.01
10 kr	103.643	86.766	90 ppm	92.613	101.726
15 kr	99.713	84.68	120 ppm	90.57	98.38
20 kr	93.933	81.796	150 ppm	90.923	94.793
F- test	NS	**	F- test	**	**
LSD 5%	—	2.378	LSD 5%	5.2	3.139
LSD 1%	—	3.402	LSD 1%	7.439	4.493

** = Highly significant at 0.01.

NS = Not significant

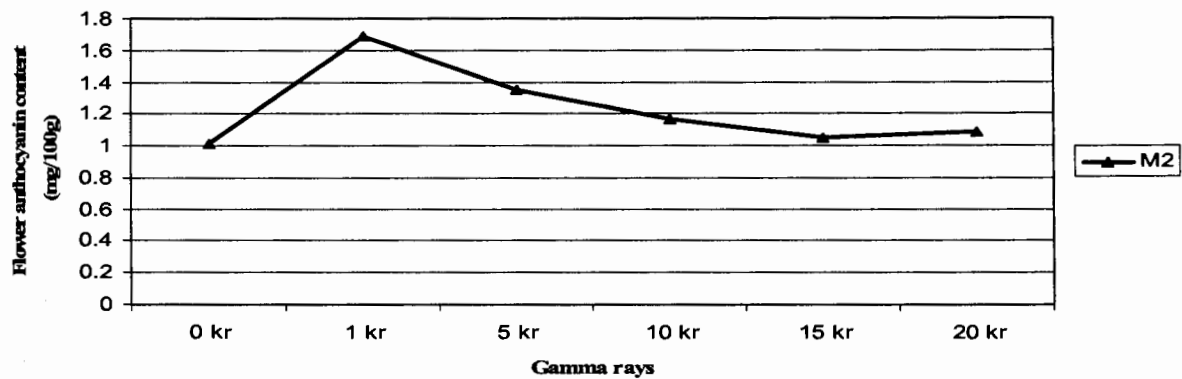


Fig. 1. Effect of physical (gamma rays) mutagen on flower anthocyanin content (mg/ 100 g) of *Lathyrus odoratus* in M_2 generation during (2013-2014)

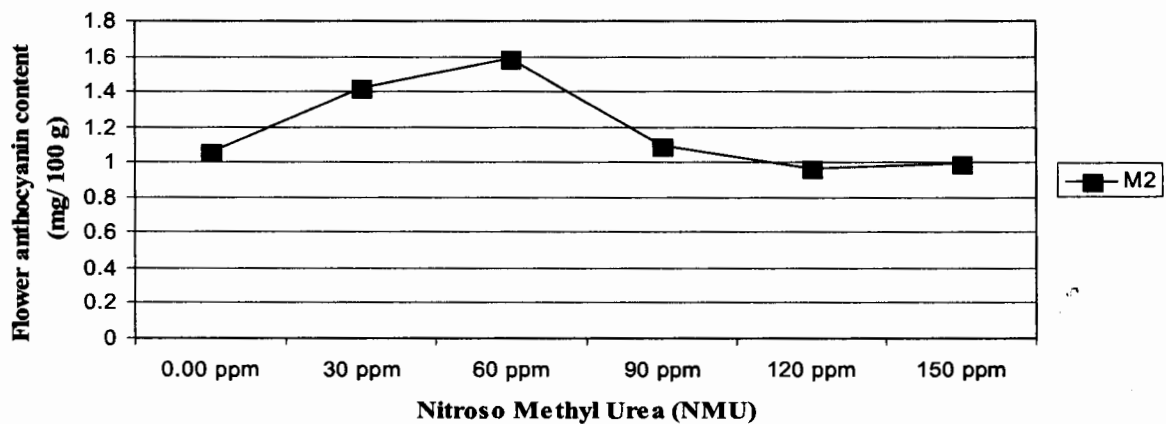


Fig. 2. Effect of nitroso methyl urea (NMU) mutagen on flower anthocyanin content (mg/ 100 g) of *Lathyrus odoratus* in M_2 generation during (2013-2014)

Table 18. Number of survival plants and mutation frequency and percentage of *Lathyrus odoratus* plants as affected by physical (gamma-rays) and chemical (NMU) mutagens in the M_1 and M_2 -generations

Treat.	Effect of [gamma -rays (krd)]				Effect of [NMU (ppm)]				
	M_1 No. of plants	(%)	M_2 No. of plants	(%)	Treat.	M_1 No. of plants	(%)	M_2 No. of plants	(%)
0 kr	85 (0)	0	82 (0)	0	0 ppm	86 (0)	0	82	0
1 kr	84 (0)	0	79 (1)	2.26	30 ppm	79 (2)	2.53	75 (6)	8.00
5 kr	82 (2)	2.43	76 (4)	5.26	60 ppm	73 (4)	5.47	68 (7)	8.82
10 kr	78 (3)	3.84	68 (5)	7.35	90 ppm	70 (2)	2.85	64 (7)	10.93
15 kr	62 (5)	8.06	53 (10)	18.86	120 ppm	68 (0)	0	63 (4)	6.34
20 kr	44 (2)	4.54	35 (9)	25.71	150 ppm	65 (1)	1.53	58 (4)	12.06
Average	2.40	3.77	5.80	11.88	Average	1.80	2.47	6.2	9.23

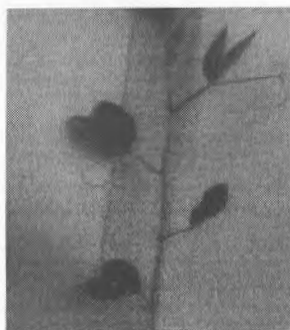


Fig. 3. Control .

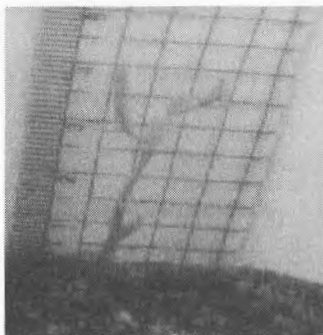


Fig. 4. Treatment of 20 kr. Gamma rays caused in plant dwarfism (mutation rate 8.57 %).

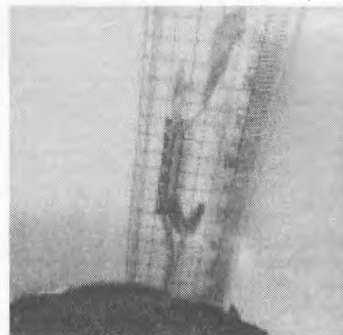


Fig. 5. Treatment of 30 ppm NMU caused in inducing plant dwarfness

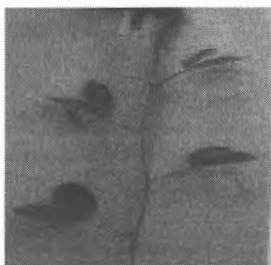


Fig. 6. Control



Fig. 7. Treatment of 1 kr. Gamma rays caused compact

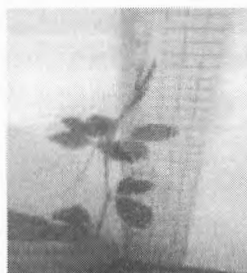


Fig. 8. Treatment of 60 ppm NMU caused compact plant

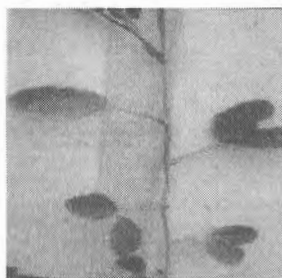


Fig. 9. Control

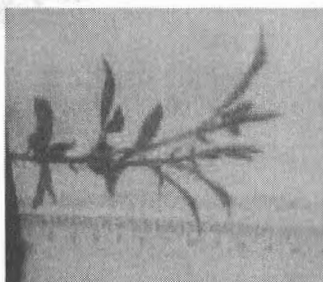


Fig. 10. Treatment of 10 kr. Gamma rays inducing mutation in the stem structure fasciata

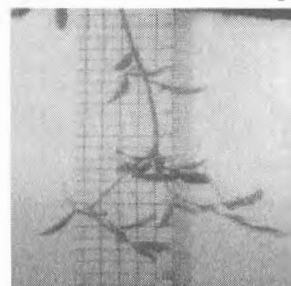


Fig. 11. Treatment of 120 ppm NMU inducing fasciata mutations

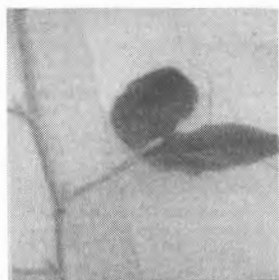


Fig. 12. Control

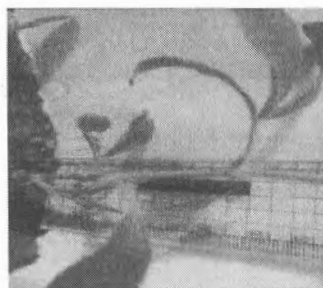


Fig. 13. Treatment of 15 kr. Gamma rays caused leaf shape (mutation rate 9.43%)

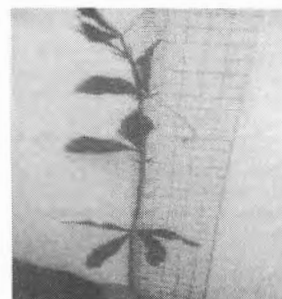


Fig. 14. Treatment of 90 ppm NMU caused leaf shape (mutation rate 7.81%).

to apical and nodal microcuttings of five species of *lonicera* (*L. xbrownii* *Dropmore* *Scarlet* *L. Periclymenum* *Serotinal*, *L. heckrotii*, *Gold flame*, *L. nitida* and *L. fragrantissima*). From 1700 plants produced from irradiated buds, only those from *L. nitida* were observed during two years. Among 200 of these plants, some compact and slender mutants were detected.

Stem structure (fasciata)

The treatment of 10 kr. gamma rays induced mutation in stem structure (fasciata) (7.35% in Table 18 and Fig. 9 and 10). Also, the treatment of 120 ppm NMU induced mutation in the fasciata (mutation rate 4.76% in Table 18 and Figs. 9 and 11).

These results agreed with the most effective treatments for increasing the leaves mutation were 1.0% EMS +10 kr by Anjalika *et al.* (2005). They also found that five mutants in the leaf shape were indentified in *Catharanthus roseus*, while 2×10^{-3} m NaN_3 + 1.0% EMS gave 15 mutant in the leaf shape of *Dimorphotica ecklonis*.

Chlorophyll mutation

The treatment of 5 kr. gamma rays induced chlorophyll mutation (5.26% in Table 18). However, the treatment of 90-ppm NMU induced chlorophyll mutation (3.12% in Table 18).

Leaf structure (Leaf shape)

The treatment of 15 kr. gamma rays caused leaf shape (9.43% in Table 18 and Figs. 12 and 13). Moreover, the treatment of 90 ppm NMU caused in change leaf shape (mutation rate 7.81% in Table 18 and Figs. 12 and 14).

Flowers (flower color)

The treatment of 15 kr. gamma rays induced mutation in flower color (9.43% in Table 18). The treatment of 30 ppm NMU caused in mutation in flower color was produced (mutation rate 5.33% in Table 18).

Flower cluster length

The treatment of 20 kr. gamma rays induced mutation in flower cluster length (2.85% in Table 18). The treatment of 150-ppm NMU induced mutation in flower cluster length (mutation rate 5.17% in Table 18).

Conclusion

Finally, it can be concluded that gamma rays irradiation and N-Nitroso -N- Methyl Urea (NMU) are powerful mutagens for the induction of mutations in *Lathyrus odoratus* plant and cause some mutation as plant dwarfism with seeds treated by 20 kr gamma rays or 30 ppm NMU, fasciata with 10 kr gamma rays or 120 ppm NMU, chlorophyll mutation with 5 kr gamma rays or 90 ppm NMU, leaf shape with 15 kr gamma rays or 90 ppm NMU and flowers color with 15 kr gamma rays or 30 ppm NMU and flowers cluster length with 20 kr gamma rays or 150 ppm NMU in M_2 generation.

REFERENCES

- Abo El-kheir, O.M. (2004). Effect of gamma irradiation and some nutrients elements on growth, yield and storagebility of Broccoil (*Brassica oleracea*). Ph.D. Thesis, Fac. Agric., Moshtohor, Benha Univ., Egypt.
- Allkin, R., J.D. Goyder, A.F. Bisby and J.R. White (1986). Names and synonym.of species and subspecies in the Viciae Database Project, 7:1 -75.
- Anjalika, M.R., N. Banerjee and S. Mandal (2005). Structural and functional parameters of mating and seed germination in mutants of *Catharanthus roseus* (Apocynaceae). J. Appl. Biosci., 31(2): 145-149.
- Ariraman, M., S. Gnanamurthy, D. Dhanavel, T. Bharathi and S. Murugan (2014). Mutagenic effect on seed germination, seedling growth and seedling survival of Pigeon pea (*Cajanus cajan* (L.) Millsp). Int. Letters of Nat. Sci., 16: 41-49.
- Badr, M., B.A. Abdel-Maksoud and S.S. omer (2004). Growth, flowrering and induced variability in *Gomphrena globosa*, L. Plant grown from dry and water-soaked seeds treated with gamma-rays. Alex. J. Agric. Res., 49 (1):49-70
- Badr, M., M. Khattab, T.Y. Al-Keay, M. Al-Keay, M. Yaqout, M.A.H. Nouh and M. Raslan (1990). Flowers, Ornamental Plants and Garden Design. 4th Ed. Dar Fagr Al-Islam. Glym, Alex., Egypt (In Arabic).

- Combacedes, J., M. Duron, L. Decourtye and R. Jalouzot (1992). Methodology of *in vitro* gamma irradiation from *Lonicera* species Mutant description and biochemical characterization. *Acta Hort.*, 320: 119-126.
- Dhakshanamoorthy, D., R. Selvaraj and A. Chidambaram (2010). Physical and chemical mutagenesis in *Jatropha curcas* L. to induce variability in seed germination, growth and yield traits. *Rom. J. Plant Biol.*, 55 (2): 113-125.
- Dilta, B.S., Y.D. Sharma, Y.C. Gupta R. Bhalla and B.P. Sharma (2003). Effect of gamma rays on vegetative and flowering parameters of chrysanthemum. *J. Ornamental Hort. New Series*, 6 (4): 328-334.
- El-Ashry, A.I., M.A. Zagloul and E. Al-Ghait (1992). Physiological studies on *Lathyrus odoratus* 2. Effect of gamma- irradiation on the growth and flowering of *Lathyrus odoratus* L. *Bull. Suez Canal Univ. Appl. Sci.*, 1: 506-521.
- El-Tony, F.H. (1999). Effect of gamma irradiation, methyl sulphonate and their combination on growth, flowering and induced variability in *Tagetes erecta* L., M. Sc. Thesis, Fac. Agric., Alex. Univ., Egypt.
- Encheva, J., F. Tsvetkova and P. Ivanov (2003). A comparison between soma clonal variation and induced mutagenesis in tissue culture of sunflower line 2-8-A (*Helianthus annuus* L.) *Helia*, 26 (38): 91-98.
- Francis, F.J. (2000). Anthocyanins and betalains composition: composition and applications. *Cereal Foods World*, 45: 208-213.
- Fuleki, T. and F.J. Francis (1968). Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. *J. Food Sci.*, 33: 72-77.
- Gaswanto, R., M. Syukur, B.S. Purwoko and S.H. Hidayat (2016). Induced mutation by gamma rays irradiation to increase chilli resistance to begomovirus. *Agrivita*, 38 (1): 24-32.
- Goyder, D.J. (1986). The genus *Lathyrus* in; kaul A.K. and Combes D. (Eds). *Lathyrus and Lathyrism*. Third World Medical Research Foundation. New York, 334: 3-7.
- Hussein, A.S. (2005). Physiological studies on growth yield and Volatile oil of Dill *Anethum graveolens* L. Ph.D. Thesis, Fac. Agric., Cairo Univ., Egypt.
- Ibrahim, H.E., F.M.H. Swaefy and A.M.H. Youssef (2009). Effect of gamma irradiation and gibberellic acid on growth and flowering of *Lathyrus odoratus* L. plants. *Egypt. J. Hort.*, 36 (2): 347-357.
- Ilbas, A.I., Y. Eroglu and H. Eroglu (2005). Effects of the application of different concentrations of NaN₃ for different times on the morphological and cytogenetic characteristics of barley (*Hordeum vulgare* L.) seedlings. *J. Integrative Plant Biol.*, 47 (9): 1101-1106.
- Karthika, R. and B.S. Lakshmi (2006). Effect of gamma rays and EMS on two varieties of soybean. *Asian J. Plant Sci.*, 5 (4): 721-724.
- Khalaf, W. (2008). Effect of gamma irradiation on growth, flowering on induced variability in *Amaranthus caudatus* L. M.Sc. Thesis, Fac. Agric, Alex. Univ., Egypt.
- Khan, S., R.M. Wani and K. Parveen (2006). Quantitative variability in mungbean induced by chemical mutagens. *Legume Res.*, 29 (2): 143-145.
- Kupicha, F.K (1983). The infrageneric structure of *Lathyrus*. *Notes by Bot. Gard. Edinb.*, 41 (2): 209-244.
- Li, R., A.H. Bruneau and R. Qu (2010). Morphological mutants of St. Augustine grass induced by gamma ray irradiation. *Plant Breed.*, 129:412-416.
- Mahmoud, F.A.N. (2002). Effect of gamma irradiation and some agrochemicals on germination and flowering of *Delphinium ajacis* and *Matthiola incana* plants. M. Sc. Thesis, Fac. Agric., Moshtohor. Zagazig Univ., (Banha Branch) Egypt.
- Mostafa, G.G. (2011). Effect of sodium azide on the growth and variability induction in *Helianthus annuus* L. *Int. J. Pl. Breed. and Genet.*, 5 (1): 76-85.

- Mostafa, G.G. (2015). Effect of some chemical mutagens on the growth, phytochemical composition and induction of mutations in *Khaya senegalensis*. Int. J. Pl. Breed. and Genet., 9 (2): 57-67.
- Naik, P.M. and H.N. Murthy (2009). Effects of gamma and ethyl methane sulphonate treatments on agronomical traits of niger (*Guizotia abyssinia* Cass). Afr. J. Biotechnol., 8 (18): 4459-4464.
- Odeigah, P.G.C., O.A. Osanyinpeju and O.G. Myers (1998). Induced mutations in cowpea (*Vigna unguiculata*) Leguminosae. Revista Biologia Tropical, 46 (3): 579-586.
- Plitman, U., R. Gabay and O. Coben (1995). Innovations the tribe Viciae (Fabaceae) from Israel J. Pl. Sci., 43:249-258.
- Quecini, V., A.S. Berenschot, M.I. Zucchi and A. Tulmann-Neto (2008). Mutagenesis in *Petunia* hybrid Vilm. and isolation of a novel morphological mutant. Braz. J. Plant Physiol., 20 (2):95-103.
- Rybinski, W. (2003). Mutagenesis as a tool for improvement of traits in grass pea (*Lathyrus sativus* L.). Lathyrus Lathyrism Newsletter 3 (1): 27-31.
- Snedecor, G.W. and W.G. Cochran (1980). Statistical Methods. 7th Ed., Iowa State Univ., Press, Ames., Iowa, USA.
- Wi, S.G., B.Y. Chung, J.H. Kim, M.H. Baek, D.H. Yang, J.W. Lee and J.S. Kim (2005). Ultrastructural changes of cell organelles in Arabidopsis stem after gamma irradiation. J. Pl. Biol., 482: 195-200.
- Yadava, U.L. (1986). A rapid and non-destructive method to determine chlorophyll in intact leaves. Hort. Sci., 21:1449-1450.

التغيرات المورفولوجية والزهرية في بسلة الزهور باستخدام بعض المطفرات الفيزيائية والكيميائية

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أجريت تجربة بالمزرعة التجريبية لقسم بحوث تربية الفاكهة ونباتات الزينة - مركز البحوث الزراعية في مصر خلال موسمي ٢٠١٣/٢٠١٢ و ٢٠١٤/٢٠١٣ لدراسة تأثير تعريض البذور لأشعة جاما بجرعات (صفر و ١ و ٥ و ١٠ و ١٥ و ٢٠ كيلو راد) ونقع البذور في تركيزات من نيتروزو ميثيل يوريا بتركيزات (صفر و ٣٠ و ٦٠ و ٩٠ و ١٢٠ و ١٥٠ جزء في مليون) على نبات بسلة الزهور، في تصميم قطاعات كاملة العشوائية في ثلاث مكررات، أظهرت نتائج الجيل الطفري الأول انخفاضاً في النسبة المئوية لإنبات البذور، ارتفاع النبات، عدد الأوراق، عدد الأزهار ومحتوى الأوراق من الكلوروفيل بزيادة الجرعات والتركيزات من المطفرات الفيزيائية والكيميائية، بينما كان تأثير المطفرات على طول الورقة ومحتوى الأزهار من الأنثوسيانين غير معنوي في الجيلين الطفرين الأول والثاني، بينما أدت المعاملة بالجرعات العالية من أشعة جاما والتركيزات من النيتروزو ميثيل يوريا إلى تأخير ميعاد التزهير في الجيل الطفري الأول والثاني، وانخفضت النسبة المئوية لإنبات البذور في الجيل الطفري الثاني، كما أدت الجرعات والتركيزات المنخفضة إلى زيادة في كل من ارتفاع النبات، عدد الأفرع، عدد الأوراق، عدد الأزهار ومحتوى الأوراق من الكلوروفيل في الجيل الطفري الأول والثاني، وأظهرت مقارنة التباين لمتوسط المربعات اختلافاً غير معنوي بين أشعة جاما والنيتروزو ميثيل يوريا في الجيل الطفري الأول والثاني لكل من النسبة المئوية لإنبات البذور، عدد الأفرع، عدد الأوراق، ميعاد التزهير وعدد الأزهار، بينما كان التباين مرتفعاً بين أشعة جاما والنيتروزو ميثيل يوريا في الجيل الأول والثاني لكل من ارتفاع النبات وطول الورقة ومحتوى الأوراق من الكلوروفيل، كما أظهرت النتائج المتحصل عليها في هذه الدراسة أن الجرعات والتركيزات المختلفة من المطفرات يمكنها إحداث تغييرات في ارتفاع النبات (نباتات متقدمة ومندمجة) وتفرع القمة النامية والطفرة الكلوروفيلية وشكل الورقة وطول الحامل الزهري لنبات بسلة الزهور.

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