Zagazig J. Agric. Res., Vol. 43 No. (4) 2016

http:/www.journals.zu.edu.eg/journalDisplay.aspx?JournalId=1&queryType=Master

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 M_1-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 M_1 and M_2-generations. The higher doses of gamma-rays and concentrations of NMU led to delay the flowering date in M_1 and M_2-generations, decrease the seed germination percentage in M_2-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 M_2-generation. The variations means squares indicated insignificant difference between gamma-rays and NMU in M_1 and M_2-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 M_1 and M_2-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 (dwarfeness 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), Dielhyl Sulphate (DES), Ethyleneimine (EI). Azides are also effective mutagens,also chochicine 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 2012/2013and 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 m^2. 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 cholorimetrically 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 M_1 and M_2 generations during (2012/2013 and 2013/2014) seasons

Seed germination percentage					
Gamma rays (kr)	**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 M_1 and M_2 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	

M_1; First mutated generation C; Chemical mutagen M_2; 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 M_1 and M_2 generations during (2012-2013 and 2013-2014) seasons

Plant height (cm)					
Gamma rays (kr)	**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 M_1 and M_2 generations during (2012/2013 and 2013/2014) seasons

Mutagen				Comparison			
Gamma		Chemical		Gamma		Chemical	
M_2.L.g	*	M_2L.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_2L.g	NS
				———	4.447	———	1.066
				M_1L.g		M_2.L.c	

M_1; First mutated generation C; Chemical mutagen M_2; Second mutated generation
NS; Not significant L; Lathyrus odoratus *; Significant at 0.05 G; Gamma - ray

Number of branches per plant

In the M_1 and M_2 –generations (Table 6) 1 and 5 krd of gamma–rays showed significant stimulation effect on branch number of *Lathyrus odorotus*, but in the M_1–generation, it was reduced with the increase of the concentration of NMU, whereas, in the M_2-generation 30 ppm of NMU had significant stimulation effect on branch number of *Lathyrus odoratus*.

Recorded data in M_1 and M_2 –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 M_1 and M_2 generation were found to be insignificant, and at this growth stage the response was similarly, and no difference was recorded between M_1 and M_2 – 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 M_1 and M_2 –generations, respectively.

Comparing the variation mean squares (Table 9) results indicate insignificant difference between gamma rays and NMU in M_1 and M_2-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 *Amarnthus caudatus*, Ibrhium *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 M_1 and M_2 – generations are listed in Table 10. In M_1 and M_2 – 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 M_1 and M_2 – generations.

Comparing variations (Table 11) showed higher significant mean square from gamma rays when compared mean square in M_1 and M_2 – generations. Such variation detected that NMU produced higher variation in M_1 and M_2 generations. However, it was observed that the variations between gamma rays and NMU were higher in M_2 and M_1 –generations, respectively on leaf length in *Lathyrus odoratus*.

The results are in accordance with those found by Dilta *et al.* (2003) on chrysanthemum, Encheva *et al.* (2003) on sunflower, Ibrahium *et al.* (2009) on *Lathyrus odoratus*, Naik and Murthy (2009) on *Guizotia abussinicia* 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 M_1 and M_2 generations during (2012/2013 and 2013/2014) seasons

Number of branches per plant					
Gamma rays (kr)	**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 M_1 and M_2 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_2L.c	NS
				———	5.769	———	2.399
				M_1L.c		M_2.L.g	

M_1; First mutated generation M_2; Second mutated generation C; Chemical mutagen
L; *Lathyrus odoratus* NS; Not significant 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 M_1 and M_2 generations during (2012/2013 and 2013/ 2014) seasons

Number of leaves per plant					
Gamma rays (kr)	**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 M_1 and M_2 generations during (2012/2013 and 2013/ 2014) seasons

Mutagen				Comparison			
Gamma		Chemical		Gamma		Chemical	
M_2.L.g	NS	M_2L.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_2L.c	NS
				———	2.638	———	4.919
				M_2L.g		M_1.L.g	

M_1; First mutated generation C; Chemical mutagen M_2; 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 M_1 and M_2 generations during (2012/2013 and 2013/2014) seasons

Leaf length					
Gamma rays (kr)	**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 M_1 and M_2 generations during (2012/ 2013 and2013/2014) seasons

Mutagen				Comparison			
Gamma		Chemical		Gamma		Chemical	
M_2.L.g	*	M_2L.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_2L.c	*
				———	1.404	———	21.387
				M_2L.g		M_1.L.g	

M_1; First mutated generation C; Chemical mutagen M_2; 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 Ibrahium *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 genration 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 mutagentic 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 M2-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 M_1 and M_2 generations during (2012/2013 and 2013/2014) seasons

Days to flowering					
Gamma ray (kr)	**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 M_1 and M_2 generations during (2012/2013 and 2013/2014) seasons

Mutagen				Comparison			
Gamma		**Chemical**		**Gamma**		**Chemical**	
M_2.L.g	NS	M_1L.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_2L.c	NS
				———	1.037	———	1.759
				M_2L.c		M_1.L.g	

M_1; First mutated generation C; Chemical mutagen M_2; Second mutated generation
NS; Not significant L; Lathyrus odoratus 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 M_1 and M_2 generations during (2012/2013 and 2013/ 2014) seasons

Number of flowers per plant					
Gamma ray (kr)	**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

Table 15. Comparison of variations resulted from (gamma rays) and (NMU) mutagens on number of flower per plant of *Lathyrus odoratus* treated seeds in the M_1 and M_2 generations during (2012/2013 and 2013/2014) seasons

Mutagen				Comparison			
Gamma		Chemical		Gamma		Chemical	
M_2.L.g	NS	M_1L.c	NS	M_2.L.g	NS	M_1.L.g	NS
———	2.809	———	1.561	———	5.441	———	1.240
M_1.L.g		M_2.L.c		M_1.L.c		M_1.L.c	
				M_2.L.9	NS	M_1L.g	NS
				———	3.484	———	1.936
				M_2L.c		M_2.L.c	

M_1; First mutated generation C; Chemical mutagen M_2; Second mutated generation
NS ; Not significant L; *Lathyrus odoratus* G; Gamma – ray

Table 16. Effect of physical (gamma rays) and chemical (NMU) mutagen on leaf chlorophyll content (SPAD-502) of *Lathyrus odoratus* in the M_1 and M_2 generations during (2012/2013 and 2013/2014) seasons

	Leaf chlorophyll content				
Gamma ray (kr)	**M_1**	**M_2**	**Nitroso methyl urea (NMU) (ppm)**	**M_1**	**M_2**
0 kr	47.67	47.993	**0.00 ppm**	39.75	50.41
1 kr	40.81	48.47	**30 ppm**	38.77	48.083
5 kr	40.886	47.513	**60 ppm**	39.00	47.66
10 kr	44.363	47.00	**90 ppm**	40.76	47.01
15 kr	38.583	41.923	**120 ppm**	39.66	46.993
20 kr	41.033	37.72	**150 ppm**	38.226	47.423
F- test	*	**	**F- test**	NS	NS
LSD 5%	4.328	3.555	**LSD 5%**	----	----
LSD 1%	6.193	5.089	**LSD 1%**	----	----

* = Significant at 0.05 ** = Highly significant at 0.01.

Table 17. Comparison of variations resulted from (gamma rays) and (NMU) mutagens on leaf chlorophyll content (SPAD-502) of *Lathyrus odoratus* in the M_1 and M_2 generations during (2012/2013 and 2013/2014) seasons

Mutagen				Comparison			
Gamma		Chemical		Gamma		Chemical	
M_2.L.g	NS	M_2L.c	NS	M_2.L.g	*	M_1.L.g	*
———	1.778	———	2.074	———	23.635	———	13.287
M_1.L.g		M_1.L.c		M_1.L.c		M_1.L.c	
				M_2.L.g	*	M_1L.c	NS
				———	11.391	———	6.403
				M_2L.c		M_2.L.g	

M_1; First mutated generation M_2; Second mutated generation C; chemical mutagen
NS; Not significant L; *Lathyrus odoratus* *; Significant at 0.05 G; Gamma-ray

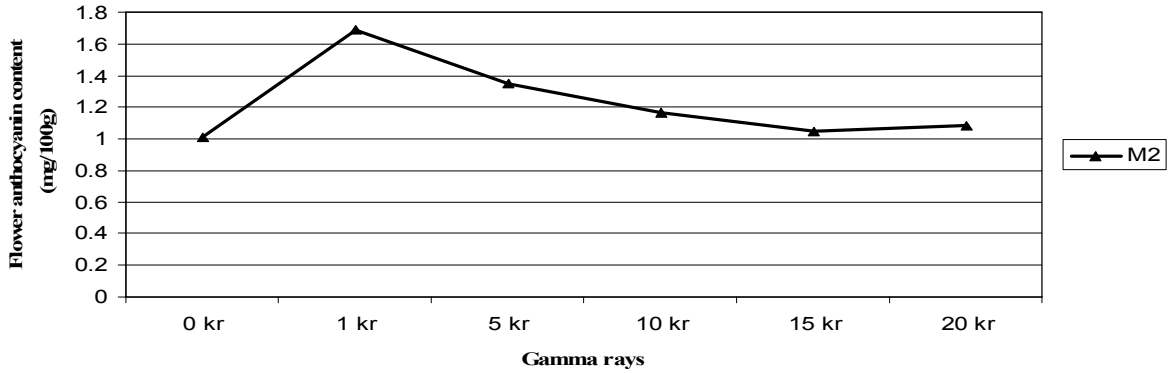

Fig. 1. Effect of physical (gamma rays) mutagen on flower anthocyanin content (mg/ 100 g) of *Lathyrus odoratus* in M_2 generation during (2013-2014)

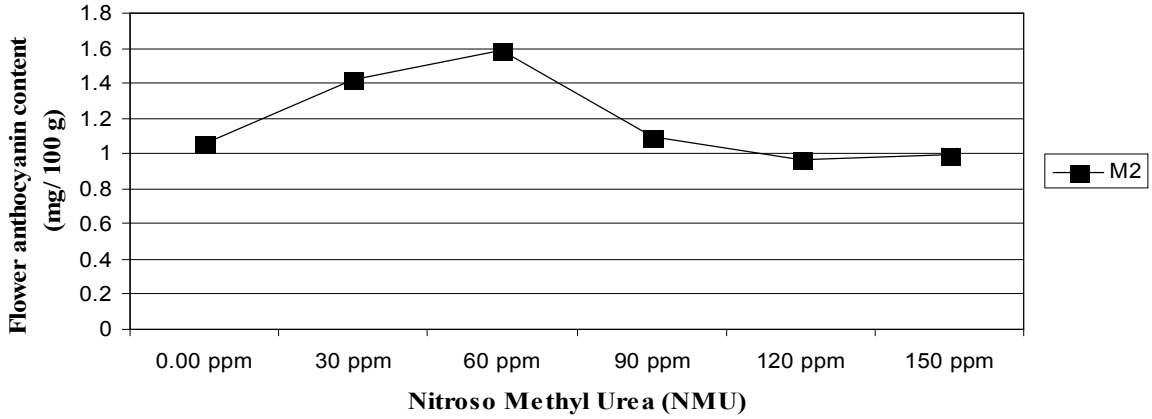

Fig. 2. Effect of nitroso methyl urea (NMU) mutagen on flower anthocyanin content (mg/ 100 g) of *Lathyrus odoratus* in M_2 generation during (2013-2014)

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

Effect of [gamma –rays (krd)]					Effect of [NMU (ppm)]				
Treat.	**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	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 indentifed 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.

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التغيـرات المـورفـولـوجية والزهرية فى 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 و٢٠١٤/٢٠١٣ لدراسة تأثير تعريض البذور لأشعة جاما بجرعات (صفر و ١ و ٥ و ١٠ و١٥ و٢٠ كيلو راد) ونقع البذور في تركيزات من نيتروزو ميثيل يوريا بتركيزات (صفر و٣٠ و٦٠و٩٠ و١٢٠و١٥٠ 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 الطفريين 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 أشعه جاما والنيتروزو ميثيل يوريا في الجيل الأول والثاني لكل من إرتفاع 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 متقزمة ومندمجة) وتفرع القمة النامية والطفرات الكلوروفيللية وشكل الورقة وطول الحامل الزهري لنبات بسلة الزهور.

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