

Effect of Gamma Radiation on Genetic Improvement Against Salinity in *Catharanthus Roseus* Plants

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ABSTRACT: The experiments were carried out during the years of 2013, 2014 and 2015 in the Flowers and Ornamental Plants Research Gardens of the Faculty of Agriculture, Alexandria University, Egypt. The objective of this research was to study the effect of treating the seeds of *Catharanthus roseus* with different gamma rays doses, i.e. 0, 5, 10, 15 and 20 kr and irrigation with saline water (0, 100 and 150 mM) on the morphological characteristics, proline content, alkaloids (vindolen and catharnthine) content and total carbohydrates content in the leaves, variations, mutations and peroxidase isozyme. Data on the effect of gamma radiation, salinity treatments and the interaction between them revealed the followings results.

- 1- Some variations in the morphological characteristics, such as habit of growth, leaf size, form and colour, stem colour and flower structure and colour.
- 2- Highly significant increases in the proline content.
- 3a- Significant increases in the leaf vindolen content.
- 3b- Significant increases in the leaf catharnthine content.
- 4- No clear effect on the total carbohydrate content.
- 5- Twelve mutated plants with variation in branching, flowering and salt tolerance.

Key words: Gamma, radiation, genetic, salinity, ornamental, plants.

INTRODUCTION

Catharanthus roseus (L.) G. Don. (Madagascar periwinkle) is a tropical and subtropical ornamental plant and one of the most important medicinal plants (also known as anticancerous drug yield in plant) and also an ornamental bedding plant belonging to the family Apocyanaceae (Jaleel *et al.*, 2008).

Catharanthus roseus contains a virtual cornucopia of useful alkaloids, used in diabetes, blood pressure, asthma, constipation, and cancer and menstrual problems. There are about two common cultivars of *C. roseus* which is named on the basis of their flower colour that is the pink flowered "Rosea" and the white flowers "Alba". *Catharanthus roseus* is found to be a species of *Catharanthus* native and also endemic to Madagascar. The synonyms of the plant name include *Vinca rosea*, *Ammocallis rosea* and *Lochnera rosea*, other English names occasionally used for the plant include Cape periwinkle, rose periwinkle, rosy periwinkle and "old maid".

Catharanthus roseus is an evergreen subherb or herbaceous plant growing to 1 m. tall. The leaves are oval to oblong, 2.5- 9.0 cm long and 1- 3.5 cm broad, glossy green hairless with a pale midrib and a short petiole about 1- 1.8 cm long arranged in opposite pairs. The flowers are white to dark pink with a dark red center; with a basal tube about 2.5- 3 cm long and a corolla about 2-5 cm diameter with five petal like lobes. The fruit is a pair of follicles about 2-4 cm long and 3 mm broad.

Catharanthus roseus possesses carbohydrates, flavinoids, saponins and alkaloids. Alkaloids are the most potentially active chemical constituents of *Catharanthus roseus*. More than 400 alkaloids are present in the plant, which are used as pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives and pesticides. The alkaloids like actineoplastidemic, Vinblastin, Vincristine, Vindesine, Vindeline Tabersonine etc. are mainly present in aerial parts whereas ajmalicine, vinceine, vineamine, raubasin, reserpine, *catharanthine* etc are present in roots and basal stem. Rosindin is an anthocyanin pigment found in the flower of *C. roseus* (Sain and Sharma, 2013).

Aim of the work:

1. Studying the effect of different doses of gamma radiation from cobalt 60 and salt water treatments on the vegetative and flowering growth of *Catharanthus roseus*, as well as on the possibility of inducing mutations, which can resist high salinity or have wider landscape value.
2. Selecting a new strain of *Catharanthus roseus*, with high alkaloid productivity.
3. Using of isozymes techniques (peroxidase enzymes) to find out the genetic relationship among the original mother plant and the mutated plants.

MATERIALS AND METHODS

The experiments were carried out in the Flowers and Ornamental Plants Research Gardens, Department of the Floriculture and Ornamental Horticulture and Landscape Gardening, Faculty of Agriculture, Alexandria University during 2013 – 2015.

Materials

Plant materials

Local cultivar of Madagascar periwinkle or rosy periwinkle (*Catharanthus roseus* (L.) G.Don) was used in this study, with purple flowers and cross-pollination. Seeds were obtained from the Flowers and Ornamental Plants Research Gardens of the Faculty of Agriculture, University of Alexandria.

Gamma radiation source

Gamma - rays doses applied in this study were generated from the Cobalt 60 Source, in Gamma – Cell installed in the Irradiation Laboratory at Middle East Regional Radio-Isotope Center for Arab Countries, El-Dokky, and Cairo, Egypt.

Methods

a. Experimental design

The effects of the two factors (irradiation and salinity) on the M₁ - plants were tested in field. The layout of the experiment was designed as factorial layout in Randomized Complete Block Design (RCBD) (Gomez and Gomez, 1984) which contained 5 radiation treatments, i.e. control (0), 5, 10, 15 and 20 kr. from gamma rays and 3 salinity levels of the irrigation water (0, 100 and 150 mM NaCl). One hundred and fifty seeds of *Catharanthus roseus* were used for

every treatment from Gamma rays, One hundred and fifty seeds for every salinity treatment within each gamma rays treatment.

b. Preparing of seeds

Lot of well developed pure seeds from healthy and abundantly fruitful plants of Madagascar periwinkle or rosy periwinkle (*Catharanthus roseus* L.) Local cultivar were collected. The total amount of seeds prepared for gamma ray treatments was divided into five equal portions; the first portion for control, while the other four portions of seeds were, paged equally in four paper bags before exposure to radiation.

c. Gamma radiation practices

On the 18th and 26th of March 2013 and 2014 in the first and second seasons; respectively, the dry seeds of *Catharanthus roseus* L. were exposed to four different doses of gamma rays as 5, 10, 15 and 20 kr from Co-60.

d. Soil analyses

Physical and chemical analyses of the used soil were carried out according to the standard methods outlined by Page *et al.* (1982) and are listed in Table (1).

Table (1). Some Physical and chemical characteristics of the used soil during 2013 and 2014.

Parameters	Value	Chemical properties	
		Soluble cations (1:2)	(cmol/kg soil)
Physical properties		Soluble cations (1:2)	
Soil texture	Sandy loam	Na ⁺	20.70
Sand %	75	K ⁺	0.50
Silt %	8	Ca ⁺⁺	7.40
Clay %	17	Mg ⁺⁺	10.80
		Available K ⁺	8.76
Chemical properties		Soluble anions (1:2)	
pH (1 : 1)	7.82	CO ₃ ⁻⁻	-
		HCO ₃ ⁻⁻	3.60
E.C. (dS/m)	3.45	Cl ⁻	21.00
		SO ₄ ⁻⁻	14.80

The experimental treatments consisted of two salinity levels of the irrigation water (100 and 150 mM NaCl) in addition to control (0.0 mM), salinity levels were obtained by addition of appropriate amount of dry NaCl to water. The salinity levels were equivalent to an electrical conductivity of 0.46, 10.9 and 15.9 dSm⁻¹, respectively using a portable EC meter instrument. To avoid an osmotic shock for seedling emergence; the salinized water was used after 45 days of sowing (Gorham and Wyn Jones, 1993).

To prepare the stock solution, a commercial sea salt (sodium chloride) without purification (contents: NaCl 98.5% , Humidity 0.3% and KIO₃ 30-70 ppm) produced by Egyptian salt and mineral company (EMISAL) was dissolved in tap water (0.46 dS/m) at (5.85 g salt per liter =100 mM and 8.775 g salt per

liter =150 mM).One month later, complete fertilizer 19-19-19 was top dressed at the rate of 1/2 g /l and this addition was repeated every two weeks.

The plants were irrigated 3 times weekly in summer with 320 ml per pot until the end of the experiment.

Cultural aspects

a. M₁ - Generation

Gamma- rays treated and non - treated seeds were sown on March 20, 2013 in the first season and on March 27,2014 in the second one. The seeds of each treatment were sown in three trays (150 seeds) filled with a mixture of equal parts of sand and clay(1/1).The trays were placed in partial shade according to the factorial experimental layout of the Randomized Complete Block Design and watered daily. On May 3 , 2013 and April 22, 2014 in both seasons , the trays were gradually transferred from shade to open place (sunny place) for one week on May 10, 2013 and April 29, 2014 in the first and second seasons, respectively. Two seedlings were transplanted into 30 cm diameter plastic pot containing sandy loam soil and reached a height of about ten cm. The pots were arranged in the three replicates according to the Randomized Complete Block Design with different numbers of pots in each treatment according to the number of the survived seedlings.

b. M₂ - Generation

For growing the M₂- generation in both seasons , seeds were collected from each treatment on March 20, 2014 and March 23, 2015 in the first and second seasons, respectively, and sown in three trays(100 seeds for each treatment). The trays contained a soil mixture of 1 sand: 1 clay by volume. The trays were placed in partial shade according to the factorial experimental layout of Randomized Completely Block Design with 3 replicates (Gomez and Gomez, 1984).The trays were watered daily. On April 24 , 2014 and April 28, 2015 in the first and second seasons ; respectively , the trays were gradually transferred from shade to sunny place along one week . On April 24, 2014 and April 8, 2015 during the first and second seasons; respectively, every two M₂- seedlings were transplanted into a plastic pot of 30 cm diameter, containing sandy loam soil and reached a height of about ten cm. The pots were arranged according to the experimental design mentioned before.

Experimental Data

The following parameters were recorded in both M₁ - and M₂-generations of the two successive experimental seasons.

1. Morphological characteristics, such as habit of growth, leaf size, form and colour, stem colour and flower structure and colour.
2. Leaf proline content (according to Bates *et al.* 1973).
3. Leaf alkaloids, vindolen and catharanthine contents (after Luo *et al.*, 2005)
4. Leaf total carbohydrates content
Total leaf carbohydrates content was determined colorimetrically as reported by Loomis and Shull (1937) and Dubios *et al.*,(1956).
5. Variations and Mutations.

All plants of the different treatments in both M_1 and M_2 experiments were examined daily to search for the variation. Changes in the vegetative or flowering growth were recorded. These changes included :

- a) Habit of growth.
- b) Leaf colour and form.
- c) Flower colour and form.

6 .Peroxidase isozyme electrophoresis

The gamma rays and salinity treatments caused variation in the flowers form and tolerance to salinity compared with the control. Leaves were used for the isozymes techniques from the control and the mutated twelve plants. The peroxidase isozymes patterns were examined after the method described by Sabrah and El- Metainy (1985).

RESULTS AND DISCUSSION

1. Effect of gamma radiation and salinity on the morphological characteristics

Some variations in seed germination percentage ,plant height, internode length, stem diameter , number of branches, number of leaves, leaf area, specific leaf weight, total leaf chlorophyll content (a,b and a+b), total carotene, fresh and dry weights of the plants, flowering date ,number of flowers per plant, flowering period, flower length and diameter, pollen viability, survival, fresh and dry weights of the roots, were recorded as a result of different treatments

2. Effect of the gamma radiation and salinity on the leaf proline content

The analysis of variance showed that the effects of the gamma radiation, salinity treatments and interaction between them were highly significant on the leaf proline content in the M_1 and M_2 generations of the second season.

Data on the effect of gamma radiation and salinity treatments on the leaf proline content of the M_1 and M_2 . generations of the second season are listed in Table 2.

In the M_1 .generation there were highly significant differences among gamma rays treatments. The highest average was at the 20 kr treatment (0.4052 g/100g) and the lowest one was at 0 kr (0.2296 g/100g).By the salinity treatments, there were also highly significant differences. The highest average was at the 150 mM (0.4497 g/100g) and the lowest one was at 100 mM (0.2376 g/100g). The interaction was also highly significant. The 20 kr with 100 mM had the highest proline content (0.7690 g/100g). The lowest one was at the treatment of 20 kr with 0 mM (0.0006 g/100g) (Table 2).

In the M_2 – generation, there were highly significant difference among the gamma rays treatments. The highest average by the gamma rays was at the 20 kr treatment (0.9993 g/100g) and the lowest average was at 5 kr (0.3579 g/100g).The salinity treatments caused highly significant differences. The highest average was at the 150 mM (1.0788 g/100g) and the lowest average was at 0 mM (control) treatment (0.3789 g/100g). The interaction was also

highly significant. The 20 kr with 150 mM treatment had the highest proline content (1.4700 g/100g). The lowest average was at the treatment of 5 kr with 100 mM (0.1818 g/100g).

The results of gamma rays were similar to those reported by Desai and Rao (2014) on *Cajanus cajan*. The results of the salinity treatments were similar to those reported by Zidan and Alzahrani (1994) on *Ocimum basilicum* L. and Heidari and Sarani (2012) on *Matricaria chamomilla*.

Generally, the treatments of gamma rays and salinity caused some increases in proline content in the M₁ and M₂ generation, which is harmony with the results of Nikam *et al.* (2015) on *Saccharum officinarum* L. which can be used for the production of mutants which have the ability for environmental stress tolerance, Desai and Rao (2014).

The results of this work revealed that the dose of 20 kr significantly increased the amount of leaf proline in the irradiated plants comparing with the control in the M₁- and M₂ – generations of the second season. Higher level of proline content in leaves may be due to irradiation at 20 kr stimulated the expression of genes encoding enzymes of proline synthesis such as pyrroline -5- carboxylate. Also, irradiation decreased enzymes of proline oxidative such as proline dehydrogenase. This explanation is similar to the opinion mentioned by Amini and Ehasapour (2005).

The results of the M₂ -generation during the second season declared that the doses of 5,10 and 15 kr significantly reduced the amount of leaf proline in irradiated plants as compared to the control. This reduction in leaf proline content could be attributed to the inhibition effect of gamma-rays doses mentioned before on the expression of genes encoding enzymes of proline synthesis and /or enhancing the activity of enzymes of proline oxidative. This declaration is nearly similar to that reported by Amini and Ehasapour (2005).

Table (2). Average values of the leaf proline content of *Catharanthus roseus*, L. as affected by gamma radiation (kr) and salinity levels (mM) treatments in the M₁ – and M₂ generations of the second season.¹⁾

Average proline content (g/100g)								
Gamma Rays (Kr)	M ₁ -2nd season			Average Gam.	M ₂ -2nd season			Average Gam.
	Salinity Levels (mM)				Salinity Levels (mM)			
	0	100	150		0	100	150	
0.0	0.3810 c	0.1980 d	0.1098 de	0.2296 b	0.4190 de	0.8310 c	1.4200 a	0.8900 b
5.0	0.3620 c	0.1740 de	0.5520 b	0.3626 ab	0.3830 de	0.1818 f	0.5090 d	0.3579 e
10.0	0.3590 c	0.0004 e	0.7030 a	0.3541 ab	0.3690 e	0.2140 f	0.8750 c	0.4860 d
15.0	0.4430 bc	0.0470 e	0.4380 bc	0.3093 b	0.3849 de	0.8650 c	1.1200 b	0.7899 c
20.0	0.0006 e	0.7690 a	0.4460 bc	0.4052 a	0.3390 ef	1.1890 b	1.4700 a	0.9993 a
Average Sal.	0.3091 b	0.2376 c	0.4497 a		0.3789 c	0.6561 b	1.0788 a	
L.S.D.0.05 for A		0.082				0.077		
L.S.D.0.05 for B		0.064				0.060		
L.S.D.0.05 for AB		0.143				0.134		

¹⁾Values marked with the same alphabetical letters, within comparable group of means, do not differ significantly, using L.S.D. at 0.05 level of probability.

It is clear that the salinity treatments of 100 and 150mM significantly increased the amount of leaf proline as compared with the control. It has been established that the plants accumulate a variety of osmoregulator solute including proline as an adaptive mechanism to environmental stress and salinity (Aspinall and Paley,1981). The use of proline as osmoregulator to overcome the bad effects of salinity, which is similar to the effect of seawater on plant growth has been reported by Lin and Kao(1996). Increase in proline content with increasing stress is one of the defense mechanisms which is used by stressed plants to reduce cell osmotic potential which resulted in increasing cell water uptake with concomitant increases in cell turgidity and activity (Khalil and El- Noemani,2012). Stressed plants diminish osmotic potential by accumulating free amino acids,ions,proline,soluble protein and carbohydrate (Salama *et al.*,1994). These osmolytes might increase the osmotic pressure of cytoplasm and enhance water flow into the different plant organs and tissues.

3. Effect of radiation and salinity on the leaf alkaloids

3a.Effect of gamma radiation and salinity on the leaf vindolen content

The analysis of variance showed that the effect of the gamma radiation alone and the interaction between gamma radiation and salinity were not significant, but the effect of salinity treatments on the leaf vindolen content was significant in the M₁-generation.

Data on the effects of gamma radiation and salinity treatments on the leaf vindolen content of the M₁ and M₂ –generations in second season are listed in Table 3a.

In the M₂-generation, the effects of the gamma radiation and that of the interaction between gamma radiation and salinity were highly significant but the effect of the salinity treatments was only significant.

Table 3a presents the mean values of the leaf vindolen content of the different treatments. In the M₁-generation, there were no significant differences between gamma rays treatments. The highest average between the gamma rays was at the 20 kr treatment (1.37 mg/g) and the lowest one was at 0 kr (control) (1.27 mg/g).The effect of the salinity treatments was significant. The highest average between the treatments salinity was at the control treatment (1.66 mg/g) and the lowest one was at 100 mM (0.97 mg/g). The 20kr with 0 mM treatment had the highest leaf vindolen content (2.12 mg/g). The lowest averages were at the treatment of 20 kr with 100 mM (0.86 mg/g).

In the M₂- generation, there were highly significant differences among the gamma rays treatments. The highest average was at the 20 kr treatment (3.35 mg/g) and the lowest one was at 10 kr (2.29 mg/g).The effects of the salinity treatments were significant. The highest average was at the 150 mM (2.94 mg/g) and the lowest one was at 0 mM (control) treatment (2.41 mg/g). The 20 kr with 150 mM treatment had the highest leaf vindolen content (4.20 mg/g).

The lowest average was at the treatment of 15 kr with 0 mM (1.65 mg/g) (Table 3a).

The results of gamma rays were similar to those reported by Abdel-Hady *et al.*(2008) on *Atropa belladonna* and Shaimaa *et al.*(2013)on *Brassica rapa* at gamma rays.

Generally, the treatments of salinity caused some increases in leaf vindolen content in the M₂- generation, which in harmony with the results of Ali (1991) on *Datura*and Heidari and Sarani (2012) on *Matricaria chamomilla*. Also the effect of gamma -rays and salinity on the vindolen was similar to the result reported by Shaimaa *et al.*(2013)on *Brassica rapa*.

Accumulation of alkaloids was considered as an adaptation to the imposed salinity stress because they have an osmoregulatory role (Elhaak and Wegmann, 1997).

William *et al* (1998) reported thatthe increase in the alkaloids content as the influence of NaCl is a combination of an osmotic effect and a specific ion effect. He added that the increase of alkaloids in response to salinity may be due to its role in the plant protection against the salt stress effects.

Table (3a). Average values of the vindolen content of *Catharanthus roseus*, L. as affected by gamma radiation (kr) and salinity levels (mM) treatments in the M₁ – M₂ generation of second season.¹⁾

Gamma Rays (Kr)	Average vindolen content (mg/g)							
	M ₁ -2nd season			Average Gam.	M ₂ -2nd season			Average Gam.
	Salinity Levels (mM)				Salinity Levels (mM)			
0	100	150	0	100	150			
0.0	1.51	1.08	1.22	1.27	2.66 bc	2.20 bc	2.89 b	2.58 b
5.0	1.82	1.06	1.11	1.33	2.95 b	2.33 bc	1.91 c	2.39 b
10.0	1.21	0.89	1.82	1.31	2.88 b	1.90 c	2.10 c	2.29 b
15.0	1.63	0.95	1.43	1.34	1.65 c	3.50 ab	3.62 ab	2.92 ab
20.0	2.12	0.86	1.12	1.37	1.94 c	3.91 a	4.20 a	3.35 a
Average Sal.	1.66 a	0.97 b	1.34 ab		2.41 b	2.76 a	2.94 a	
L.S.D.0.05 for A		N.S.				0.445		
L.S.D.0.05 for B		0.485				0.345		
L.S.D.0.05 for AB		N.S.				0.771		

¹⁾Values marked with the same alphabetical letters, within comparable group of means, do not differ significantly, using L.S.D. at 0.05 level of probability.

3b. Effect of the gamma radiation and salinity on the leaf catharanthine content

The analysis of variance showed that the effects of the gamma radiation, salinity treatments and the interaction between them on the leaf catharanthine content were highly significant in the M_1 -generation in the second season.

Data on the effect of gamma radiation and salinity treatments on the leaf catharanthine content of the M_1 and M_2 -generations of the second season are listed in Table (3b).

In the M_2 -generations, the effect of the gamma radiation was not significant, but that of the salinity treatment was significant, while the interaction between them was highly significant.

Table 4 presents the mean values of the leaf catharanthine content of the different treatments. In the M_1 -generation, there were highly significant differences among the gamma rays treatments. The highest average was at the 10kr treatment (0.413 mg/g) and the lowest one was at 15 kr (0.104 mg/g). The effects of the salinity treatments were highly significant. The highest average was at the control treatment (0.372 mg/g) and the lowest one was at 100 mM (0.079 mg/g). The 10 kr with 150 mM had the highest leaf catharanthine content (0.890 mg/g). The lowest average was at the treatment of 20 kr with 100 mM (0.002 mg/g).

In the M_2 - generation, there were no significant differences among the gamma rays treatments. The highest average between the gamma rays was at the 15 kr treatment (0.186 mg/g) and the lowest one was at 10 kr (0.116 mg/g). The effects of the salinity treatments were significant. The highest average was at the 100 mM (0.177 mg/g) and the lowest one was at 0 mM (control) treatment (0.128 mg/g). The 0 kr with 100 mM treatment had the highest leaf catharanthine content (0.316 mg/g), while the lowest averages was at the treatment of 10 kr with 150 mM (0.080 mg/g).

The results of this work indicated that the gamma doses of 5 and 10kr significantly increased the leaf catharanthine content compared with the control during the M_1 -generation of the second season. It was also noticed that the dose of 20kr significantly increased the leaf vindolen content as compared with the control during the M_2 -generation of the second season. This means that radiation supported accumulation of alkaloids in the irradiated plants. The response of plants against radiation induced reproductive and metabolic disorder may be due to the accumulation of several bioactive constituents like alkaloids (Padhya, 1986), which may act through different mechanisms such as inhibition of lipid peroxidation (Goel *et al.* 2004).

Alkaloids are end products for the reaction of toxic components in plants and they are harmless for plants (Hossien, 1987). The radiation may stimulate this reaction which resulted in accumulation of alkaloids in the irradiated plants.

Regarding the salinity treatments, it was noticed that the treatment of 10mM in the M₂-generation of the second season significantly reduced the amounts of leaf catharnathine and vindolen compared with the control. It is known that under stress condition plants generally shift a major portion of their metabolic activities towards secondary metabolite synthesis, so an increase in alkaloid contents was expected (Ali,1991;Moons *et al.* 1997;Wu *et al.*,2004;Pandey *et al.*, 2007 and Shaimaa *et al.* ,2013).

But in the case of the treatment of 100mM during the M₁-generation the decrease in alkaloids was recorded and it was unexpected. During the M₂-generation of the second season, it was clear that the treatment of 10mM significantly increased the amount of the catharnathine and both treatments of 100 and 150mM significantly increased the amount of leaf vindolen as compared with the control. It has been mentioned before that under stress condition as salinity stress plants generally shift a major portion of their metabolic activities towards secondary metabolite synthesis, as alkaloids (Ali,1991;Moons *et al.*, 1997;Wu *et al.*,2004;Pandey *et al.*, 2007 and Shaimaa *et al.* ,2013). A biotic stresses as salinity stress may result in an increase in the level of endogenous methyl jasmonate, which can stimulate the activity of enzymes involved in the biosynthesis of alkaloids leading to enhanced alkaloids accumulation (Moons *et al.*, 1997).

Table (3b). Average values of the leaf catharntine content of *Catharanthus roseus*, L. as affected by gamma radiation (kr) and salinity levels (mM) treatments in the M₁ – M₂ generation of second season.¹⁾.

Gamma Rays (Kr)	Average leaf catharntine content (mg/g)							Average Gam.
	M ₁ -2nd season			Average Gam.	M ₂ -2nd season			
	Salinity Levels (mM)				Salinity Levels (mM)			
	0	100	150		0	100	150	
0.0	0.326 cd	0.093 e	0.065 e	0.161 bc	0.050 c	0.316 a	0.190 b	0.183
5.0	0.603 b	0.210 de	0.300 d	0.371 a	0.051c	0.130 bc	0.270 ab	0.150
10.0	0.293 d	0.056 e	0.890 a	0.413 a	0.180 b	0.089 c	0.080 c	0.116
15.0	0.183 de	0.036 e	0.093 e	0.104 c	0.210 b	0.170 bc	0.180 b	0.186
20.0	0.453 c	0.002 e	0.103 e	0.186 bc	0.150 bc	0.190 b	0.091 c	0.143
Average Sal.	0.372 a	0.079 b	0.290 a		0.128 b	0.177 a	0.162 ab	
L.S.D.0.05 for A		0.080				N.S.		
L.S.D.0.05 for B		0.062				0.040		
L.S.D.0.05 for AB		0.139				0.090		

¹⁾ Values marked with the same alphabetical letters, within comparable group of means, do not differ significantly, using L.S.D. at 0.05 level of probability

4. Effect of the gamma radiation and salinity on the total carbohydrate content

The analysis of variance showed that the effects of the gamma radiation, salinity and the interaction between them on the total carbohydrate content was not significant in the M_1 -generation in the second season. In the M_2 , the effects of the gamma radiation and salinity were significant but the interaction between them was not significant. Table 4 presents the mean values of the total carbohydrate content of the different treatments in M_1 -and M_2 of the second season. In the M_1 -generation, there were no significant differences among the gamma rays treatments. The highest average was at the 0 kr (control) treatment (5.39 %) and the lowest one was at 20 kr (4.68 %). The effects of the salinity treatments were also not significant. The highest average between the salinity was at 150 mM (5.38 %) and the lowest one was at 0 mM (control) treatment (4.90 %) and the interaction between radiation and salinity was not significant. The 10 kr with 150 mM had the highest total carbohydrate content (6.72 %) and the lowest average was at the treatment of 10 kr with 0 mM (4.04 %). In the M_2 - generation, there was significant difference among the gamma rays treatments. The highest average was at the 15 kr treatment (8.96 %) and the lowest one was at 5 kr (6.50 %). The effects of the salinity treatments were significant. The highest average was at the 100 mM (8.50 %) and the lowest one was at 0 mM (control) treatment (7.10 %). The 15kr with 100 mM and 15 kr with 150 mM treatment had the highest total carbohydrate content (9.50 %). The lowest average was at the treatment of 5 kr with 0 mM (4.70 %). These results were similar to those reported by Rashad (1995) on *Tagest erecta*, El-Sharnouby *et al.* (1997) on *Hibiscus sabdariffia* and Farid *et al.* (1999) on the sweet marjoram. The results of the other workers are not in harmony Kandeel *et al.* (1991) reported on *Ocimum basilicum* that the high gamma dose of 12000 r caused a slight decrease in comparison with the control. These results were similar to those reported by Zidan and Alzahrani (1994) on *Ocimum basilicum*.

The obtained results of salinity treatments during the M_2 -generation of the second season indicated that the treatments of 100 and 150mM increased the amount of carbohydrate contents and the increase was significant at the treatment of 100mM compared with the control. Many plants, which are stressed by Na Cl salinity, accumulated starch and soluble carbohydrates (Greenway and Munns, 1980 and Rathert, 1984). This accumulation has been attributed to impaired carbohydrate utilization (Munns and Termaat, 1986). Dhanapackiam and Ityas (2010) reported that the soluble and total carbohydrates content in leaves were higher in salt stress plants compared with the control. This is strong evidence that photosynthesis is the main source of accumulating carbohydrates under water stress. The accumulation of organic solutes (soluble and insoluble carbohydrates) might play an important role in increasing the internal osmotic pressure (Zidan and Alzahrani, 1994). This has been widely regarded as response to salinity stress condition. Munns (1993) reported that the concentration of sugars and reserve polysaccharides always rise after plants are exposed to salinity in both growing and fully expanded tissues. This is consistent with a blockage in utilization of sugars in the growing tissues and a subsequent build-up in the rest of the plant.

Table (4). Average values of the total carbohydrates content of *Catharanthus roseus*, L.as affected by gamma radiation (kr) and salinity levels (mM) treatments in the M₁– M₂ generation of second season.¹⁾.

Average total carbohydrates content (%)								
Gamma Rays(Kr)	M ₁ -2nd season			Average Gam.	M ₂ -2nd season			Average Gam.
	Salinity Levels (mM)				Salinity Levels (mM)			
	0	100	150		0	100	150	
0.0	6.53	4.67	4.99	5.39	7.79	7.99	7.40	7.73 ab
5.0	4.79	5.17	5.59	5.18	4.70	7.93	6.88	6.50 b
10.0	4.04	5.13	6.72	5.29	7.60	8.60	7.99	8.06 a
15.0	4.22	4.70	5.47	4.79	7.88	9.50	9.50	8.96 a
20.0	4.92	5.00	4.12	4.68	7.54	8.50	8.20	8.08 a
Average Sal.	4.90	4.93	5.38		7.10 b	8.50 a	7.99 ab	
L.S.D.0.05 for A		N.S.				1.48		
L.S.D.0.05 for B		N.S.				1.15		
L.S.D.0.05 for AB		N.S.				N.S.		

¹⁾Values marked with the same alphabetical letters, within comparable group of means, do not differ significantly, using L.S.D. at 0.05 level of probability.

5. Effect of gamma rays and salinity on the induction of variations (Aberrations)(Mutations)

5.1.Growth habit changes

Some treatments caused changes in the habit of growth in some plants resulting in fasciated, dwarfed, creeping and conical forms. Changes in growth habit may be due to the effect of radiation on genetic factors controlling the normal growth habit of the plant. The dwarfed growth can be attributed to the effect of radiation on the apical bud which inhibited its growth. It is a fact that most genetic changes in plants result from chromosome aberrations rather than single gene change (Broertjes *et al.*, 1976).

The dwarfed plant lost its ability to grow and was associated with inhibition of flowering. The observed effects in this dwarf plant could be separated as primary and secondary effects. The secondary effects are totally depending upon the primary effects (Donnini *et al.*, 1984).

The dwarfed growth in the M₁-generation may be due to physiological damage resulting in the alteration from normal to dwarf growth (Abd El-Maksoud and El-Mahrouk, 1993).

The fasciated growth occurred when a bud had been injured or splitted by radiation, which resulted in many breaks (instead of one break) to come from the apical point. This result is in agreement with that reported by Badr and Etman (1976) and Abdel-Maksoud (1980).

5.2. Leaf changes (shape and colour)

All treatments caused a wide range of leaf deformities during the M₁-generation of the two seasons. Leaf abnormalities included dwarfing, prolonging, slanting, diminishing. Some leaves were linear, lanceolate, oblong, elliptic, obovate and spatulate. Other leaves had oblique bases. Some leaves had obtuse, marginate and cuspidate tips. There were changed margins included dentate, undulate, sinuate, incised, lobed and deeply lobed margins. Some plants had curly leaves. Some leaves had the bell-shape. There were some leaves with two midribs. It was noticed in some leaves that the midrib divided the lamina into unequal parts.

The leaf abnormalities were found in the control plants, as well as in the other treatments. In general the frequency of the leaf form changes in the control was less than that of any other treatment. Selfing was carried out in the plants which had the leaf form changes and the seeds of each plant were sown. The inheritance of these changes were obvious in the M₂- generation and there was a wide range of variation between the M₂- plants.

In this experiment, variation in leaf size and shape suggested that more than one effect may be responsible for the modified leaf patterns. One possible explanation would be the alteration in the ontogeny of leaf tissues through the selective destruction of 1 or more cell layers in the shoot meristem (Skirvin and Janick, 1977 and Abdel-Maksoud, 1980). Second explanation can be given through genetic changes or chromosomal disturbances, as a result of primary effect of radiation, which may occur

and caused a decrease in the leaf size in irradiated plants (Kaicher and Swarup,1972 and Evans,1984). Third possibility is that the cell number per unit area and the length of cells may be altered in the leaf area of irradiated plants as a result of the primary effect of radiation. From the number of cells per unit leaf area and the cell length it could be concluded that broader leaves had a decreased number of cells and /or length of cells.

Some leaf changes, especially those with distorted patterns of development,may be resulted in as induced polyploidy which was also reported by love(1966). Also, these changes could be referred to the layer rearrangements as a result of irradiation effect (Kaicher and Swarup,1978 and Abdel-Maksoud,1980 and 1988). These results were in agreement with those reported by Sorour (2011) on *Farfugium japonicum* and Minisi *et al.*(2013) on *Moluccella laevis* at the effect of gamma and Khayamim *et al.* (2014) on sugar beet at the effect of salinity.

The leaf variegation which appeared in the M₂-generation could be attributed to one of the following reasons:

- 1) The epidermal layer lacked chlorophyll and the internal tissues also showed lack of chlorophyll - because epidermal cells have displaced inner cells in particular regions , the result was creamy green colour.This explanation is supported by those mentioned by Watts(1980),Irvine(1984) and Abdel-Maksoud (1988),who have stated that when the plant is irradiated,the cell layer L_I is easily destroyed and this urges the epidermis or the tissue beneath it to substitute the cell layer L_{II} and then the variegation type appears.
- 2) The variegation may be caused by gene and / or plastid changes as a results of the irradiation (Borner *et al.*,1976; Walbot and Thompson,1982 and Preil,1985).

Regarding the M₂-generation dwarfed albino plant, it could be concluded that this plant suffered from chlorophyll deficiency which might be due to chromosomal breaks induced by the mutagen (Abd El-Maksoud and El-Mahrouk, 1992).

5.3. Stem colour changes

During the M₁- generation of the first season, one changed plant was found at the combined treatment of 5kr+100mM NaCl. The phenotypic change was restricted in the stem and branches colour. The base of the stem was green, while the rest of the stem had a light purple colour, also,all branches of the plant had a light purple colour.The exact mechanism of the induction of the light purple and green colours cannot be explained with certainly. Both gene and chromosomal structural change has been responsible for the induction of this light purple on the stem of irradiated *Catharanthus roseus* (L) G. Don plant(Sparrow,1961 and Gupta and Shukla,1971).

It is suggested that the appearance of light purple may be due to one or more of the following suggestions:

1. During the biosynthesis of purple pigments, radiation may decrease the methylation of one or more hydroxyl groups by affecting the gene controlling this process, which consequently decreased the purple colour (Wagner, 1975).
2. The co-pigmentation may be changed as a result of radiation effect and this may dilute the purple colour and changed it to light purple (De Vries *et al.*, 1974 and Chaleff and Torrey, 1981).
3. The radiation may affect one of the genes which determine the quantity of pigments responsible for the purple colour which consequently decreased the quantity of the whole pigments (Wagner, 1975). This came to the agreement with that reported by Adachi and Katayama (1970).

The role of salinity in the production of the light purple colour cannot be neglected, where it may be decreased the methylation of hydroxyl groups and/or the degree of co-pigmentation, consequently the appearance of light purple on the stem and branches. According to Adachi and Katayama (1970), the pigment of betacyanin causes the purple in plant. Radiation may reduce the biosynthesis of betacyanin which resulted in appearance of the light purple.

Regarding the appearance of green colour on the base of plant which subjected to the treatment of 5kr+100mM. Scott-Moncrieff (1936) reported that there are intensifying and diluting genes whose action is not effective over the whole, but is restricted to certain areas. It can be suggested that radiation depressed or inhibited the action of the genes which control the purple colour of the stem or determine the extension of purple colour all over the stem. So, the purple colour withdraw from the base of stem while the green colour spread over the stem base.

5.4. Flower changes

5.4.1. Changes in the number and size of petals

The different treatments caused different changes in the number and size of petals. The normal corolla of *Catharanthus roseus*, (L.) G. Don consists of separated and equal five petals. The changes in petal numbers were classified into four types:

Type 1. The corolla contained two separated petals and this type was found at the treatments of 5kr+0mM and 10kr+100mM during the M₁- generation of the first season.

Type 2. The corolla contained three separated petals and this type was found at the treatments of 15kr+0mM, 5kr+100mM and 20kr+100mM during the M₁- generation of the first season.

Type 3. The corolla contained four separated petals (crucifer form). This type was found during the M₁- generation of the first season at the treatments of 5kr+100mM, 10kr+100mM and 15kr+100mM and in the second season at the treatment of 20kr+150mM. Also, this type was found during the M₂- generation of the first season at the treatments of 5kr+0mM, 10kr+0mM, 15kr+0mM and 20kr+0mM.

Type 4. The corolla contained six separated petals. This type was found during the M_2 - generation of the first season at the treatments of 5kr+0mM, 10kr+0mM, 15kr+0mM and 20kr+0mM. There was one flower with six petals one of them was very small at the treatment of 5kr+0mM. Also, there was one flower with six petals, but one of them was above other petals and this form was detected at the treatment of 10kr+0mM. It was found during the M_2 - of the second season some flowers with six separated petals at the treatments of 5kr+100mM, 10kr+100mM, 15kr+100mM and 5kr+150mM. One flower at the treatment of 15kr+100mM and other one at that of 5kr+150mM had unequal petals.

The flower is a modified stem and the floral whorls are modified leaves and these whorls are appendages similar to the normal leaves in its initiation. Therefore, petal deformities can be attributed to the effect of radiation on flower bud during its initiation. The changes in the number of petals can be postulated that these changes are a result of chromosomal deletion, or changes of the factors governing the normal form or structure, as well as according to the effect of radiation on the ontogeny of flower organ tissues through the selective destruction of one or more cell layer in the apical floral meristem (Abd El-Maksoud, 1980 and 1988).

Bidwell (1979) reported that the initiation and development of flower depends upon the balance of hormones or growth factors. Regarding the type of six petals, it is probably to assume that the gamma-rays had stimulation effect on the initiation and development of petals from the meristematic apex, since gamma doses may affect the balance of growth hormones which in turn may result in an increase in the number of petals.

The reduction in the number of petals (two, three and four petals) could be attributed to the damage effect of gamma-rays and/or salinity on the primordia of petals or on the cells in the shoot growing point, and were later activated and become involved in flowering (Bidwell, 1979).

The flowers with changes in the number of petals were selfed. The type 1 (two petals) and type 2 (three petals) did not form seeds. The types 3 and 4 (four and six petals; respectively) formed seeds and their plants produced normal flowers.

5.4.2. Changes in flower colour

Four types of flower colour changes were observed in the treated *Catharanthus roseus*, (L.) G. Don plants during the M_1 - M_2 - generations of both seasons (pale (light) purple, white, variegated and striped flowers).

The induced changes at the treatments of 5 kr + 0 mM, 10 kr + 0 mM, 15 kr + 0 mM and 20 kr + 0 mM were:

1. Pale purple (5 kr + 0 mM, 10 kr + 0 mM and 15 kr + 0 mM),
2. Purple variegated with white (15 kr + 0 mM),
3. Purple striped with (15 kr + 0 mM).

These flower colour changes appeared through the M₁- generation of both seasons.

Three types of induced flower colour changes were recognised at different treatments.

1. White flowers (5 kr + 100 mM and 10 kr + 100 mM),
2. Pale (light) purple flowers (5 kr + 100 mM, 10 kr + 100 mM, 15 kr + 100 mM and 20 kr + 100 mM),
3. Variegated flowers (5 kr + 100 mM, 10 kr + 100 mM, 15 kr + 100 mM and 20 kr + 100 mM)

In the M₂- generation of the second season, there was a new variegated at the treatment of 15kr+0mM. The flowers were purple variegated with yellow colour and the yellow areas were at the margins of four petals, while on the fifth petal the yellow colour was extended to the flower centre.

In order to give a general interpretation for the appearance of the pale(light)purple flowers, it should be outlined that:

1. Adachi and Katayama (1970) mentioned that betacyanin pigment causes the purple colour.
2. Glycosides of the betacyanins and their co-pigmentation with several other substances are responsible for innumerable variation in the purple colours (Asen *et al.*, 1972 and De Vries *et al.*, 1974). Glycosides type and probably the degree of methylation are each determined by simple gene (Wagner, 1975). The methylation of one or more hydroxyl groups will increase the colour (Wagner, 1975).
3. The different combinations of the pigments are principally responsible for variation in flower colour (De vries *et al.*, 1974 and Chaleff and Torrey, 1981).
4. The presence of the pigment may be controlled by a single gene, while the quantitative effect of genes in pigment production refers to the effect of multigenes responsible for the amount of pigment in the floral parts (Wagner, 1975).

Accordingly it is suggested that the appearance of light purple flowers in *Catharanthus roseus*, (L.) G. Don plants may be due to one or more of the following suggestions:

- i. During the biosynthesis of betacyanin, radiation may decrease the methylation of one or more hydroxyl groups by affecting the gene controlling, this process, consequently decreased the purple colour.
- ii. The co-pigmentation may be changed as a result of radiation and /or salinity effects and this may dilute the purple colour and changed it to light purple.
- iii. The radiation may affect one or more of the genes which determine the quantity of purple pigments which consequently decreased the quantity of the whole pigments. This came to agreement with what was reported by Adachi and Katayama (1970).

These results were in agreement with those reported by Sorour (2011) on *Ligularia japonica*, Minisi *et al.* (2013) on *Moluccella laevis* and Khayamim *et al.* (2014) on sugar beet.

5.5. Mutated plants

The gamma rays and salinity treatments caused branching, flower texture, form and colour and salt tolerance mutations in twelve plants in the M₁ compared with the control as follows:

- T₀ —————> 0 Kr Gama ray + 0 mM salinity (control).
- T₁ —————> 5 Kr Gama ray + 0 mM salinity (petals texture).
- T₂ —————> 10 Kr Gama ray + 0 mM salinity (flower form).
- T₃ —————> 15 Kr Gama ray + 0 mM salinity (flower colour).
- T₄ —————> 20 Kr Gama ray + 0 mM salinity (little branching).
- T₅ —————> 0 Kr Gama ray + 100 mM salinity (salt tolerate).
- T₆ —————> 5 Kr Gama ray + 100 mM salinity (salt tolerate).
- T₇ —————> 10 Kr Gama ray + 100 mM salinity salt (tolerate).
- T₈ —————> 15 Kr Gama ray + 100 mM salinity (salt tolerate).
- T₉ —————> 0 Kr Gama ray + 150 mM salinity (salt tolerate).
- T₁₀ —————> 5 Kr Gama ray + 150 mM salinity (salt tolerate).
- T₁₁ —————> 10 Kr Gama ray + 150 mM salinity (salt tolerate).
- T₁₂ —————> 15 Kr Gama ray + 150 mM salinity (salt tolerate).

The leaves of the control and mutated plants were used for the isozymes techniques and the separation of the peroxidase isozymes of the control plant and the twelve mutants were carried out.

5.6. Peroxidase isozyme

It is important to notice that isozyme analysis using electrophoresis offers a very well define effective tool for the detection of genetic differences among individuals. This makes electrophoresis a useful tool for plant breeders (Arulsekhar and Parfitt, 1986). The study of isozyme electrophoretic patterns can offer a rapid method for the identification of different genotypes without carrying out field experiment, which saves time and money (Bailey, 1983). Moreover, the analysis of a protein can be reflected to its gene (Gottlieb, 1977).

The similarity values, (Table 5) showed that the control plants were more genetically distinct to the plants treated with 150 mM NaCl (similarity value equal to 50), while high similarity value (100) was found between the control plants and the plants treated with 5, 10 kr, 5kr+ 150 mM and 15 kr+ 150 mM NaCl (Table 5).

Table (5). Similarity value among the control and all mutants of *Chatharanthus roseus*, L. produced by gamma rays and salinity.

	0	1	2	3	4	5	6	7	8	9	10	11	12
0	100												
1	100	100											
2	100	100	100										
3	66.6	66.6	66.6	100									
4	80	80	80	88.8	100								
5	66.6	66.6	66.6	75	66.6	100							
6	80	80	80	88.8	100	66.6	100						
7	66.6	66.6	66.6	100	88.8	75	88.8	100					
8	66.6	66.6	66.6	50	66.6	75	66.6	50	100				
9	50	50	50	57.1	50	75	50	57.1	85.7	100			
10	100	100	100	66.6	80	66.6	80	66.6	66.6	50	100		
11	88.8	88.8	88.8	75	66.6	75	66.6	75	66.6	57.1	88.8	100	
12	100	100	100	66.6	66.6	66.6	66.6	66.6	66.6	50	100	88.8	100

The separation of the peroxidase isozyme of the control plant and the twelve mutants of *Catharanthus roseus* illustrated in Figure 1. These results were in agreement with those reported by Jaleel *et al.* (2007) on *Catharanthus roseus*.

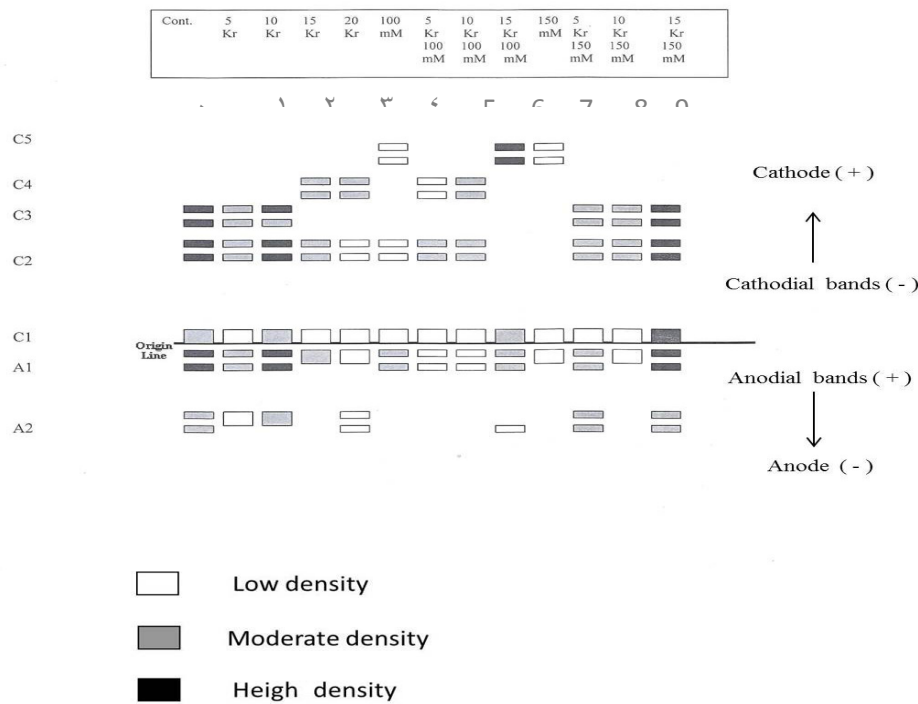


Figure (1). Zymogram of electrophoretic separation samples of peroxidase isozyme of the control and the twelve mutated plants of *Catharanthus roseus*.L.

The electrophoretic banding patterns indicate different profiles among gamma rays doses and salinity concentrations. It can be noticed that a total number of seven loci control the production of peroxidase in the *Catharanthus roseus*. Five bands migrated toward the cathode (-) and designed as C1 to C5, while, two bands migrated toward the anod (+) in the electrophoresis field and were designed as A1 and A2.

The bands of the loci C1 and A1 were presented in all the treatments. Bands of the loci A1 differed in the intensity and homogeneity among treatments. This locus was presented by one homozygous allele in the treatments of 15, 20 kr, 150 mM NaCl and the treatment with 10 kr +150 mM saline water, while this locus showed heterozygous profile in all other treatments. The locus A2 disappeared from the samples treated with 15 kr, 100 mM, 150 mM NaCl and the treatments of 10 kr+150 mM NaCl.

The locus C5 was found only in the samples treated with 100 mM, 150 mM NaCl in low intensity and in the samples treated by 15 kr+ 100 mM in high intensity. The locus C4 was found only in the treatments of 15, 20 kr, 5 kr+ 100 mM and 10 kr + 100 mM NaCl with low intensity. On the other hand the locus C2 was absent in the 150 mM and 15 kr+ 100 mM NaCl treatments.

Phylogenetic tree classified the studied plants into three groups. The control plants (T₀) and the treatments of T₁, T₂ and T₁₂ were classified in cluster I, plants of T₃, T₄, T₆ and T₇ were classified in the cluster II and plants of T₅, T₈ and T₉ were grouped in the cluster III (Figure 2).

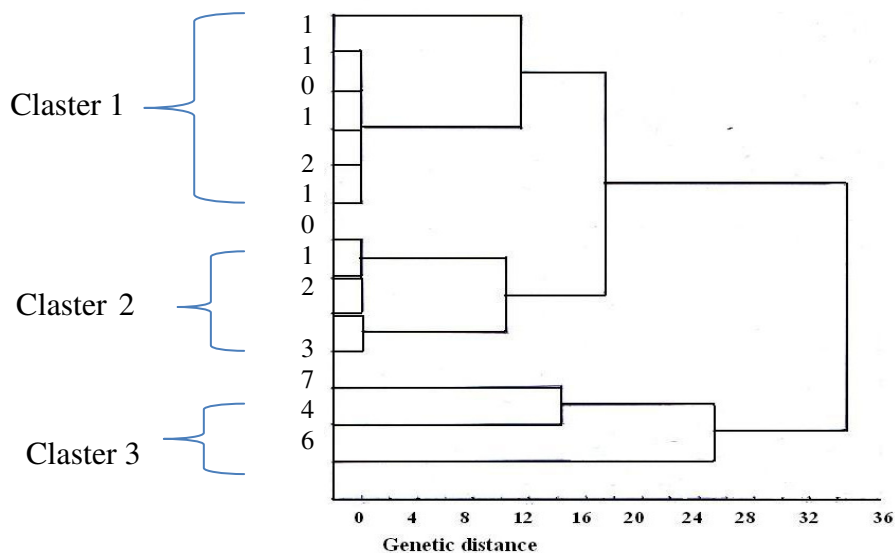


Figure (2). Genetic relationship among the control and the twelve mutants *Catharanthus roseus*, L. based on peroxidase isozymes patterns and similarity values.

CONCLUSION

The obtained results indicated that different doses of gamma radiation cause some morphological variations in the vegetative and flowering growth of *Catharanthus roseus* Linn. and induced salt – tolerant plants with high alkaloid content, which can be grown in saline soils. It can be also concluded that peroxidase isozyme could act as a useful biochemical marker in *Catharanthus roseus*, L. to study the genetic relationship between the mother plant and the induced mutations.

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الملخص العربي

تأثير إستخدام أشعة جاما علي التحسين الوراثي ضد ظروف ملوحة التربة في نبات الونكا

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تم إجراء البحث في حدائق أبحاث الزهور ونباتات الزينة بكلية الزراعة- جامعة الإسكندرية خلال الاعوام ٢٠١٣، ٢٠١٤، ٢٠١٥ علي نبات الونكا *Catharanthus roseus*. كان الهدف من البحث هو دراسة تأثير الجرعات المختلفة من أشعة جاما ومعاملات الري بالماء المالح علي الصفات الخضرية والزهرية للونكا فضلا عن إمكانية إحداث الطفرات، التي يمكن أن تقاوم الملوحة العالية أو لها قيمة تنسيقية.

هذا وقد تم زراعة البذور المعاملة بأشعة جاما بجرعات صفر، ٥، ١٠، ١٥، ٢٠ كيلو راد وذلك في ٢٠/٣/٢٠١٣ بالنسبة للجيل الأول للموسم الأول و٢٧/٣/٢٠١٤ بالنسبة للجيل الأول للموسم الثاني. وكان تصميم التجربة في صورة قطاعات عشوائية كاملة بعاملين. مشتملة على ٥ معاملات تشيع و ٣ مستويات ملوحة وخصص لكل معاملة ١٥٠ بذرة في الجيل الأول للموسمين و ١٠٠ بذرة للموسمين من كل معاملة في الجيل الثاني. ويمكن تلخيص اهم النتائج التي تم التوصل إليها فيما يلي:

١ - الصفات المورفولوجية (التغيرات المورفولوجية والإختلافات الظاهرية)

لوحظت تغيرات مورفولوجية في النباتات تبعاً لجرعات اشعة جاما وتركيزات الملوحة في كلا الجيلين والموسمين وذلك في عدة صفات هي :

١. طبيعة النمو (بعض النباتات المتقزمة وعديمة الكلوروفيل).
٢. لون وشكل الورقة (أوراق ذات أشكال غير منتظمة ومصفرة).
٣. طبيعة الساق (سيقان فاتحه اللون).
٤. لون وشكل الزهرة (أزهار قصيرة وأخري مبرقشة وأزهار ذات أعداد بتلات مختلفة).

تقدير البرولين في الاوراق - تقدير القلويدات (محتوي الفاندولين والكاثرانثين) - محتوى الكربوهيدرات - حدوث طفرات- تقدير البيروكسيديز .

٢- محتوى الأوراق من البرولين

وجدت فروق معنوية بين المعاملات في محتوى الاوراق من البرولين (جرام/١٠٠ جرام) نتيجة المعاملات بأشعة جاما والملوحة و التأثير المشترك بينهما في الجيل الطفوري الاول ، وكان اعلي تاثير لاشعة جاما عند ٢٠ كيلو راد (٠.٤٠٥٢ جرام/١٠٠ جرام) و اقل تاثير عند صفر كيلو راد (كنترول) (٠.٢٢٩٦ جرام/١٠٠ جرام) ، واعلي تاثير للملوحة

عند ١٥٠ ملي مول (٠.٤٤٩٧ جرام/١٠٠ جرام) وللتأثير المشترك بين الملوحة والاشعة عند ٢٠ كيلو راد مع ١٠٠ ملي مول (٠.٧٦٩٠ جرام/١٠٠ جرام) كأعلي متوسط واكل متوسط عند ٢٠ كيلو راد مع صفر ملي مول (٠.٠٠٠٦ جرام/١٠٠ جرام).

الجيل الطفوري الثاني كان هناك فروق معنوية بين المعاملات في محتوى الاوراق من البرولين بالنسبة لاشعة جاما وتأثير الملوحة و التأثير المشترك بين الملوحة والاشعة وكان اعلي تأثير لاشعة جاما عند ٢٠ كيلو راد (٠.٩٩٩٣ جرام/١٠٠ جرام) واكل تأثير عند ٥ كيلو راد (٠.٣٥٧٩ جرام/١٠٠ جرام) ، و اعلي تأثير للملوحة عند ١٥٠ ملي مول (١.٠٧٨٨ جرام/١٠٠ جرام) وللتأثير المشترك بين الملوحة والاشعة عند ٢٠ كيلو راد مع ١٥٠ ملي مول (١.٤٧٠٠ جرام/١٠٠ جرام) كأعلي متوسط واكل متوسط عند ٥ كيلو راد مع ١٠٠ ملي مول (٠.١٨١٨ جرام/١٠٠ جرام).

3a - محتوى الأوراق من الفندولين

وجدت فروق معنوية بين المعاملات بالنسبة لمحتوي الاوراق من الفندولين (مللجرام/جرام) نتيجة لتأثير الملوحة فقط عند صفر ملي مول (كنترول) (١.٦٦ مللجرام/جرام) في الجيل الطفوري الاول . أما في الجيل الطفوري الثاني فقد كانت هناك فروق معنوية بين المعاملات في محتوى الاوراق من الفندولين بالنسبة لاشعة جاما و التأثير المشترك بين الملوحة والاشعة وكان اعلي تأثير لاشعة جاما عند ٢٠ كيلو راد (٣.٣٥ مللجرام /جرام) واكل تأثير عند ١٠ كيلو راد (٢.٢٩ مللجرام/جرام). اما تأثير الملوحة فقد كان الاعلي عند ١٥٠ ملي مول (٢.٩٤ مللجرام /جرام) والاكل عند صفر ملي مول (كنترول) (٢.٤١ مللجرام /جرام) . أما التأثير المشترك بين الملوحة والاشعة عند ٢٠ كيلو راد مع ١٥٠ ملي مول (٢.٤٠ مللجرام /جرام) كأعلي متوسط واكل متوسط عند ١٥ كيلو راد مع صفر ملي مول (١.٦٥ مللجرام /جرام).

3b-محتوي الأوراق من الكاثرانثين

في الجيل الطفوري الاول وجدت فروق معنوية بين المعاملات في محتوى الاوراق من الكاثرانثين (مللجرام/جرام) نتيجة لمعاملات اشعة جاما والملوحة والمشاركة ، وكان اعلي تأثير لاشعة جاما عند ١٠ كيلو راد (٠.٤١٣ مللجرام/جرام) واكل تأثير عند ١٥ كيلو راد (٠.١٠٤ مللجرام/جرام) ، و اعلي تأثير للملوحة عند صفر ملي مول (كنترول) (٠.٣٧٢ مللجرام/جرام) والاكل عند ١٠٠ ملي مول (٠.٠٧٩ مللجرام/جرام) و تأثير مشترك بين الملوحة والاشعة عند ١٠ كيلو راد مع ١٥٠ ملي مول (٠.٨٩٠ مللجرام/جرام) واكل تأثير عند ٢٠ كيلو راد مع ١٠٠ ملي مول (٠.٠٠٢ مللجرام/جرام) . وفي الجيل الطفوري الثاني لم يكن هناك فروق معنوية بين المعاملات في محتوى الاوراق من الكاثرانثين بالنسبة لاشعة جاما ، لكن تأثير الملوحة كان معنوي عند ١٠٠ ملي مول (٠.١٧٧ مللجرام/جرام) و زاد التأثير المشترك بين الملوحة والاشعة معنوياً عند صفر كيلو راد مع ١٠٠ ملي مول (٠.٣١٦ مللجرام/جرام) كأعلي متوسط بينما كان اقل متوسط عند ١٠ كيلو راد مع ١٥٠ ملي مول (٠.٠٨٠ مللجرام/جرام).

٤- محتوى الاوراق من الكربوهيدرات الكلية

لم يكن هناك فروق معنوية بين المعاملات لمحتوي الاوراق الكربوهيدرات الكلية (%) في الجيل الطفوري الاول . أما في الجيل الطفوري الثاني فقد كان هناك فروق معنوية بين المعاملات لمحتوي الاوراق الكربوهيدرات الكلية بالنسبة لاشعة جاما و تأثير الملوحة ، وكان اعلي تاثير لاشعة جاما عند ١٥ كيلو راد (٨.٩٦ %) و اقل تاثير عند ٥ كيلو راد (٦.٥٠ %) ، كما كان اعلي تاثير للملوحة عند ١٠٠ ملي مول (٨.٥٠ %) . أما عن التأثير المشترك بين الملوحة والاشعة لم يكن هنالك فروق معنوية بين المعاملات .

٥- إنتاج الطفرات

تم الحصول علي ١٢ نبات مُطَفَّر مقارنة بالنبات الام (المقارنة control) منها ٣ نباتات ذات ازهار مختلفة ونبات ذو تفريع مختلف وثمانية نباتات تتحمل الملوحة وهي

- T_0 = المعاملة صفر كيلو راد + صفر ملي مول (كنترول).
 T_1 = المعاملة ٥ كيلو راد + صفر ملي مول (طفرة ملمس البتلات).
 T_2 = المعاملة ١٠ كيلو راد + صفر ملي مول (طفرة شكل الزهرة).
 T_3 = المعاملة ١٥ كيلو راد + صفر ملي مول (طفرة لون الزهرة).
 T_4 = المعاملة ٢٠ كيلو راد + صفر ملي مول (طفرة طريقة تفريع).
 T_5 = المعاملة ٠ كيلو راد + ١٠٠ ملي مول (طفرة تَحْمَل للملوحة).
 T_6 = المعاملة ٥ كيلو راد + ١٠٠ ملي مول (طفرة تَحْمَل للملوحة).
 T_7 = المعاملة ١٠ كيلو راد + ١٠٠ ملي مول (طفرة تَحْمَل للملوحة).
 T_8 = المعاملة ١٥ كيلو راد + ١٠٠ ملي مول (طفرة تَحْمَل للملوحة).
 T_9 = المعاملة ٠ كيلو راد + ١٥٠ ملي مول (طفرة تَحْمَل للملوحة).
 T_{10} = المعاملة ٥ كيلو راد + ١٥٠ ملي مول (طفرة تَحْمَل للملوحة).
 T_{11} = المعاملة ١٠ كيلو راد + ١٥٠ ملي مول (طفرة تَحْمَل للملوحة).
 T_{12} = المعاملة ١٥ كيلو راد + ١٥٠ ملي مول (طفرة تَحْمَل للملوحة).

٦- إنزيم البيروكسيداز

أظهرت نتائج دراسة المشابهات الإنزيمية لإنزيم البيروكسيداز تحكم سبعة مواقع وراثية في إنتاج إنزيم البيروكسيداز في نبات الونكا . ظهرت خمسة مواقع سالبة متجهة نحو القطب الموجب (الكاثود) وموقعين موجبين متجهين نحو القطب السالب (الانود) . وقد تم التمييز بين الطفرات ومعاملة المقارنة بإستخدام إختبار تحليل المشابهات الإنزيمية لإنزيم البيروكسيداز . كما تم دراسة قيم التشابه بين نباتات المقارنة ونباتات الطفرات الناتجة من المعاملات. وقد امكن وضع المقارنة والطفرات الناتجة في ثلاث مجموعات شملت الاولي منها المقارنة وأربعة طفرات T_4 و T_3 و T_2 و T_1 و T_0 والثانية اربع طفرات (T_8 و T_7 و T_6 و T_5) والثالثة أربع طفرات (T_{12} و T_{11} و T_{10} و T_9).

