The Use of Genetic Variation Induced by Gamma Radiation for Genetic Improvement of *Mirabilis jalapa* L. Plants against Salinity

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ABSTRACT

This study was carried out throughput two successive seasons during the period 2008 - 2009 in the experimental farm of the faculty of Agriculture, Saba Basha, Alexandria University. Seeds of *Mirabilis Jalapa* L were exposed to four different doses of gamma rays (0.5, 1.0, 1.5 and 2.0 Kr) from Co-60 in Gamma–Cell in addition to treatment on March 2008. After germination, the transplants of 30 days old were transferred into plastic pots 25cm in diameter and 30 cm in height and watered with different salted solutions at consternations of 0.0,50,100,150 and 200mM NaCl. The effect of gamma radiation and salinity, as well as, their combinations on plant parameters; like plant height (cm), , total chlorophyll content of leaves (SPAD units), internodes length (cm), stem diameter (cm), number of branches, leaf area per plant (cm^2), leaf petiole length, root fresh weight (gm) were determined. In addition, Peroxidase isozyme patterns were detected in leaves of *Mirabilis jalpa* grown under experiment conditions .The obtained results showed that the highest dose of gamma radiation (2.0Kr) has affected significantly and adversely in most studied characteristics .Meanwhile, the other doses had no significant effect compared to control treatment. All the biometric parameters experienced gradual decrease with increasing salinity levels during the first and second seasons. On the other hand, the interaction of the first season interaction was not significant at all characters except the root fresh weight(g) and leaf area (cm^2),while during the second season all characters were significantly affected . At 1.0 Kr, plant height, total chlorophyll content, internodes ' length, stem diameter , number of branches and leaf petiole length were increased. In conclusion, *Mirabilis jalpa* plants could be improved *via* gamma radiation at the range from 0.5 to 1.5 Kr. All the biometric parameters experienced gradual decrease with increasing salinity levels. The interaction between salinity levels and gamma radiation led to increase in the mean value of the plant height at (1.0Kr +50mM), total chlorophyll content (1.0Kr+100mM), internodes length at (1.5Kr+100mM), number of branches at (1.0Kr+100mM) and leaf area per plant at (0.5Kr+100mM).Isozyme banding pattern of peroxidase displayed more activity during the second season at 2.0Kr combined with 50mM NaCl.

__Additional index words__: *Mirabilis Jalapa* L, gamma radiation, salinity, genetic improvement, morphological characters, peroxidase isozyme banding pattern .

1. INTRODUCTION

The genus *Mirabilis* (family: Nyctaginaceae), has several common names, like Clavillia, four O'clock, Jalap, Maravilla (Hutchinson, 1969). The species of the present study, *Mirabilis jalapa,* is a perennial herb that reaches 50-100 cm height from a tuberous root. It produces beautiful flowers that usually open around 4 O'clock afternoon, hence its common name was derived from(Bailey, 1941; Heath and Manukian, 1994). In addition, it is a popular ornamental plant, grown worldwide for the beauty of its flowers (which can be white, red, pink, purple, or multicolored) and their sweet fragrance. The full morphological description of the plant was given by Bailey (1941). There are dwarf and compact varieties; also forms with variegated foliage. Self pollination is common (Cruder, 1973; Del -Rio and Burquez, 1986).

Also,the plant considers as a food source and in preparing human foods (Manandhar,2002), in decoction and traditional medicine (Walker *et al.*, 2008; Bolognesi ,2008). It can be treated as a tender annual, thriving in any garden soil (Bailey, 1941), often persisting even after the garden has been abandoned, and can be used in beds, and could be planted near the front door (Manandhar ,2002). However, salinity is one of the major environmental stresses affecting plant productivity, growth and yield of most crops including *Mirabilis.* However, the combination use of mutagenesis and tissue culture can, greatly, facilitate the selection and isolation of useful salt tolerant lines. Nevertheless, attempts to improve the salt tolerance of *Mirabilis jalapa* through conventional breeding programmers have met with very limited success, due to the complexity of the trait of salt tolerance ,whereas ,it is a complex genetically and physiologically (Khaing *et al.*,2007).

Mutagenic agents have been used to induce useful phenotypic variation in plants for more than 70 years (Foster and well,1996). However, the physical mutagens like ionizing radiations (X- rays, gamma rays and neutrons) and UV light , in addition to a series of chemical agents are common mutagenesis agents that have a high efficiency to generate mutations in plants, animals and bacteria. Nevertheless, gamma rays play a great role in inducing genetic changes in DNA in different crop plants. It has, successfully, used in several crop species to induce variation, in quantity and qualitative traits. Mutation techniques using ionizing radiations and other mutagens have, successfully, produced and commercialized a large number of new promising varieties in different crops including ornamental plants worldwide (Datta,2007).This work was conducted to (1)select a new strain of the plants, *Mirabilis jalapa* , able to resist high salinity , (2) select some new mutant strains with the most economically acceptable phenotypes, (3) compare the genetic characteristics of the new mutants with the original plant using peroxidase enzyme.

2. MATERIALS AND METHODS

The present study was conducted at the experimental farm of the faculty of Agriculture, Saba Basha, Alexandria University, during the period from 2008 to 2009. A local cultivar of four o'clock-flower *(Mirabilis jalapa* L.*)* was used in the present study, with crimson flowers and self pollination, as obtained from the Flowers and Ornamental Plants Research Gardens of the Faculty of Agriculture, El Shatby , University of Alexandria.

The seeds of the plant prepared for gamma rays treatments was divided into five equal portions; the first portion for the control, while the other four portions of seeds were exposed to four different doses of gamma rays as 0.5, 1.0, 1.5 and 2.0 Kr from Co-60 in Gamma–cell in the Irradiation laboratory at Middle East Regional Radio-Isotope Center for Arab countries at El-Dokky, Cairo, Egypt.

The treated seeds with gamma –rays were germinated in translucent plastic bags filled with sandy soil (Page,1982) (Table1) and peatmoss medium (2/1,v/v).After 30 days, when seedlings reached approximately 5cm height, one seedling was transplanted into plastic pot of 25 cm in diameter and 30 cm height, filled with the same medium. The measurements of morphological parameters were carried out on the produced plants. The seeds produced by the plants of the first season were collected, germinated and transplanted in mid of March 2009. The plants morphological parameters of the second season were measured. The seedlings were irrigated by four levels of salinity [50, 100, 150 and 200 mM NaCl], in addition to the control [0.0 mM]. The salinity levels were obtained by addition of appropriate amount of dry NaCl to water to be equivalent to an identified electrical conductivity using a portable EC meter (Table 2).Morphological measurements were determined for three replicates of the cultivated plants for two successive seasons, including plant height (cm), total chlorophyll content of leaves (SPAD unites) according to Yadava (1986), internodes length (cm), stem diameter (cm), number of branches, leaf area per plant (cm^2), according to Zidan (1962), root fresh weight (gm). In addition, the activity of peroxidase isozymes banding pattern was measured under the effect of gamma rays and salinity. Peroxidase isozyme banding patterns were detected in leaves of *Mirabilis jalpa* grown in saline condition treated with doses of gamma radiation at 0.0, 0.5, 1.0, 1.5, 2.0 Kr., using Total Lab program (version 2.2). The Tris-citrate buffer (0.23 M Tris- Citrate, pH 8.0) was prepared, and the solution was adjusted to pH 8.0. (Sabrah, 1980). Agar- Starch- Polyvinyl pyrolidine (PVP) gel was cooked (Sabrah and El- Metainy, 1985). Approximately 0.5g of plant tissue (leaves) was ground with purified sea sand in cold mortar and pestle to which 0.5-1.0 ml of the running buffer was added with continued grinding until the tissue was well macerated and the mixture was homogenous. The homogenate was absorbed into small stripes of filter paper and the wet filter papers were then, placed on the agar gel plates for 30 min, at 4 °C. The filter papers were removed and a constant current of 13-14 V/ cm was applied for 90 min, at 4°C using running buffer as electrode buffer. The plates were stained with peroxidase staining solution (Palanichamy and Siddig, 1977). Staining solution was prepared using 100 ml of 0.01 ml sodium acetate –acetic acid buffer (pH5.0), containing 0.1 gm Benzidine and 0.5 ml 5% hydrogen peroxide (H_2O_2) to be used as staining solution of peroxidase isozymes.

Table (1): Chemical and physical properties of the studied soil medium under investigation during 2008 and 2009 germinations.

Parameters	value
pH	7.8
E.C. (1.5) (ds/m)	1.2
O.M. %	0.35
Available N %	0.82
Available P %	9.30
Available K %	8.76
Soluble cations (mg/L)	1.5
Ca^{++}	4.2
Mg^{++}	5.1
Na^{++}	4.9
K^{+}	
Soluble anions (mg/l)	
HCO_3^-	1.6
Cl^-	4.2
Soil texture	Silty clay loam

Table (2): Salt levels of irrigation water and its counterparts of electric conductivity

Sodium chloride levels(mM)	0	50	100	150	200
EC (dSm^{-1})	0.55	4.3	8.2	12.3	17.5

Experimental design

Complete randomized design was followed in all experiments. All the experiments were of factorial type Data Collected and statistically analyzed according to Gomez and Gomez (1984) using SAS (Statistical Analysis System) computer program. Least significant different (LSD) was used to determine the significant difference between means . Probability levels Probability levels of 1 and 5 present were taken ,generally ,for this respect .The significance levels were represented as follows : probability at 1% =**, 5% =* and not significant=NS.

3. RESULTS AND DISCUSSIONS

Biometric measurements of the studied plant demonstrated pronounced variations under the effect of gamma radiation and salinity during the two seasons.

A- Plant height (cm.)

Results outlined in Table (3) revealed that during the first season, the plant height was affected significantly by both main factors under the study, but the interactions between them was not so. The given trait showed gradual significant decrease with increasing salinity levels, reaching the minimum mean value at the maximum level (200mM) compared to the control treatment (Fig. 1). Similar results were obtained for the strawberry plants (Ebida, 1988). This reduction could be attributed to the toxic effects of Na^+ and Cl^- in the physiologically active parts of tissues, and to inefficient compartmentation for these ions in vacuoles (Munns (1993; 2002). On the other hand, the gamma radiation had significant but a limited effect on the plant height. These results seem to agree with those reported by Sareen and Koul (1994) and Chowdhury and Sharma (1985). The combined effect of salinity and gamma radiation did not exert significant effect on the given trait.

Fig (1): Arrow indicates to the change in the growth habit reduction (right) in the first season of *Mirabilis jalapa*, L. plants (after cut-back) as a result of the treatments of salinity at 200 mM and 1.5 Kr compared with the control(left).

Results listed in Table (4) disclosed that, in the second season; the plant height was affected significantly by both factors and their interactions were not. Such trend was similar to that reported during the first season, where the given trait was decreased with increasing salinity levels. On the other side, the given trait showed significant increase with increasing gamma radiation doses and reached the maximum height at 1.0Kr.These results were similar to those mentioned by Dilta *et al.* (2003) for *Chrysanthemum morifolicm*. The interaction between salinity and gamma radiation exerted highly significant effects on the given trait, where significant decrease in the plant height occurred, particularly at both 1.5 and 2.0 Kr with 200mM. Flowers (1977), Greenway and Munns (1980) and Maas (1983) found that the growth of cells is primarily correlated with turgor potential, in which decreasing the turgor is the major cause of inhibition of plant growth under saline conditions. The absence of variation in the plant height in the second season could be due to the adaptation of the plants produced from the seeds of the first season, as it adapted to salinity variation, or due to the incidence of epigenetic variation (temporary variation) in the first season, which diminished in the second season. The recovery of changes induced by high levels of salinity is limited by somatic effects, such as reduced growth in the first season only. These results are in agreement with Rhoades and Loveday (1990); Munns (1993; 2002). The adapted (survived) plants were nearly similar in their heights which resulted in narrow ranges, not enlarged variances and low coefficient of variation values, compared with the control treatment. Consequently, the obtained results showed that gamma rays doses decreased the height of different plants. However, one of the main effects of ionizing radiation is the suppression of cell division activity, which is responsible partially for the reduction of vegetative growth. Low and intermediate of used gamma rays doses stimulated the plant height (Kothekar 1989 a; Arnold *et al.*, 1998; Badr *et al.*, 2000). On the other hand, Gorden (1958) has emphasized its negative changes in amount of endogenous auxin occurring, as a possible factor responsible for the decrease of growth, whereas the reduction in plant height is known to be caused by the reduction in the endogenous level of IAA auxin due to its biosynthesis disrupting by gamma radiation (Chandorkar and Dengler, 1987). The results of the present study are not in agreement with those for *Vigna aconitifolia*as (Ahloowalia, 1992), and for *Chrysanthemum* (Banerje and Datta, 2002; Dilta *et al.*, 2003). But Omar (1996) did not found significant differences among the different gamma radiation treatment on plant height of *Gomphorena glbosa*

B-Stem diameter

Results of Table (3) disclosed that during the first season, the stem diameter was affected significantly by both studied factors, but the interaction between them was not so .However, the given trait demonstrated gradual decrease with increase salinity levels, reaching its minimum level at 200mM. For gamma radiation doses, the stem diameter showed irregular decrease, but it reached the minimum at the highest dose (2.0Kr). The previous result may be attributed to decrease in the cell number and /or cell size (Gorden, 1958). Results outlined in Table (4) reveal that stem diameter of *Mirabilis jalapa*, L. in the second season was affected significantly by both studied factors and their interactions. The given trait was affected adversely and significantly by salinity levels, gamma radiation and their interactions. However, the reduction in stem diameter may be attributed to the effects of both variables and their interactions on the syntheses of growth regulators such as gibberellins, cytokinins and abscisic acid, which play important role in controlling cambial activity (Chandorkar and Dengler, 1987). Macroscopically colour changes of some plant stems were observed (Fig 2).

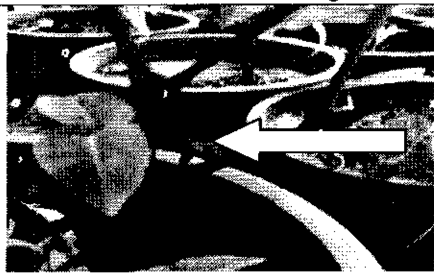

Fig (2): The arrow indicates to the stem change in second season of *Mirabilis jalapa* , L. as a result of the treatment with 1.0 Kr+200mM.

Moreover, one of the main effects of ionizing radiation is the suppression of cell division activity, which responsible partially for the reduction of variation has been suggested by many workers (Gunckel 1957; Gorden; 1958 Pelc and Haward 1995) .They have emphasized the changes in amount of auxin occurring as a result of radiation, as a possible factor responsible for the decrease of growth. On the other extreme, it is known that salinity creates a specific problem of ion toxicity, whereas the high concentration of sodium is not favorable for the cells.

C- Internode lengths (cm).

Results listed in Table (3) showed that during the first season, that the internode length of before cut –back was affected significantly by both factors ,but their interaction was not so. However, there is an inverse relationship between the applied salinity levels and the given trait. Whereas, the minimum level of salinity coincided with the highest mean value of the given trait, while the highest salinity (200mM) led to the shortest internode. This finding could be attributed to the osmoregulatory role of Na^+ as suggested by Huq and Larher (1983). This result was in conformity with those reported by Valdez *et al.*(2009) who reported that salinity tended to reduce internode elongation. The increase of gamma radiation doses to 1.0Kr led to the highest mean value of given characters .On the other hand, the internode showed gradual decrease with increasing gamma radiation doses.

During the second season, the internode length of *Mirabilis jalapa*, L. was affected significantly by gamma radiation doses and salinity levels and their interactions. (Table, 4). Nevertheless, the effects of both studied factors and combinations on the given trait of plants were, more or less, similar to those exhibited during the first season. Also, such significant increase occurred at 1.5 Kr gamma radiation compared to the control treatment. In general, these reductions in the plant height, internode length and stem diameter may be due to the physiological damage produced by gamma radiation doses and its hydrolysis products.

D- Number of main branches

Results outlined in Table (3) showed that in the first season, the number of the branches was affected significantly by gamma rays and salinity but their interactions was not so. It is noticeable the gradual decrease of the mean values with increasing salinity levels. This reduction in the number of the branches may be due to the effect of salinity on the endogenous hormones (gibberellins, cytokinins and auxins) which play important roles on the growth assembly of the plant. Meanwhile, tested gamma rays showed no significant difference in their effect up to1.5 Kr but the highest dose significantly reduced the number of branches as compared with the other ones. This finding could be due to releasing sufficient supply of endogenous auxin and cytokinins which increased the activity of cambial cells and consequently the branches production, then suppressed at the highest dose.

Results of the second season (Table, 4) demonstrated that the number of branches of Mirabilis *jalapa*, L. was affected by gamma radiation doses (Kr) and salinity levels (mM) and their interaction. As salinity levels increased, the mean number of branches significantly decreased significantly .On the other hand, similar performance was observed for the effect of gamma ray doses on the given trait except that of 1.5 Kr.. These performances could be taken place due to the negative effects of both factors on biosynthesies of plant phytohormones of the given trait especially at the elevated levels and doses. Dilta *et al.* (2003) noticed that gamma rays at 2.0 kr decreased the number of branches of *Chrysanthemum* .On the other hand, gamma radiation doses at 0.5 Kr showed the highest mean value of number of branches of *Chrysanthemum* plants. However, the interaction effect, as the combinations between the highest levels and doses of salinity and gamma rays, each in turn, decreased the given trait.Gorden (1958) indicated that the change in the amount of auxin occurring as a result of radiation, as a possible factor responsible for the decrease of growth .So, this reduction may be attributed to the effect of radiation on growth regulators such as gibberellins, cytokinins and abscisic acid which play important roles in controlling cambial activity; hence, radiation effect resulted in a reduction of these growth regulators which reduced the mitotic effect of the cambial cells, consequently suppressed the branching as reported by Chandorkar and

Dengler (1987); El-Mahrouuk (2000), reported that; gamma rays increased the mitotic activity of cambial cells which led to branches production . Gamma rays suppressed gibberellins, cytokininis and abscisic acid which may played important roles in decrease branches production (Chandokar and Dengler, 1987; El- Mahrouk, 2000). Gamma rays increased nutrients which were not utilized in the stem elongation, but utilized in branches induction. (Chandrokar and Dengler, 1986).

E- leaf area

Results outlined in Table (3) indicated that leaf area of *Mirabilis jalapa,* L. was affected by both factors under the study and their combinations. However, during the first season, the leaf area of plants experienced clear and significant decreases with increasing salinity levels. The inverse relationship between the applied salinity levels and the given trait could be attributed to the severe water stress conditions caused by salinity which decreased the plant growth and accumulate solutes in cells in order to maintain the cell volume against dehydration as shown in grapevines (Patakas *et al.*, 2002). Likewise, the decreased rate of leaf growth with increasing salinity is primary due to reduction in water potential in the root zone which reduced the transmit it *via* the xylem to the leaves , causing leaf cells to lose water and reduced its elongation rates (Fricke and Peters,2002). On the other hand, the gamma ray doses caused significant differences among the means values of the leaf area, whereas the highest mean value was obtained upon using 1.0 Kr, meanwhile the lowest one was recorded at 2.0 Kr. This may be related to the effect of ionizing radiation on cell cycle and expansion. Approximately similar results were obtained by Dilta *et al.* (2003) for *Chrysanthemum morifolicm*; which experienced decrease in leaf number and size at 2.0 kr. The present study revealed that the combined effect of both salinity levels and gamma radiation doses led to increase in the leaf area at 0.5, 1.0 and 1.5 Kr at both 50 and 100 mM of salinity levels, but as both factors increased, the given trait decreased.Results of Table (4) indicated that leaf area of *Mirabilis jalapa,* was affected significantly by both factors and their interactions regarding the plant and showed a similar trend of variation with salinity levels and gamma radiation individually, in addition to the combined effect of both factors similar to those observed during the first season. There was a clear inverse relationship between salinity levels and the given trait. Also, such obvious significant differences were detected among the mean values of the given trait and gamma radiation doses, where the greatest leaf area was observed at 0.5 Kr but the lowest mean value was at 2.0 Kr. However, the interaction between 50 mM salinity and 0.5 Kr resulted in the highest mean value of the given trait, while the smallest leaf area was observed at the highest salinity and gamma dose (2.0kr and 200mM).

F- Root fresh weight

Result of Table (3) pronounced that the root fresh weight of *Mirabilis jalapa,* L. plants was affected by both factors under the study and their interactions during the first season. An inverse relationship was observed between salinity levels and root fresh weight, which decreased gradually with increasing salinity. This reduction may be attributed to suppression of plant growth under salinity stress during developmental growth stages. Caparadeh (2003) found that the high salinity levels caused a great reduction of plant growth parameters like the roots and shoots fresh and dry weights. On the other side, the given character showed significant increase at 0.5 Kr compared to the other doses. The highest dose (2.0Kr), produced the lowest mean value and significantly differed with the other doses. This inhibitory effect may led to reducing mitotic activity in meristemic tissues (Abdul Majeed et al.,2010).The interaction between salinity levels and gamma radiation doses caused significant decrease in root fresh weight, particularly at 2.0 Kr with all experienced salinity levels, while the root fresh weight increased by different ratios under all salinity levels of 50 and 100mM at 0.5,1.0 and 1.5 Kr. The root fresh weight of *Mirabilis jalapa,* L. plants was affected significantly by both salinity and gamma radiation under the study and their interaction during the second season (Table 4). The given trait decreased significantly with increasing salinity level to the minimum at 200 mM. High salt concentrations inhibited enzymes by impeding the balance of forces controlling the protein structure (Serrano *et al.*, 1999). On the other extreme, the gamma radiation doses increased the root fresh weight significantly at 0.5, 1.0 and 1.5 Kr; while it decreased it at high dose (2.0 Kr). The combined effect of both salinity and gamma radiation at 50 and 100mM and 0.5 Kr caused a significant increase of the given trait, meanwhile at higher levels of both factors resulted reduction in the given trait.

G- Leaf petiole length

Results of Table (3) disclosed that leaf petiole length of *Mirabilis jalapa*, L. was affected significantly only by salinity levels, but neither gamma ray doses nor their interactions with salinity levels affected it during the first season. There was an inverse relationship between salinity levels and the given trait. This finding could be attributed to the adverse effects of salinity on the mode of action of growth regulators such as gibberellins and auxins, which play important roles in cell elongation; hence, salinity resulted in a reduction in these growth regulators which caused reduction of cell elongation and consequently suppressed the petiole length(Fricke and.Peters, 2002). As shown in table (4), leaf petiole length of *Mirabilis jalapa* , L. was affected by both factors and their interactions during the second season of the experiment. The leaf petiole length of plants experienced gradual and significant decrease with increasing salinity levels. Meanwhile, with gamma radiation doses, the leaf petiole length significantly exhibited significant effect on the trait, especially at 1.5 Kr, where the highest mean value was recorded; and significant reduction was reported at various used doses. Also, the interaction between both factors showed highly significant effect on the given trait. It is noticeable that the shock effect of the high salinity (200mM) during the first season led to lose of large amount of water of the plants through an osmoregulation process. It was reported that the decrease in the root fresh weight may be due to osmotic stress under saline condition which caused a rapid and potentially lasting reduction in rate of growth (Munns, 1993). From the biophysical point of view, Cosgroves (1993) ascertained that cell of a NaCl –treated plant can expand at reduced rates because of reduced uptake rates of water or osmolytes, because of hardened walls, or because of lowered turgor.

H- The total leaf chlorophyll content

Results of Table (3) disclosed that the total leaf chlorophyll content of *Mirabilis jalapa* L. during the first season was affected significantly by both factors under the study, but their interaction was not so. Salinity levels affected adversely the given trait, especially at the higher levels (150 and 200mM) compared to the other treatments (i.e. 0.0, 50 and 100 mM). Such finding may be attributed to decrease of photosynthetic rate because of salt osmotic and toxic ionic stress. This is in agreement with Rai (1990) and Rai and Abraham (1993) who found that chlorophyll is the primary target to salt toxicity limiting net assimilation rate, resulting in reduced photosynthesis and reduced growth. The present study showed that gamma radiation doses exhibited an adverse effect on the given trait especially at the highest dose (2.0Kr), while doses of 0.5 up to 1.5 Kr enhanced the given trait without significant differences from the control, followed by obvious significant decrease at the highest dose (2.0 Kr). This could be due to enhancement of the photosynthetic rate by the gamma ray at the given doses (0.5 -1.5Kr), and the inhibiting effect at the highest dose (2.0 Kr).

During the second season, the total leaf chlorophyll content of *Mirabilis jalapa* L. was affected by both factors under the study and their interaction (Table, 4), similar to those observed in the first season. The alteration in chlorophyll synthesis of irradiated leaves may be due to effect of the auxin synthesis (Hagen and Gunckel, 1958; Kuzin, 1956) and the primary effect of radiation occurs on the development of merisematic cells and on auxin supply (Giacomellis, 1967). In the meantime, radiation may affect chloroplasts and consequently affect the chlorophyll syntheses (Evens, 1984). On the other side, increasing salinity levels caused significant decrease in the chlorophyll content of leaves (Huany, 1987). This supports our findings which indicate decrease of chlorophyll content with high salinity in the first and second seasons. Furthermore, the chlorophyll content expressed on leaf area basis increased under conditions of salinity due to a reduction in leaf tissue water content (Jacobsen *et al*,.2001), while increasing EC reduced leaf and turned yellowing in *Tagetes erecta* (Huany,1987), and rice chlorophyll content was decreased in proportion to increase salt concentration (Alam *et al*.,2001).

Biochemical parameters

<u>Peroxidase Isozyme banding patterns **of *Mirabilis jalapa*** , L.</u>

1 (0and 2.0Kr)	2 (50mM and 2.0Kr)	3 (100mM and 2.0Kr)
4 (0mM and 1.5Kr)	5 (50mM and 1.5Kr)	6 (100mM and 1.5Kr)
7 (0mM and 1.0Kr)	8 (50mM and 1.0Kr)	9 (100mM and 1.0Kr)
10 (0mM and 0.5Kr)	11 (50mM and 0.5Kr)	12 (100mM and 0.5Kr)
13 (0 mMand 0Kr)	14 (50mMand 0Kr)	15 (100mM and 0Kr)

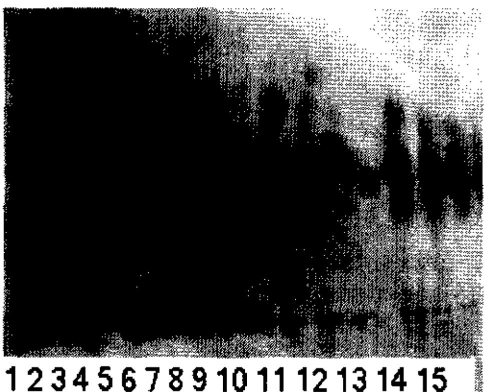

Fig:(3)_Activity of Isozyme banding Patterns of Peroxidase

As shown in figure (3) the activity of peroxidase could be indicated from the density of the bands, which reveal that the maximum activity of peroxidase was reported at 50 mM and 2.0 Kr compared to other gamma doses and salinity levels. Chen *et al.*, (2003) found that under salt stress, peroxidase activity maintained high levels. Enzymes are nuclear encoded and the enzyme is present in all aerobic organisms and in all subcellular compartments susceptible of oxidative stress. Zahed *et al.* (2006) found that on *Chrysanthemum morifolium*, enhanced salt tolerance of the R1 mutant was attributed to increased activities of reactive oxygen species (ROS)scavenging enzymes, namely superoxide dismutase (SOD). The RI mutant developed by gamma ray treatment can be considered a salt-erant mutant showing all the positive characteristics of tolerance to NaCl stress Zahed *et al.* (2004) mentioned that all the positive responses towards biochemical change under stress condition indicate possibilities towards inducing salt tolerance through *in vitro* adaptation of whole plant. Plants posses very efficient defense systems that allow their detoxification and protect plant cells from oxidative damage .Among these defense system,the enzyme piroxidase (Dat *et al* .,2000)

<u>Genetic relationship among samples based on Peroxidase isozymes patterns</u>

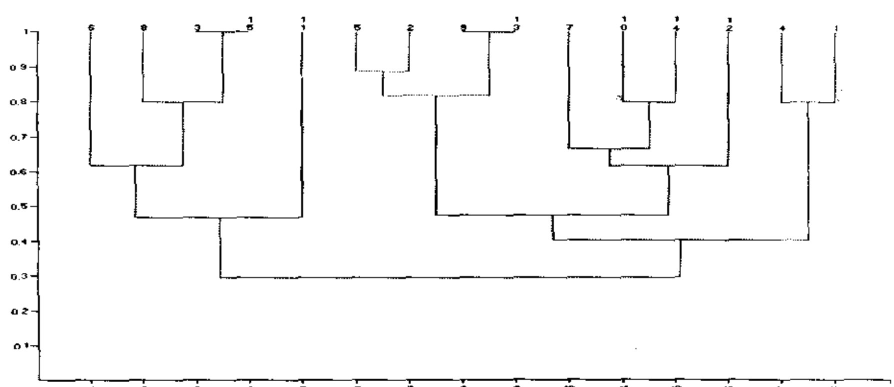

Fig (4): Genetic relationship among *Mirabilis jalapa* , L. samples based on Peroxidase isozymes patterns :

The cluster analyses of different treatment demonstrated that two mean groups the small group compress five treatments with different degrees of similarity, the highest of which was between treatment (3) and (15) ,while the second group is divided into three sup cluster with the highest degree of similarity between treatment (9) and (13).Other similarity were also observed between treatment (5) and (2) by (90%),treatment (4) and (1) by (80%)and treatment (10) and (14)by (80%).Meanwhile , all the treated samples were different in the values of genetic similarity which was due to exposure to different doses of gamma radiation doses . Also, the different levels of salinity Unexpectically, all the treatment samples were different in values of genetic variation which may be due to exposure to different doses of gamma radiation. Also, the different levels of salinity (Fig .4).

Table(3): Mean values of vegetative parameters of *Mirabilis jalapa* , L. as affected by gamma radiation doses (Kr) and salinity levels(mM) during the first season .

Char.	Gam Rays (Kr)	Salinity levels(mM)					Mean Gam.	Significance
		0	50	100	150	200		Gam. xSal.
Plant Height (cm)/plant								
	0.0	23.23	21.93	20.67	16.88	11.3	18.80a	NS
	0.5	22.17	20.53	19.52	19.50	11.1	18.56a	
	1.0	24.93	21.91	22.03	14.83	10.96	18.93a	
	1.5	24.03	22.80	22.26	13.03	11.00	18.63a	
	2.0	21.76	20.63	19.23	11.73	10.00	16.67b	
Mean Sal.		23.23a	21.56b	20.7b	15.19c	10.86d		
L.S.D(50.0)								---- ---
Stem diam(cm)/plant								
	0.0	0.923	0.847	0.794	0.488	0.398	0.690a	NS
	0.5	0.796	0.747	0.809	0.581	0.547	0.696a	
	1.0	0.817	0.800	0.721	0.552	0.423	0.660a	
	1.5	0.795	0.821	0.787	0.515	0.430	0.669a	
	2.0	0.430	0.695	0.624	0.4363	0.343	0.505b	
Mean Sal.		0.752a	0.782ab	0.74b	0.514c	0.429d		
L.S.D(50.0)								--------
Internode length								
	0.0	2.606	2.433	2.303	1.703	1.43	2.09 a	NS
	0.5	2.416	2.343	2.156	1.266	1.140	1.864b	
	1.0	2.73	2.633	2.526	1.680	1.42	2.20a	
	1.5	2.406	2.296	2.163	1.513	1.213	1.91b	
	2.0	2.136	2.026	1.626	1.146	1.033	1.59c	
Mean Sal.		2.46a	2.34a	2.15b	1.46c	1.24d		
L.S.D(50.0)								-----------
Number of branches								
	0.0	7.03	6.16	5.65	3.46	2.45	4.95a	NS
	0.5	8.00	7.26	5.60	3.40	1.16	5.08a	
	1.0	7.30	6.60	4.50	4.03	1.31	4.74a	
	1.5	5.70	6.06	6.36	3..5	1.27	4..57a	
	2.0	4.93	4.43	3.20	1.17	1.05	2.95b	
Mean Sal.		6.59a	6.102a	5..06a	3.11b	1.44c		
L.S.D(50.0)								----------

To be cont..............

Cont...................

Leaf area								
	0.0	16.17	15.14	14.86	12.08	9.98	13.64 b	**
	0.5	14.47	15.96	15.15	11.32	10.07	13.39b	
	1.0	20.50	15.55	16.96	13.52	10.05	15.31a	
	1.5	16.57	16.31	15.13	11.10	9.21	13.66b	
	2.0	13.26	12.67	11.48	10.76	9.50	11.53c	
Mean Sal.		16.19a	15.12b	14.72b	11.76c	9.78d		
L.S.D(50.0)								0.9543
Root fresh weight(g)								
	0.0	61.17	50.07	42.24	31.34	21.70	41.30 c	**
	0.5	73.70	61.20	52.31	31.82	22.81	48.36a	
	1.0	61.29	53.25	61.31	33.70	20.05	45.93b	
	1.5	61.22	50.20	62.15	41.18	31.90	49.33ab	
	2.0	43.97	42.27	31.67	30.03	21,21	33.83d	
Mean Sal.		60.28a	51.39b	49.39b	33.61c	25.14d		
L.S.D(50.0)								1.321
Leaf petiole length								
	0.0	2.36	2.17	2.09	1.57	1.36	1.91a	NS
	0.5	2.52	2.26	1.93	1.60	1.27	1.91a	
	1.0	2.73	2.56	1.95	1.56	1.12	1.98a	
	1.5	2.52	2.58	2.39	1.51	1.29	2.05a	
	2.0	2.31	2.57	2.11	1.42	1.11	1.90a	
Mean Sal.		2.49a	2.43a	2.09b	1.53c	1.23d		
L.S.D(50.0)								
Total chlorophyll								
	0.0	23.51	23.07	22.67	17.43	16.30	20.60b	NS
	0.5	25.71	26.27	23.67	18.43	16.84	22.18ab	
	1.0	28.30	26.57	28.92	16.91	15.74	23.29a	
	1.5	26.70	25.74	26.08	17.01	13.42	21.79ab	
	2.0	23.19	22.12	21.44	16.36	10.99	18.82c	
Mean Sal.		25.48a	24.75a	24.55a	17.23b	14.66c		
L.S.D(50.0)								-------

Table(4): Mean values of vegetative parameters of *Mirabilis jalapa*, L. as affected by gamma radiation doses (Kr) and salinity levels(mM) during the second season .

Char.	Gam Rays (Kr)	Salinity levels(mM) 0	50	100	150	200	Mean Gam.	Significance Gam. xSal.
Plant Height (cm)/plant								
	0.0	23.1	21.5	20.3	18.2	11.7	18.9 c	**
	0.5	25.8	23.0	20.4	15.9	11.6	19.4 cb	
	1.0	28.9	25.2	21.3	21.5	17.3	22.8a	
	1.5	26.5	23.0	26.8	11.9	11.1	19.8b	
	2.0	23.8	23.1	19.6	12.6	10.9	17.9b	
Mean Sal.		25.6a	23.2b	21.6	16.6d	12.5e		
L.S.D(50.0)								1.3028
Stem diam(cm)/plant								
	0.0	0.927	0.858	0.851	0.453	0.327	0.683b	**
	0.5	0.743	0.745	0.895	0.662	0.431	0.695ab	
	1.0	0.823	0.874	0.724	0.575	0.429	0.685b	
	1.5	0.832	0.839	0.877	0.511	0.450	0.702a	
	2.0	0.773	0.770	0.768	0.460	0.336	0.621c	
Mean Sal.		0.820a	0.817a	0.823a	0.530b	0.390c		
L.S.D(50.0)								0.0369
Internode length								
	0.0	2.74	2.50	2.28	1.35	1.23	2.02b	**
	0.5	2.46	2.31	2.28	1.29	1.13	1.89c	
	1.0	2.79	1.68	2.50	1.55	1.25	1.96cb	
	1.5	2.82	2.39	2.86	1.96	1.45	2.24a	
	2.0	2.64	2.66	2.23	1.12	1.09	1.95cb	
Mean Sal.		2.69a	2.30c	2.43b	1. 46d	1.24e		
L.S.D(50.0)								0.218
Number of branches								
	0.0	6.16	5.76	4.53	4.63	4.80	5.01ab	**
	0.5	8.83	5.93	4.60	3.50	2.76	4.72 bc	
	1.0	6.00	4.86	4.40	3.96	3.00	4.44c	
	1.5	6.83	5.56	6.83	4.10	2.76	5.05a	
	2.0	5.46	5.10	4.36	2.16	1.80	3.77c	
Mean Sal.		6.49a	5.44b	4.94c	3.67d	2.86e		
L.S.D(50.0)								0.432

To be cont..............

Leaf area								
	0.0	16.37	14.09	11.03	10.13	10.55	12.47c	**
	0.5	18.6	19.57	19.35	11.63	12.69	16.37a	
	1.0	19.31	17.55	12.54	14.51	11.14	15.01b	
	1.5	17.43	19.27	18.58	11.30	8.83	15.08b	
	2.0	18.3	16.35	12.13	8.08	7.30	12.45c	
Mean Sal.		18.05 a	17.37 B	14.73 c	11.13 d	10.10 E		
L.S.D(50.0)								1.495
Root fresh weight(g)								
	0.0	63.42	54.26	31. 45	23.29	21.03	38.69c	**
	0.5	82.51	64.33	54.79	42.19	3132	55.06a	
	1.0	82.34	65.56	56.35	42.07	30.12	55.28a	
	1.5	54.92	71.32	81.18	41.64	40.33	57.87a	
	2.0	51.50	51.35	51.15	37.57	25.32	35.78b	
Mean Sal.		66.94a	61.36b	56.98c	37.35d	29.62e		
L.S.D(50.0)								2.531
Leaf petiole length								
	0.0	2.63	2.45	2.35	2.16	2.07	1.93c	**
	0.5	2.47	2.29	2.51	1.63	1.41	2.06b	
	1.0	2.79	2.66	1.71	1.35	1.08	1.92c	
	1.5	2.70	2.66	2.67	1.49	1.38	2.18a	
	2.0	2.26	2.58	2.12	1.28	1.08	1.87c	
Mean Sal.		2.57a	2.53a	2.27b	1.38c	1.20d		
L.S.D(50.0)								0.217
Total chlorophyll								
	0.0	23.26	23.31	22.27	17.40	16.40	20.64b	**
	0.5	26.26	26.40	23.76	18.38	16.94	22.35ab	
	1.0	28.52	26.73	29.24	16.99	15.77	22.54a	
	1.5	27.04	26.78	26.22	17.12	13.03	22.05ab	
	2.0	26.79	27.98	27.84	16.54	11.34	22.10ab	
Mean Sal.		26.3 a	26.2 a	26.0 a	17.29 b	14.7 c		
L.S.D(50.0)								2.231

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.
** = Highly significant at the 0.01 level of probability.
NS = Not Significant .

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الملخص العربي

استخدام التباين الوراثي المستحدث بأشعه حاما من اجل التحسين الوراثي لنبات شب الليل في مقاومه الملوحة

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**كلية الزراعة –الشاطبى– جامعة الإسكندرية

أجريت هذه الدراسة، في خلال موسمي الزراعة 2008/ 2009 بالمزرعة التجريبية لكلية الزراعة – سابا باشا – جامعة الإسكندرية حيث تم تعريض بذور نباتات شب الليل لأربع جرعات من أشعة حاما قدرها 0.5، 1.0، 1.5، 2.0 كيلو راد من وحده التشعيع Co-60 بالاضافه الى المعاملة الشاهد (الكنترول) وذلك في شهر مارس لعام 2008.بعد الإنبات تم نقل النبتات عند عمر 30 يوم إلى أصص بقطر 25 سم

وارتفاع 30 سم ثم رويت بمحاليل مائية مملحه بكلوريد الصوديوم بتركيزات [صفر (الكنترول) 50، 100، 150، 200 ملليمول]. ولقد تم تقدير تأثير كل من عاملي الدراسة: التأثير المنفرد لكل من مستويات الملوحة و أشعة حاما وكذلك تأثيرهما المشترك على الصفات النباتية مثل: ارتفاع النبات(سم) ، عدد الأوراق ، عدد الأفرع الرئيسة ، المحتوى الكلوروفيللى للأوراق،المساحة الورقية، قطر الساق، الوزن الرطب للجذور(جم)، طول عنق الورقة ، طول السلاميات ، والنسبة المئوية لحيوية حبوب اللقاح. هذا بالاضافه إلى اكتشاف نموذج المشابه لإنزيم البيروكسيديز فى أوراق النبات النامي تحت ظروف التجربة . أوضحت النتائج المتحصل عليها ان الجرعة الكبرى من اشعه جاما 2كيلو راد قد أثرت معنويا و بصورة سلبيه على معظم الصفات تحت الدراسة بينما لم يكن للجرعات الأخرى من أشعه جاما تأثير معنويا مقارنه بمعامله الشاهد (الكنترول). ولقد أوضحت الصفات المورفولوجيه نقصا تدريجيا مع زيادة مستويات الملوحة أثناء موسمي الزراعة. ومن ناحية أخرى لم يكن التفاعل التداخلى بين عنصري الدراسة في الموسم الأول تأثيرا معنويا على كل الصفات المدروسة ماعدا على كل من الوزن الطازج للجذور (جم) والمساحة الورقية (سم^2). بينما أثناء الموسم الثاني كانت على كل الصفات المدروسة قد تأثرت معنويا وعند جرعه أشعه حاما 1كيلو راد، زادت متوسطات طول النبات والمحتوى الكلوروفيلى و طول السلاميات و قطرا لساق و عدد الأفرع الرئيسة و كذلك طول عنق الورقة.

والخلاصة يمكن تحسين نباتات شب الليل باستخدام أشعه حاما عند مدى من 0.5 – 1.5 كيلو راد. وان كل الصفات المورفولوجيه المدروسة قد أوضحت نقصا تدريجيا مع زيادة مستويات الملوحة المستخدمة وكان التفاعل التداخلى بين عنصري الدراسة قد أدى إلى زيادة متوسط قيم طول النبات عند (50ملليمول مع 1.0كيلو راد) والمحتوى الكلوروفيللى عند (100ملليمول مع 1.0كيلو راد) وطول السلاميات عند(100ملليمول مع 1.5كيلو راد)و عدد الأفرع عند(100ملليمول مع 1.0كيلو راد) و المساحة الورقية عند(100ملليمول مع 0.5كيلو راد) وأوضح نموذج المشابه لإنزيم البيروكسيديز المزيد من النشاط أثناء الموسم الثاني من الدر اسه وبصفه خاصة عن (2كيلو راد مع 50ملليمول).