INDUCTION OF MUTANT BY GAMMA RAY IN *CALENDULA OFFICINALIS* PLANTS IRRIGATED WITH SALINE WATER.

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ABSTRACT

This study was carried out through two successive generations (M1 and M2) during 2004 and 2005, in the greenhouse of the Faculty of Agriculture, Alexandria University. Seeds of Calendula were treated by gamma radiation at doses of 5, 10, 15 and 20 Kr. After germination, the plantlets were transferred into a sandy soil, and watered by different salt concentrations (0, 50, 100, 150 and 200 mM NaCl), in order to investigate the effect of gamma radiation and salinity on the plant, as well as their combined effect. The following parameters were studied for each plant: plant height, number of leaves, number of main branches, Chlorophyll content, leaves fresh and dry weights, inflorescence number, diameter, and fresh and dry weight, morphological changes, and activity of peroxidase enzyme in leaves. The results showed that at 5 Kr, the leaves fresh weight and the number, diameter and fresh weight of inflorescence were increased during the first generation, while the inflorescence diameter increased during the second generation at 10 Kr. Also, at 5 Kr and 50 mM NaCl inflorescence number, dry weight and diameter were increased during the first generation. Enzyme peroxidase displayed more activity during the second generation at 10 and 20 Kr combined with 50 mM NaCl.

INTRODUCTION

There are global constraints on fresh water supplies, and this has led to a surge of interest in reusing water (Shannon and Grieve, 1999). However, in many cases the value of water has decreased because the water is salty. Salt stress can be a major challenge to plants. It limits agriculture all over the world, particularly on irrigated farmlands (Rausch, 1996).

Calendula is a genus that belongs to the Family Composite with 15 species of herbs chiefly from the Mediterranean region, Canary island to Iran. In Egypt there are two species, the common cultivated species *C.officinalis* and the wild type species *C.micrantha*. The common cultivated plant is one of the most universal garden flowers running into many varieties, distinguished by size, colour, and degree of doubling the color varies from white-yellow to deep orange. The pot marigold is a favorite of children gardening for its showy flowers. Its pet als can be used fresh in salad, or dried to flavor rice. It has been researched for immune system activity and was initially determined to have some potential therapeutic activity against the human immuno deficiency virus (HIV).

Generally, the most likely effect of salinity on plants is stunted growth. Growth parameters such as plant height and number of branches per plant weredecreased with increasing soil salinity in *Tagetes erecta* (Reedy *et al*., 2001). Sharma and Vanda (1989) reported that relative water content and leaf water potential were declined as soil EC increased, but leaf diffusive resistance increased for both adaxial and abaxial leaf surfaces in Calendu!a, Chrysanthemums, Dimorphotheca, Petunia, Gomphrena and Zinnia plants. Also, Chaparzadeh, (2004) found that high salinity caused a great reduction in relative growth rate and leaf area ratio on *calendula officinals*. High salinity is known to cause both hyper ionic and hyper osmotic effects in plants, leading to membrane disorganization, increase in activated oxygen species production and metabolic toxicity imbalance between production and quenching of ROS (Oxygen Reactive Species) leads to plant damage. Antioxidant resistance mechanisms may provide a strategy to enhance salt tolerance (Charparzadeh *et al*., 2003).

Hence, developing salt-tolerant crops is essential for sustaining food production. Progress in breeding for salt-tolerant crops has been hampered by the lack of understanding of the molecular basis of salt tolerance and lack of availability of genes that confer salt tolerance, (Flowers, 2004).

Gamma irradiation has successfully been used in several crop species to induce variation in quantities and quantitative traits. The effect of gamma radiation on the expression of genes controlling protein sub fractions of electrophoresis spectra could enhance selection programs executed to screen and maintain those mutants that have: (1) avoidance of stress effects through earliness and (2) high biological stress tolerance, or (3) high potential yield (Stoeva, 2002).

Enhanced formation of ROS under stress conditions induces both protective responses and cellular damage inefficiency of ROS-detoxifying enzymes. Under salt stress, reactive oxygen species including superoxide, and hydrogen peroxide. Nakano *et al*. (1981) found that to remove reactive oxygen species, plant cells possess an antioxidant system. These include super oxide dismutase for scavenging the super oxide radicals. José et al., (2001) reported that NaCl-induced oxidative stress in the apoplasts might be related to the appearance of highly localized O_2/H_2O_2 and induced necrotic lesions in the minor veins in NaCl-treated pea plants. Under salinity, the isozymes of superoxide dismutase which is localized in the chloroplast are activated (Dhindsa and Matowe, 1981) . In *Calendula officinalis* under salinity stress, high salinity caused reduction in lipid peroxidation and hydrogen peroxide accumulation (Chaparzadeh, 2004).

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Mutation is the ultimate source of all heritable variation in organisms variability caused by induced mutation is not essentially different from variability caused by spontaneous mutation during evolution. We have today a relatively good understanding of the processes of mutation induction by UV, by ionizing radiation and also by certain chemical mutagens. Rahi *et al.* (1998) found that on *Tagetes erecta* , and 3 Gladiolus cultivars grown under alkaline soil conditions and were treated with gamma rays, the performance of the treated plants in salt affected soils indicated the possibility of selecting stable strains from treated populations which can grow well under stress soil.

Zahed et al., (2004) found that a stable NaCl-tolerant mutant (R1) of *Chrysanthemum morifolium* Ramat has been developed by *in vitro* mutagenesis with gamma radiation (5 Gy). Salt tolerance was evaluated by the capacity of the plant to maintain both flower quality and yield under NaCl stress. Enhanced salt tolerance of the R1 mutant was attributed to increased activities of reactive oxygen species (ROS)-scavenging enzymes, namely superoxide dismutase (SOD). The Rl mutant developed by gamma ray treatment can be considered a salt-tolerant mutant showing all the positive characteristics of tolerance to NaCl stress.

Xiao and Zhao (1988) treated six spring wheat cultivars with 1-2 kr and were cultured in medium containing 0, to 2.5% NaCl, they concluded that 1 kr and 1% NaCl were the optimum doses. Salam and Ismail (1991) treated wheat varieties with gamma rays to obtain mutant genotype tolerant to environmental stresses (salinity and drought). He found that the best dose was 7.5Kr for Saka8, while 10Kr dose was the best dose for Giza 157. Both doses gave increase in yield and yield rated traits under salinity and drought stress. Rascio *et al.* (2001) used sodium azide as a mutagen for durum wheat. The mutants germinated better than the wild under series stress imposed by salts.

The present study aims to produce a mutant capable to tolerate high salinity. It also followed the effect of gamma rays and salinity on some morphological parameters and peroxidase enzyme activity in Calendula.

MATERIALS AND METHODS

A greenhouse experiment was conducted in the Research Station of Faculty of Agriculture, University of Alexandria, Eygpt. Two series of experiments were conducted during the period of 2004 and 2005. The first one dealt with the M_1- generation, while the second one dealt with the M_2 - generation.

1-Plant

Seeds of *Calendula officinalis* were obtained from Floriculture and Ornamental Horticulture Division, Horticulture, Research Institute, Cairo, Egypt.

2- Radiation

Gamma –rays used 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 at El-Dokky, Cairo, Egypt.

3-Salinity.

Surface soil (0-15cm) was collected from Burg El Arab area West Alexandria city. The soil was air dried, passed through a 5mm mesh screen, thoroughly mixed and analyzed for some physical and chemical characteristics according to the standard methods outlined by Page (1982) and are listed in Table 1.

Table (1) Main chemical characteristics of the studied soil

Parameters		Parameters	
pH (soil paste)	7.9	Soluble ions($meqL^{-1}$)	
EC (dSm^{-1})	1.2	Na^+	4.4
Soil texture	Silty clay loam	K^+	5.1
% O.M	0.39	Ca^{++}	1.7
% T.N	0.84	Mg^{++}	4.4
P(olsen) μg^{-1}	10.3	HCO_3^-	1.8
K (avai.) μg^{-1}	8.89	Cl^-	4.8
		SO_4^{--}	0.0

4-Experimental Design

The lay-out of the two experiments was designed to provide complete randomized blocks in factorial experiment containing three replicates

5-Preparing seeds .

The total amount of seeds was divided into five equal parts for treating with gamma ray. The first part was specified only for soaking treatment. The other four parts of seeds were paged in paper bags before exposure to radiation

The dry seeds were exposed to different doses of gamma rays (0, 5, 10, 15 and, 20Kr) from Co-60 in gamma rays on October 10th, 2004.

Saline treatments

For the first season, the treated seeds with gamma-rays were planted in seed-pans of 1-2 cm deep filled with sandy soil. When seedling reached approximately 5 cm height, one seedling was transplanted into plastic pots of 25 cm in diameter, and

35 cm height, packed with 3.0 kg soil. Before planting, uniform rates of NPK fertilizers were added at the rate of 150 kg fed^{-1} as super phosphate and 50 kg fed^{-1} potassium sulfate. Nitrogen was added at the rate of 300 kg fed^{-1} as ammonium sulfate in three doses (initially, 25 and 50 days) from cultivation date.

Five salt levels (0, 50, 100, 150 and 200 mM NaCl) were used as irrigation water. The salinity levels were equivalent to an electrical conductivity of 0.55, 4.3, 8.2, 12.3 and 17.5 dSm^{-1}, respectively to avoid an osmotic shock for seedling emergence; the salinized water was used after 45 days of sowing.

During the experiment, plants were watered to achieve the filed capacity. All treatments were replicated three times.

Experimental Data

The following characteristics of the cultivated plants were recorded for two successive seasons.

A -Vegetative parameters

1-Plant height (cm)

Height of plant was measured in centimeters from the soil surface to the highest point of the plant. Four plants, from each treatment were used to determine this character at the end of the first and second season.

2-Number of leaves per plant

The total number of leaves per plant was counted at the end of the first and second generations.

3-Total chlorophyll content of leaves (SPAD).

At the harvest time, the total chlorophyll content was measured in three plants from each treatment for the two generation. The total chlorophyll content was determined using Minolta machine with SPAD unit's caporal leaf chlorophyll meter of Minolta crop.

4-Fresh and dry weight

At the end of the experiment (at harvest time), plants were cut at 1 cm above the soil. The leaves were washed with tap waterthen, distilled water and air dried to determine the fresh weight then dried at 60 °C for 24 hours to determine the dry weight in the first and second seasons.

B-Flowering parameters

1-Number of flowers per plant

The number of flower was calculated from the average number of three plants of the treatment.

2-Diameter of flower

The diameter of flowers was measured in centimeter, this measurement was carried out for three flowers chosen from one plant from each treatment within replicate, and the average diameter was calculated.

3-Number of branches per plant

Number of branches per plant was recorded from the average number of three plants for each treatment.

4-Fresh and dry weight

At harvest time, plants were cut at 1 cm above the soil. The flowers were washed with a tap water and distilled water and air dried to determine the fresh weight then dried at 60 °C for 24 hours to determine the dry weight

C-Biochemical parameters

Isozymes techniques

1-Peroxidase isozymes

a- Running buffer:- This buffer was prepared by dissolving 270.7 gm of Tris-HCl dissolved in 200 ml distilled water and 11.0 gm citric acid were added and completed to 1000 ml volume, then the solution was adjusted to pH 8.0. (Sabrah, 1980).

b- Gel Media:- Agar- Starch- Polyvinyl Pyrolidine (PVP) gel (1 gm Agar; 0.5 gm PVP and 0.3 gm of hydrolyzed starch) was added to 100 ml of -0.1 running buffer- (Sabrah and El- Metainy, 1985).

c- Peroxidase staining solution

It was prepared by 100 ml of 0.01 m sodium acetate –acetic acid buffer (pH5.0), containing 0.1 gm benzidine and 0.5 ml 5% hydrogen peroxide (H_2O_2).

d- Procedures

Approximately 0.5g of plant tissue (leaves) was ground with purified sand in cold mortar and pestle to which 0.5-1.0 ml of the running buffer was added, The homogenate was absorbed into stripes of filter paper (0.5 X 0.2cm). Filters were placed on the agar gel plates for 30 min, at 4 °C. The filter paper was placed on the original line of the gel plates and stored at 4°C for 30 minutes. 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 according to Palanichamy and Siddig, (1977).

Effect of radiation on morphological variations

All plants were examined daily to follow up changes in flowers' color, leaves' shape, color, and growth.

D-Statistical Analysis

Data were statistically analyzed according to Gomez and Gomez (1984) using SAS (Statistical Analysis System) computer program.

RESULTS & DISCUSSIONS

1-Effect of salinity on vegetative parameters

1-1-Plant height

It appeared in Table (2) that the response of the plant height showed more pronounced response to salinity effect in the first generation than did in the second generation, particularly at 200mM. At this level of salinity the decrease in height compared to the control was about 30 and 16% during the first and second generation, respectively. This means that the shock effect of salinity on plant height was stronger during the first generation than in the second generation. However at lower salinity the decrease in the plant height was small during both seasons. Generally, increasing salinity decreased plant height

(Huany, 1987; Khalil 1991; Sharma et al., 2001 and Haouala, 2002). 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. These results are in agreement with those of Munns (2002) who reported that salinity imposes two major stresses on plant: one is high osmotic pressure in soil solution making it hard for the plant to extract, the second is high NaCl. The osmotic effect reduces stomata conductance which lead to reduced photosynthesis. Thus there are three somewhat independent processes being affected i.e. new leaf formation, old leaf death and photosynthetic activity that all contribute to a reduction in the net assimilation rate of the plant.

1-2-Number of leaves per plant.

The data in Table (2) showed that the number of leaves in the second generation was decreased as the salinity increased. This decrease may be due to the osmotic effects of specific ions and nutritional imbalance (Flower, *et al.*, 1991).

1-3- Number of the main branches.

In the first generation the number of the main branches showed low similar value at both 50 &100mM while it increased at higher salinity levels (150, and 200 mM (Table 2). Similar trend was observed in the second generation. It could be supposed that the interaction of number of main branches may be related to the promotion effect of salinity on the enzyme responsible for branching.

Table 2: Effect of salinity levels on vegetative and flowering parameters of *Calendula officinalis*.

Salinity levels	Plant height cm	No of Leaves No/plant	Leaves F.W gm	Leaves. D.W gm	No Infl. No/plant	Infl. F.W gm	Infl. DW gm	No.M bran No/plant	Chl.	Infl. diam. cm
	First generation									
0	33.12a	166.1a	14.1a	1.61 a	11.13 a	0.59 a	0.13	3.5b	32.86a	3.56a
50	31.7a	167.8a	12.9b	1.43 b	10.66 a	0.60 a	0.11	3.06c	31.30ab	3. 14b
100	32.1a	163.9a	12.2bc	1.41 bc	10.53ab	0.63 a	0.13	3.06b	31.60a	2.82c
150	29.10b	157. 0b	11.80c	1.25 d	9.60 b	0.53 b	0.12	3.8ab	29.6bc	2.78c
200	23.02c	141 .0c	10.36d	1.31 cd	6.00 c	0.49 b	0.11	4.5a	28.68c	2.51d
L.S.D	1.6174	4.514	0.81	0.109	0.982	0.0572	N.S	0.795	1.69	0.27
	Second generation									
0	32.9a	173.1a	16.78a	0.56 a	11.33 a	0.59 a	0.13 a	4.13a	30.34a	3.39
50	31.84ab	161.6b	16.22ab	0.54 a	10.33 a	0.54 b	0.11 a	3.40a	30.47a	3.31
100	32.9a	164.4b	16.04b	0.52 ab	11.06 a	0.49 c	0.13 a	3.40a	30.75a	3.66
150	30.4b	155.6c	14.62c	0.47 bc	9.06 b	0.51 bc	0.12 a	3.66a	28.99ab	3.63
200	27.2c	141.6d	14.49c	0.46 c	8.13 b	0.44 d	0.11 a	43.60a	27.21b	3.24
L.S.D	1.778	5.010	0.630	0.048	1.103	0.04	0.02	0.737	1.895	N.S

1-4-Total chlorophyll content of leaves

The chlorophyll content was significantly decreased with the different salinity levels in both first and second generations (Table 2). These results are in agreement with those of Huany, (1987) who found that increasing salinity reduced leaf and turned yellowing in *Tagetes erecta*. Alam *et al.* 2001 found that chlorophyll was decreased proportionally to the increasing salt concentration in rice.

1-5- Leaves fresh & dry weight

A clear effect of salinity on the leaves fresh weight (Table2) was observed in the first and second generation. It is obvious that salinity reduced leaves' fresh and dry weight. This may be due to osmotic effect. Munns (2002) reported that the osmotic effect reduces stomata conductance which leads to reduced photosynthesis. Also, Chaparzadeh et al., (2004) stated that high salinity caused reduction in growth parameter like the roots and the shoots fresh and dry weight of Calendula.

1-6- Number of inflorescences

As shown in (Table2) the number of inflorescence was decreased significantly with increasing salinity and the lowest number of inflorescence was recorded at the highest salinity of 200mM. These results are in agreement with Haouala, (2002). However in the first generation the decrease in number of inflorescence was about 46 % comparing with 29 % in the second generation relative to control. Such difference may be attributed to the shock effect of salinity during the first season, as the plant was exposed to high salinity for the first time while during the second generation the plant became adapted to high salinity. These results are in agreement with Zahed *et al.*(2004) on *Chrysanthenum morifolium*.

1-7- Inflorescence diameter

In the first generation (Table 2) the inflorescence diameter was significantly decreased with increasing salinity while, in the second generation, the decreasing was insignificantly.

1-8- Inflorescence fresh & dry weight

The inflorescence fresh and dry weights were decreased significantly during the first and second generation (Table 2). Chakraborty andSadhu (1990) reported that with increasing salinity, flower diameter, weight and longevity were most reduced in *Callistephus chinensis, Calendula officinails and Tagetes signata.*

2- Effect of gamma radiation on Calendula

2-1- Plant height

It was noted in Table (3) that the plant height was increased by about 6.17 and 26.6 % at 10 kr in the first and second generations, respectively relative to the control and this was followed by gradual decrease of plant height by increasing gamma radiation doses.

Radiation affected the genetic component of the treated plants which increased the genetic variation and caused greater frequencies of unadaptive (including lethal) genotypes, which have been kept adaptive to the environment, and reduce adaptability function of the plant (Ichikawa, 1981). The adapted (survival) 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.

These results are in agreement with some investigators Banerje and Datta (2002) on *Chrysanthemum morifolium*, Dilta (2003) on *Chrysanthemum*, and Bader *et al* (2000) on *Gomphrena glbosa.*

Table 3: Effect of gamma levels on vegetative and flowering parameters of Calendula officianils.

Gama level	Plant height cm	No of Leaves No/plant	Leaves F.W gm	Leaves .D.W gm	No Infl. No/plant	Infl. F.W gm	Infl. DW gm	No.M bran No/plant	Chl.	Infl. diam. cm
	First generation									
0	30.72b	166.3a	12.66a	0.56a	8.73b	0.61 ab	0.129	4.20	34.26a	2.86bc
5	30.44b	163.4ab	13.1a	0.51ab	10.20a	0.63 a	0.123	3.13	32.25b	3.58a
10	32.72a	160.1bc	12.66bc	0.482b	10.86a	0.59 ab	0.130	3.73	32.00b	3.14b
15	28.74c	156.13c	11.72b	0.460b	10.13b	0.57 b	0.130	3.53	28.98c	2.76c
20	26.56d	149.8d	10.93b	0.457b	8.00b	0.45 c	0.110	3.40	26.60d	2.46d
L.S.D	1.617	4.51	0.81	0.061	0.982	0.057	N.S	N.S	1.69	0.27
	Second generation									
0	32.21a	165.2a	16.94a	0.560a	10.6ab	0.56 a	0.128	4.13	31.84a	3.39ab
5	31.3ab	166.3a	16.11b	0.553a	10.93a	0.54 ab	0.123	3.40	31.29a	3.31ab
10	32.9a	158.4b	15.58bc	0.553a	10.3ab	0.55 a	0.130	3.40	30.85a	3.66a
15	29.9bc	159.7b	14.98bc	0.485b	9.66b	0.51 b	0.118	3.66	27.74b	3.63a
20	28.9c	146.7c	14.48d	0.428c	8.40c	0.43 b	0.114	4.60	25.40c	3.24b
L.S.D	1.778	5.010	0.64	0.048	1.103	0.043	N.S	N.S	1.895	0.372

2- 2- Number of leaves per plant.

The number of leaves was decreased with all doses of gamma radiation in the first and second generations, except at 5Kr in the second generation, the number of leaves was insignificantly increased (Table 3).

It seems that the gamma radiation caused depression in number of leaves in both generations at all doses of gamma radiation, while the slight increase appeared during the second generation may be attributed to the adaptation of the plants produced from the seeds in the first generation. This result is in agreement with Datta et al. (2001), Lamseejan et al. (2000) on *Chrysanthemum morifolium.* Gordon (1958) has emphasized the changes in amount of auxin occurring as a result of radiation effect, as a possible factor responsible for decrease of growth, while Pelc and Howard (1955) regarded the inhibition of DNA synthesis due to radiation to be more important. Wada *et al* (1998) reported that radiation can cause indirect damage in living systems by various radical in irradiated cells.

2- 3- Number of the main branches.

In the first and second generations, the number of the main branches decreased non-significantly with increasing gamma radiation, while it showed pronounced increase at 20 Kr in the second generation (Table 3).

Similar results were mentioned by Misra (1998) who reported that the number of shoots was found to be non-significant after irradiation with gamma- rays.

The stimulation of the main branching in the second generation at 20Kr may be due to: 1-Gamma rays increased the mitotic activity of cambial cells which led to branches production increase (El Mahrouk, 2000). 2-Gamma rays supplied gibberellins, cytokininis and abscisic acid which may play important roles in stimulating branches production (Chandokar and Dengler 1987 and El Mahrouk, 2000).

3- Gamma rays increased nutrients which were not utilized in the stem elongation (Chandrokar and Dengler, 1987).

2-4- Total chlorophyll content of leaves

A gradual decrease in the total chlorophyll content was observed with the increase in gamma radiation in the two generations (Table 3). On the other hand, many workers reported that the largest amount of chlorophyll was found in leaves treated with gamma radiation at dose of 15 kr in M_1-generation, (El –Mahrouk, 2000 and Badr *et al*., 2004. However, Badr et al (2000) found that gamma –rays had insignificant effect on leaf chlorophyll content in M_1-generation.

The alteration in chlorophyll synthesis of irradiated leaves may be due to auxin synthesis (Hagen and Gunckel, 1958). Giacomelli *et al.* (1993) found that the primary effect of radiation was on the development of meristematic cells. It is known also, that the change in chlorophyll content is associated with the changes in the chloroplasts (Evens, 1984).

2- 5- Leaves fresh & dry weight

In the first generation (Table 3) the leaves fresh weight showed an increase of about 3.5% at 5Kr relative to control, but it decreased with increasing gamma radiation in both generations. (Table3).

The leaves dry weight significantly decreased with all doses of gamma radiation (5-20Kr) in the first and second generations. One of the main effects of the ionizing radiation is the suppression of cell division activity, which is responsible partially for the reduction of vegetative growth. Various possible explanations of inhibition in growth after irradiation have been suggested by many workers (Gunckle, 1957; Pelc and Howard 1955; and Wada et al., 1998) .

2-6- Number of inflorescences

In the first generation, increasing doses of gamma radiation between 5 and 15 Kr led to higher numbers of inflorescence by about 17.2, 24.1 and 16.1% than the control (Table3). But the dose of 20 Kr was associated with a decrease of about 8% than the control. This pattern appeared to be pronouncedly different in the second generation, when the number of inflorescences was decreased with increasing the doses of gamma radiation, with the exception of slight increase at 5Kr. This means that the 5 Kr promoted the production of inflorescences during the first generation while the increase of production in the second generation was slight and may be due to the adaptation of the second generation, to the gamma radiation which caused slight promotion to the production of the inflorescences.

Bidwell (1979) mentioned that all steps in flowering process are programmed in totiopotent cells of the meristems. All that needed a release that sets these cells on the way in the program for flowering. The capacity to flower is inherent. In this work, the dose of 10 kr in the first generation and 5 kr in second one stimulated the totipotent cells of meristems to change the developmental pattern from vegetative to floral and this might increase the number of inflorescences. The effect of 20 kr dose was inhibiting of the number of inflorescence in the first generation.

Badr *et al.* (2000); El Mahrouk (2000) and Przybyla (2000) stated that the flower and inflorescence number was increased or decreased with the different doses of gamma –rays during the second generation .

2-7-Inflorescence diameter

The inflorescence diameter displayed different behaviors with gamma radiation in the two generations. In the first generation it increased at 5 and 10Kr by about 25% and 9.9% respectively than the control (Table3). In the second generation, the inflorescence diameter showed an increase relative to control at 10 and 15 Kr by about 8 and 7.1% respectively. This result may be attributed to an increase in cell number and /or cell size. However, at other gamma doses, the inflorescence diameter showed pronounced decrease in the first and second generation. Similar results were reported by Banerji and Datta (2002) .

2-8 Inflorescence fresh & dry weight

In the first generation, the inflorescence fresh weight at 5Kr was higher than the control by about 3.8% (Table 3), but it decreased continually with increasing the doses of gamma radiation to be a minimum at 20 Kr by about 75% relative to the control. However, the inflorescence fresh weight in the first generation recorded the highest value at 5 Kr, in the second generation it decreased with increasing gamma radiation.

The inflorescence dry weight (Table 3) showed insignificant increase in the first and second generations at 10&15kr than the control.

2-9- Effect of gamma radiation on the induction of variation in shape and colour

Treatment of doses 15 and 20 kr caused some changes in inflorescence shape and colour and growth habit in the first and second generations such as the colour was changed from yellow to orange and the pet als became thinner and longer than control in the second generation. These results are in agreement with Vieira *et al.* (1996). Also, irradiation of *Chrysanthemum morifolium* by 20 Gy. caused some changes in the M1 generation, and reoccurred in the M2 and M3 generations, Zalewska *et al* (2001). Meanwhile, Banerji and Datta, (2002) recorded different types of morphological abnormalities in leaves and flowers of chrysanthemum treated with different doses of gamma rays.

3-Combined effect of gamma radiation and salinity on Calendula

3-1- plant height

The analysis of variance indicates a significant correlation between gamma radiation and the plants grown in soil salinity in the first generation (Table 4),

while the correlation was not significant in the second generation (Table 5).

In the first and second generations (Tables 4 and 5) showed a decrease in all gamma radiation combined with different salinities. These results seem to contradict with those reported by Salam (1991) on wheat, Rahi et al. (1998) on *Tagetes erecta* and Zahed et al. (2006) in *Chrysanthemum morifolium*.

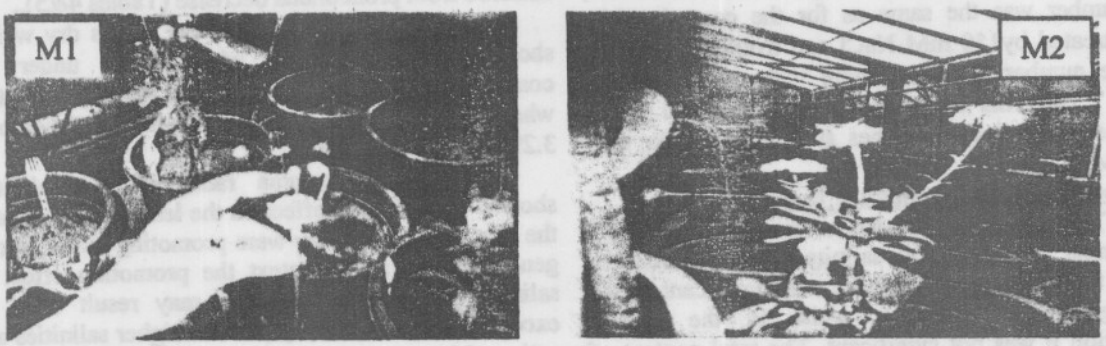


Fig.1: Different inflorescence colour and shape abnormalities of *Calendula officinalis* in first generation

3-2-Number of leaves per plant.

As shown in (Table 4&5) the number of leaves in the plant grown at different salinity levels and exposed to different doses of gamma radiation suffered from clear decrease during the first and second generations.

Table 4: Effect of combined effects of gamma and salinity levels in vegetative and flowering parameters of *Calendula officinalis* in the first generation.

Salinity level	Gama level	Plant height cm	No of Leaves No/plant	Leaves F.W gm	Leaves. D.W gm	No Infl. No/plant	Infl. F.W gm	Infl. DW gm	No.M bran No/plant	Chl.	Infl. diam. cm
0	0	35.10ab	181.66	18.93a	0.859	11.00ab	0.642ab	0.126	4.66	37.0a	3.13bc
	5	32.83ab	173.00	17.26ab	0.699	10.00b	0.588ab	0.109	3.33	34.10ab	4.23a
	10	34.96ab	177.66	16.80ab	0.618	12.66a	0.681ab	0.139	3.33	32.20b	3.73ab
	15	30.90bc	175.00	15.80bc	0.558	12.66a	0.581ab	0.139	3.33	30.56bc	2.90c
	20	30.70bc	158.33	15.90bc	0.613	10.33ab	0.484bc	0.164	3.00	30.40bc	2.96c
50	0	31.23bc	167.33	17.93ab	0.617	10.33ab	0.591ab	0.119	2.66	32.33b	3.03bc
	5	31.26bc	175.00	17.00ab	0.574	12.00ab	0.540b	0.163	3.33	31.03bc	3.93ab
	10	33.10ab	161.66	16.00bc	0.463	10.66ab	0.618ab	0.178	4.66	33.66ab	3.70ab
	15	32.33bc	157.00	14.80c	0.521	9.66bc	0.520bc	0.115	2.33	30.03bc	3.16bc
	20	31.26bc	147.00	14.78c	0.421	9.00bc	0.454bc	0.110	2.33	29.46bc	2.70c
100	0	36.40a	176.33	15.60bc	0.502	12.66a	0.515bc	0.118	4.00	36.70a	4.23a
	5	34.16ab	175.33	17.13ab	0.534	12.33ab	0.501bc	0.105	2.33	33.10b	3.60b
	10	34.63ab	148.66	16.63bc	0.538	10.33ab	0.572ab	0.119	3.33	31.16bc	4.06ab
	15	30.30bc	172.67	14.36c	0.525	10.33ab	0.479bc	0.110	2.33	30.06bc	3.23bc
	20	29.13bc	149.33	14.26c	0.512	9.67bc	0.418bc	0.104	3.33	26.96cd	3.20bc
150	0	31.56bc	161.33	14.90c	0.477	10.00b	0.586ab	0.101	4.00	35.33ab	3.66ab
	5	29.70bc	157.1`0	15.33bc	0.434	10.67ab	0.600ab	0.130	2.66	30.26bc	4.16ab
	10	32.53bc	164.00	15.93bc	0.432	9.00bc	0.497bc	0.121	3.33	32.63b	3.80ab
	15	29.96bc	152.33	14.52c	0.414	8.33bc	0.449bc	0.109	5.00	27.90cd	3.70ab
	20	28.23cd	143.33	14.26c	0.405	7.32c	0.421bc	0.101	4.00	22.16d	2.83c
200	0	26.76d	139.66	16.60bc	0.354	9.00bc	0.461bc	0.122	5.66	29.93bc	2.86c
	5	28.96cd	151.33	14.10c	0.327	9.67bc	0.503bc	0.110	4.00	32.76b	3.63ab
	10	28.56cd	140.00	14.80c	0.358	9.00bc	0.408c	0.106	4.00	30.33bc	3.20bc
	15	26.30d	141.67	13.60c	0.284	7.33c	0.510bc	0.107	4.66	26.33cd	2.90c
	20	25.54d	135.66	13.26c	0.702	5.66c	0.354c	0.110	4.33	24.03d	3.63ab
L.S.D		3.6	N.S	1.80	N.S	2.4	0.13	N.S	N.S	3.8	0.61

3-3-Number of main branches .

According to the analysis of variance (Tables 4&5) no significant variations were reported in the number of the main branch under the combined effect of gamma-radiation and salinity during the first generation but highly significant variation was observed in the second generation.

Number of the main branches in the first generation increased by about 7.3% at 15 kr and 150 mM. Meantime, number of the main branches did not

differ from the control at 10Kr &50mM and 15Kr & 200mM. at all other doses of gamma radiations and salinity, the number may be decreased (Table 4).

In the second generation the number of main branches showed different behaviors with salinity and gamma radiation. At 50mM the gamma dose of 10 Kr, the number was the same as for the control, while plant treated by150 mM NaCl and irradiated with 15 kr, the number increased more than the control by about 7.3%. This increase was also observed in the number of the main branches at 5, 15 & 20 kr with 200mM.

3-4-Total chlorophyll content of leaves

The analysis of variance of the combined effect of gamma rays and soil salinity indicated that the leaves total chlorophyll content was significant in the first generation (Table4), while in the second generation it was not significant. The total content of chlorophyll displayed a decrease at all doses of gamma radiations and salinities in the first and second generations (Tables 4&5).

3-5-Leaves fresh & dry weight

Under the combined effect of all salinity and gamma radiation levels, the leaves fresh weight suffered from pronounced decrease (Tables 4&5)..

In the second generation, the leaves dry weight showed different trends of variation under the combined effect of salinity and gamma radiation whereas at 15 Kr and 50 mM it was increased by about 3.2% relative to control.

Although the gamma radiation and salinities showed an inhibiting effect on the leaves dry weight in the first generation, they were promoting in the second generation. In such context the promotion effect of salinity and gamma radiation may result from an excess of salt observed by plant at higher salinities and activated by gamma radiation.

Table 5: Effect of combined effects of gamma and salinity levels in vegetative and flowering parameters of *Calendula officinalis* in the second generation.

salinity	Gamma Level	Plant height cm	No of Leaves No/plant	Leaves F.W gm	Leaves. D.W gm	No Infl. No/plant	Infl. F.W gm	Infl. DW gm	No.M bran No/plant	Chl.	Infl. diam. cm
0	0	35.83	173.33a	14.73a	0.60 ab	12.00	0.66	0.13	4.66c	33.56	3.20
	5	32.20	173.00a	15.20a	0.56 bcd	9.33	0.59	0.11	3.33e	33.50	5.03
	10	37.90	164.00cd	14.73ab	0.68 a	13.66	0.54	0.17	3.33e	31.43	3.73
	15	32.73	161.66de	13.13bc	0.56 bcd	12.00	0.64	0.17	3.33e	27.23	3.06
	20	27.20	158.66e	12.70bc	0.42 cd	8.66	0.53	0.10	3.00f	26.00	2.80
50	0	31.56	173.66a	12.26bc	0.50 bcd	8.00	0.69	0.13	2.66g	29.83	2.56
	5	30.90	170.66ab	14.33ab	0.56 bcd	12.00	0.68	0.09	3.33e	31.66	4.73
	10	35.16	169.66bc	13.33bc	0.53 bcd	12.00	0.63	0.10	4.66c	31.86	3.16
	15	30.63	165.00cd	12.83bc	0.62 ab	12.00	0.52	0.13	2.33g	29.46	2.70
	20	30.23	160.00e	11.96c	0.52 bcd	9.33	0.49	0.12	2.33g	29.46	2.53
100	0	35.20	167.00c	13.30bc	0.61 ab	7.00	0.67	0.13	4.00d	35.40	3.16
	5	33.76	164.33cd	10.66cde	0.62 ab	12.00	0.75	0.16	2.33g	31.76	3.06
	10	34.86	164.00cd	14.60ab	0.51 bcd	12.33	0.69	0.15	3.33e	31.16	2.86
	15	29.13	163.66d	11.86cd	0.46 bcd	12.33	0.59	0.12	2.33g	29.63	2.60
	20	28.00	160.66e	10.80cde	0.43 cd	9.00	0.47	0.12	3.33e	25.80	2.40
150	0	29.53	165.66cd	12.06bc	0.54 bcd	10.00	0.44	0.13	4.66c	32.43	2.73
	5	30.43	162.00de	13.96ab	0.55 bcd	10.66	0.59	0.14	3.00f	30.06	2.66
	10	30.20	158.00e	11.73cd	0.52 bcd	10.00	0.55	0.13	2.33g	31.60	2.96
	15	29.20	152.33f	11.03cde	0.39 cd	9.00	0.69	0.12	5.33a	27.90	3.16
	20	26.16	147.00g	10.23de	0.39 cd	8.33	0.40	0.12	4.33c	22.96	2.41
200	0	21.50	152.00f	10.96cde	0.56 bcd	6.66	0.58	0.12	4.66c	28.13	2.66
	5	24.90	147.33g	11.63cd	0.49 cd	7.00	0.55	0.12	5.00b	32.46	2.40
	10	25.46	145.00g	10.46de	0.54 bcd	6.33	0.53	0.11	3.33e	28.23	2.96
	15	22.03	138.00h	9.76e	0.39 cd	5.33	0.41	0.12	5.00b	24.23	2..30
	20	21.20	122.66h	6.96f	0.37 d	4.66	0.39	0.11	5.00b	22.76	2.20
L.S.D		N.S	3.1	1.4	0.1	N.S	N.S	N.S	0.23	N.S	N.S

3-6-Number of Inflorescences

The analysis of variance (Table 4 &5) indicated that the number of inflorescence reported highly significant variations under the combined effect of gamma rays and salinities in the first generation but not significant in second onen.

The number of inflorescences in the first generation mostly decreased with the combined effect of salinity and gamma radiations. However at 50 mM and 100 mM, the number of inflorescences was increased more than the control at gamma radiation of 5 kr by about 9 and 12%, respectively (Table 4).

In the second generation, the number of inflorescence showed no variation under the effect all gamma radiation of 5, 10 and 15kr with 50mM and at 5kr and 100mM. But at 10kr and 15kr with 100mM, the number of inflorescence increased by about 2.8%,

while at other salinities and gamma radiations the number of inflorescence decreased.

3-7-Inflorescence diameter

According to the analysis of variance the inflorescence diameter showed highly significant variation with the combined effect of salinities and gamma radiation in the first generation but no significant in the second generation (Tables, 4&5).

In the first generation, (Table 4) the inflorescence diameter at 50 mM increased pronouncedly than the control at gamma radiation doses of 5, 10 and 15Kr by about 1% to 25.6%. At 100 mM the diameter decreased than the control at all gamma doses by about 2.2% to 29.7% ,while at 150mM the inflorescence diameter increased with gamma radiation of 5, 10 and 15Kr by about 18.2%, 32.9%. In addition, 200mM and 5,10and 20Kr caused increase of the inflorescence diameter by about 2.2% and 16% respectively.

In the second generation, (Table5) the inflorescence diameter decreased at all doses of gamma radiations and salinities, except at5Kr and 50mM, whereas it showed an increase of about 47.8% . From the results mentioned above it is clear that the combined effect of gamma at 5Kr and salinity 50 mM increase the diameter in the M2 than the same cases in M1, while increasing in salinity in M2 lead to inhibition in diameter than at other cases in M1(Table 5).

These results are in agreement with Haouala, (2002). However the adaptation of the plant to the height salinity during the second season showed less effect of salinity on the production of the inflorescence and consequently its diameter. These results are in agreement with Zahed et al., 2004. .

3-8-Inflorescence fresh & dry weight

The combination of gamma radiation with salinity in the first generation significantly decreased the inflorescence fresh weight (Table 4).

In the second generation, the inflorescence fresh weight increased at 100mM irradiated by 5 Kr (Table 5).

In the first generation, the inflorescence dry weight was insignificantly decreased at all exposed treatments of the combined effect of gamma radiation and salinities (Table 4).

In the second generation, the trend of variation in the inflorescence dry weight with salinities and gamma radiation appeared to be more or less different from the first generation. It decreases at all doses of gamma radiation with different salinities (Table5). While with 100mM and 5 and 10kr the inflorescence dry weight increased by about 23 and 12.4% respectively and at 150 mM and 5kr by about 5 %.

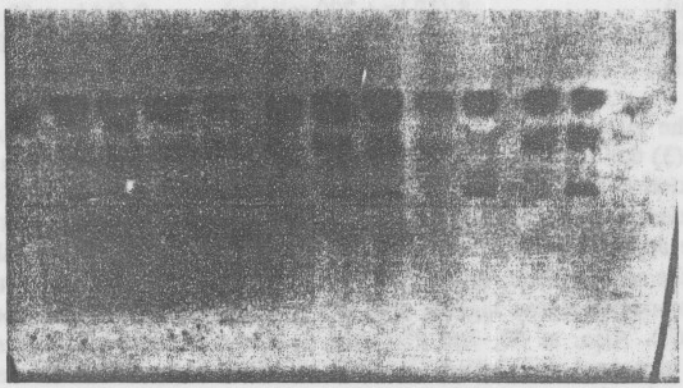


Fig: (2) photograph of peroxidase isozymes patterns

3-9- Biochemical parameters

Peroxidase isozymes

The peroxidase isozyme pattern of Calendula grown under the effect of different salinities and gamma radiations are given in Fig.2. The cathodal isozymes bands were designated C1, C2, and C9, while the anodal isozymes ones were designated A1, A2, and A4 according to their mobility from the original line.

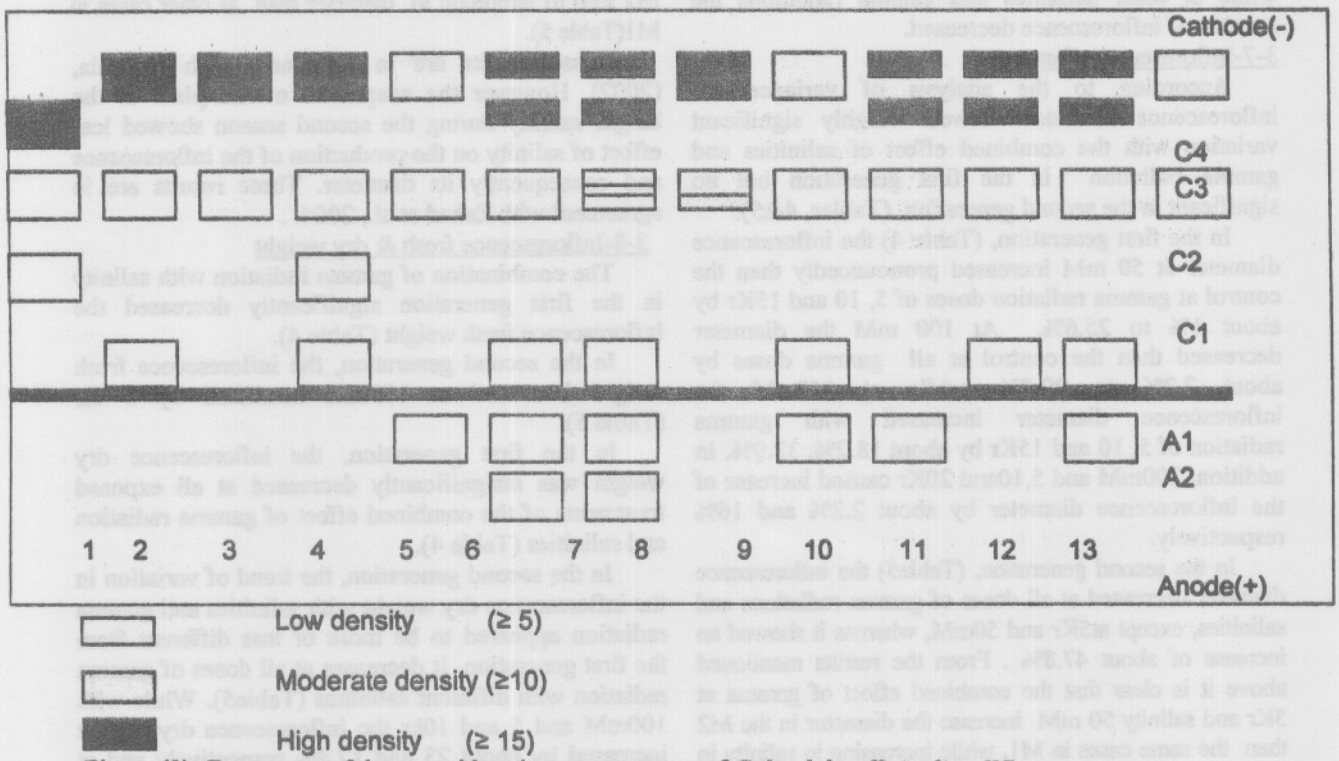


Figure (3): Zymogram of the peroxidase isozyme patterns of *Calendula officinalis* Where:

1- (0 kr + 0 salinity)	2-(5 kr + 50 salinity)	3-(5 kr + 100 salinity)
4-(5 kr + 150 salinity)	5-(5 kr + 200 salinity)	6-(10 kr + 50 salinity)
7-(10 kr + 100 salinity)	8-(10 kr + 200 salinity)	9-(15 kr + 50 salinity)
10-(15 kr + 100 salinity)	11- (15 kr + 200 salinity)	12-(20 kr + 50 salinity)
13- (20 kr + 100 salinity)		

It is shown in the zymogram of the peroxidase isozyme pattern Fig. (3) that the first sample (control) had three isozyme patterns called C2, C3, and C4, and they differed in their densities. It is clear that the stress of salinity caused the appearance of new band called C5 (high density) at the second, third, fourth and fifth sample, but it was moderate at the highest dose of salinity (200mM NaCl). Also, at 50, and 150 mM NaCl, two isozymes appeared and called C1. It is noted that A1 appeared only at the highest salinity levels.

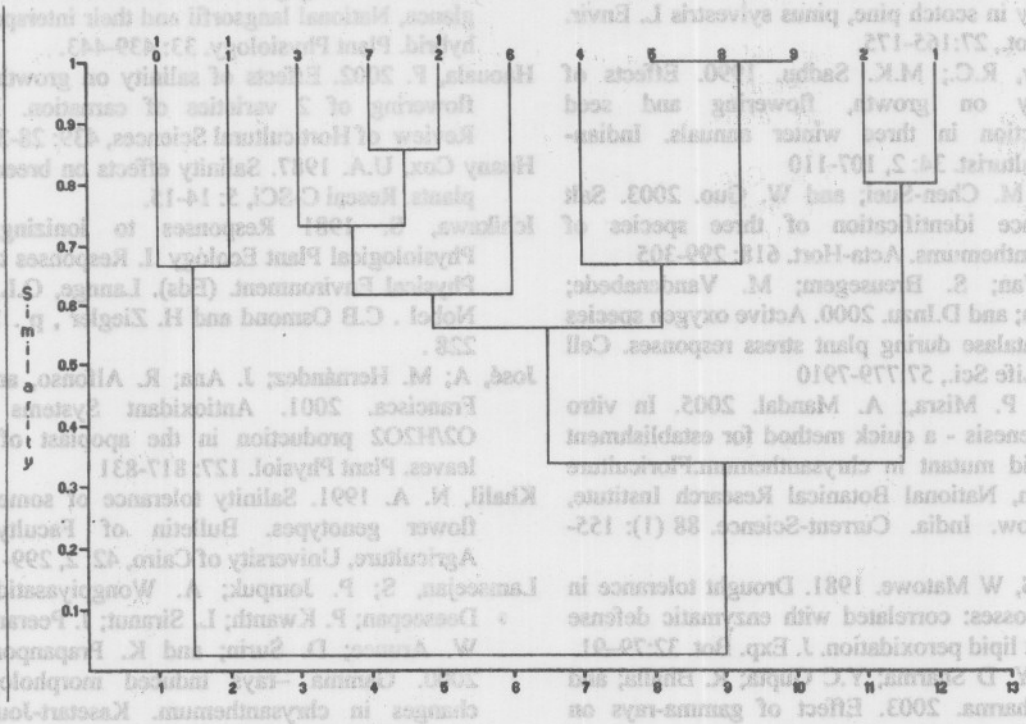
At higher doses of gamma radiation (10kr), the zymogram illustrated two different anodal pattern appeared at 50 and 100 mM NaCl, while disappeared at 200 mMNaCl. The same concentration showed two heterozygous bands at C5 location.

In contrary, increasing gamma radiation to 20kr induced absence of anodal bands, C1, and C2. Moreover, the density of C3 and C5 became relative to control.

Zahed *et al.* (2006) found that on *Chrysanthemum morifolium*, the enhanced salt tolerance of the R1 mutant was attributed to increased activities of reactive oxygen species (ROS)-scavenging enzymes, namely superoxide dismutase (SOD). The R1 mutant developed by gamma ray treatment can be considered a salt-tolerant mutant showing all the positive characteristics of tolerance to NaCl stress.

Chen *et al.* (2003) found that under salt stress, peroxidase activity maintained high levels. Plants require oxygen for efficient production of energy. During the reduction of O_2 to H_2O,active oxygen radicals, namely superoxide radical(O), hydrogen peroxidase (H_2O_2) and hydrogen radical(OH) can be formed. Plants possess very efficient defense systems that allow their detoxification and protect cells from oxidative damage. Among these defense system, the enzyme piroxidase (Dat *et al.*, 2000). Poontariga et al., (2003) suggested that total peroxidase activity is enhanced under 150mM NaCl in mulberry, which indicate that peroxidase activity appears to play an active in scavenging reactive oxygen species.

Genetic relationship among *Calendula officinalis* samples based on peroxidase isozyme patterns



In this analysis, samples are divided into two main clusters for 0.05% genetic similarity. The first cluster contained sample No. 10 and 11. On the other hand, another cluster had two main sub clusters, at 33 % of genetic similarity. Sub cluster No. 1 had samples No 1 and 2, whereas second sub cluster had two main groups at 55 % genetic similarity. The first group is composed of two sub groups A and B. Sub group A had two main forms, at 66 % of genetic similarity, the first form contained samples No. 5, 8 and 9, by contrary, the second form contain sample No. 4. The second group contained two main clusters at 60 % of genetic similarity. Sample No. 6 form the first cluster, on the other hand two sub clusters form the second cluster at 75% of genetic similarity. The first sub cluster contained samples No. 7 and 12 (85% of genetic similarity), on the other hand, sample No. 3 form the second sub cluster. Unexpectically, all the treated samples were different in the values of genetic similarity which may be due to exposure to different doses of gama ray. Also, the different levels of salinity.

From the previous statistical cluster, we can group the control with the treatment of 50 mM NaCl irradiated with 5 Kr in the same class, which means that the irradiation treatment could help plants to adapt with salinity stress. All the positive responses towards cytological, physiological and biochemical change under stress condition indicate possibilities towards inducing salt tolerance through *in vitro* adaptation of whole plant.

REFERENCES

Alam S.M., R. Ansari; S.M. Mujtaba and S. Aisha. 2001. Salinization of millions hectares of land continues to reduce crop productivity severely worldwide NIA,TandoJam, Pakistan Apr.23-29.

Badr , M., O. EL- Shennaway , M . Mostafa and F.EL-Tony. 2000. Effect of gamma irradiation, ethyl methane sulphonate and their combinations on growth, flowering and induced variability in Tagetes erecta L. J. Agric. Sci. Mansoura univ. 25:3587-3604.

Badr, M; B.A.Abdel-Maksoud and Salwa S.Omer. 2004. Growth, Flowering and induced Variability in Gomphrina globosa,L. Plant grown from dry and water-soaked seed treated with gamma rays. Alex. J. Agric. Res. 49(1).

Banerji-BK., and S.K. Datta. 2002. Induction and analysis of somatic mutation in chrysanthemum. Journal-of-Ornamental-Horticulture-New-Series. 5: 1-7.

Bidwell, R.G.S. 1979. Plant Physiology. Second Edition, p.446-449. Macmillan Publishing Co., Inc. New York.

Chaparzadeh N; N.Khavari; I. Navari. 2003.Water relations and ionic balance in calendula officinals under salinity conditions. Agrochemical, 47: 69-79.

Chaparzadeh N. 2004. Antioxidative responses of Calendula officinalis under salinity conditions." Plant Physiology and Biochemistry. 42: 695-701.

Chandorkar, K.R. and N.G. Dengler . 1987. Effect of low level of continuous on vascular cambium activity in scotch pine, pinus sylvestris L. Envir. Exp.Bot., 27:165-175.

Chakraborty, R.C.; M.K. Sadhu. 1990. Effects of salinity on growth, flowering and seed production in three winter annuals. Indian-Agriculturist. 34: 2, 107-110

Chen, F; M. Chen-Suei; and W. Guo. 2003. Salt tolerance identification of three species of chrysanthemums. Acta-Hort. 618: 299-305

Dat.J.; F.Van; S. Breusegem; M. Vandenabede; M.Van; and D.Inzu. 2000. Active oxygen species and catalase during plant stress responses. Cell Mol. Life Sci., 57:779-7910

Datta,-S-K; P. Misra,; A. Mandal. 2005. In vitro mutagenesis - a quick method for establishment of solid mutant in chrysanthemum.Floriculture Section, National Botanical Research Institute, Lucknow. India. Current-Science. 88 (1): 155-158

Dhindsa, RS, W Matowe. 1981. Drought tolerance in two mosses: correlated with enzymatic defense against lipid peroxidation. J. Exp. Bot. 32:79–91.

Dilta, B.S; Y. D Sharma; Y.C Gupta; R. Bhalla; and B.P Sharma. 2003. Effect of gamma-rays on vegetative and flowering parameters of chrysanthemum. J. Orn. Hort., New-Series. 6 (4): 328-334

El–Mahrouk,M.E.M.(2000). Introduction of Genetic Variability in Gomphrena gloposa L. Plant by Gamma Rays. M.Sc. Thesis in Floriculture and Ornamental Horticulture, Faculty of Agri., Tanta Univ., Kafer El–Sheikh, A.R.E.

Evens, D. 1984. Genetic basis of some colonel variation in tomato in plant tissue and cell culture, Application to Crop Improvement Proceeding of International Symposium, p. 259-265. Published by Inst. of Experimental Botany, Cezechsolvak Academy of Sci., Prague.

Flowers T. J.; M.A. Hajibagheri; and A.R. Yeo. 1991. Ion accumulation in the cell walls of rice plants growing under saline conditions. Plant, Cell and Environ. 14, 319-325.

Flowers T. J. 2004. Improving crop salt tolerance. J. Exp. Bot. 55: 307-319.

Giacomelli, G.A; D.H. Royal; and W.I. Roberts. 1993. Photometric, radiometric, and quantum light units of measure. Hort. Sci. 18: 818-822

Gorden, S. 1958. Intracellular localization of the tryptophane indole acetate enzyme system. Plant Physiology. 33, 23-27.

Gomez, K. A., and A.A. Gomez. 1984. Statistical Procedures for Agricultural Research. 2nd ed. John Wiley and Sons, Inc. New York.

Gunckel, J. 1957. The effect of ionizing radiation on plant. Quart. Rev. Bio. 32, 46-56.

Hagen, L. and E. Gunckel. 1958. Free amino acid levels following gamma irradiation of Nicotiana glauca, National langsorfii and their interspecific hybrid. Plant Physiology. 33: 439-443.

Haouala, F. 2002. Effects of salinity on growth and flowering of 2 varieties of carnation. PHM Review of Horticultural Sciences, 439: 28-32;7

Huany Cox, U.A. 1987. Salinity effects on breedding plants. Reseai C-SCi, 5: 14-15.

Ichikawa, S. 1981 Responses to ionizing. In Physiological Plant Ecology .I. Responses to the Physical Environment. (Eds). Lannge, O.L, p.s. Nobel . C.B Osmond and H. Ziegler , p . 199 - 228 .

José, A; M. Hernández; J. Ana; R. Alfonso, and S. Francisca. 2001. Antioxidant Systems and O2/H2O2 production in the apoplast of pea leaves. Plant Physiol. 127: 817-831

Khalil, N. A. 1991. Salinity tolerance of some sun flower genotypes. Bulletin of Faculty of Agriculture, University of Cairo, 42: 2, 299- 310.

Lamseejan, S; P. Jompuk; A. Wongpiyasatid; S. Deeseepan; P. Kwanth; L. Siranut; J. Peeranuch; W. Arunee; D. Surin; and K. Prapanpongse. 2000. Gamma –rays induced morphological changes in chrysanthemum. Kasetart-Journal, Natural Sciences. 34: 417-422.

Misra, R. 1998. Radiation induced variability in gladiolus. Indian J. Genet. . 58:237-239.

Munns R. 2002. Comparative physiology of salt and water stress. Plant, Cell and Env. 25: 239-250.

Nakano Y and Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology, 22: 867-880.

Page A.L., R.H. Miller, and DR Keency 1982. Chemical and Microbiological Properties. Part 2 Madison, Wisconsin U.S.A.

Palanichamy, K. and Sidding. E.A. 1977. Study of inter relationship among A-genome species of the genus Oriza through isozume. Thror. APPL. Genet.50:201-210.

Pelc S. and A. Howard. 1955. Effects of various doses of X-rays on the number of cells synthesizing deoxyribose nucleic acid. Radiation Res. 3:135-142. .

Pootntariga, H.; P. Darinee, R. Kannarata; and C.Rangsi. 2003. Salinity effects on antioxidant enzymes in mulberry cultivar. Science Asia. 29:109-113.

Przybyla, A.A. 2000. Mutagenesis in creation of new Alstroemeria genotypes. Acta Hort. 508:351-353.

Rahi, T; R. Shukla; R. Pandey; and S. Datta. 1998 Performance of ornamental crops in salt affected soils and use of gamma rays to develop salt resistant strains. Journal of Nuclear –Agriculture and Boiology.27:4,253-263.

Rascio, A., M.R. Susso, L.Mazzucco, C.planti, G.Nicastro and N.Difonzo (2001). Enhanced osmo-tolerance of wheat mutant selected for plotassium accumulation. Plant Sci., 160:441-448.

Rausch, T.; M. Kirsch; A.Lehr; R.Viereck; and A. Zhigang. 1996. Salt stress responses of higher plants: the role of proton pumps and Na+/H+ antiporters. J. Plant Physiol. 148: 425-433.

Reedy, K.M; C.C. Rao; G. Padjama; and P. Goud. 2005. Screening of African marigold (Tagetes erecta L.) cultivars for flowers yield and cartenoids pigments. Indian J. Hort. 62: 276-279.

Sabrah, N.S. 1980. Genetical and cytological studies on Maize. Ph.D.Thesis, Faculty of Agriculture, University of Alexandria .Egypt.

Sabrah ,N.S., and A.Y. El-Metainy. 1985. Genetic Distances between local and exotic cultivars of Vicia faba based on esterase isozyme variation. Egypt J. genetic Cyto. 14:301-307.

Salam, H. M.; and S.M. Ismaeil. 1992. Interaction of salinity and temperature on germination, seedling growth and some chemical constituents of two sun Flower cultivars. Annuals of Agricultural Science Cairo. 37: 411-424.

Shannon, M.C., and C.M. Grieve. 1999. Tolerance of vegetable crops to salinity. Scientia Hort. 78: 5-38.

Sharma, SS; R.K. Vanda. 1989. Effect of salinity levels on physiological aspects of garden annuals. South Indian Horticulture. 37: 337-340.

Sharma S.S.; R. Tamdagni; T. Abha; and A. Tikkoo. 2001. Effect of salinity on vegetative growth of winter garden. Haryana Journal of Horticultural Sciences. 30: 57- 59.

Stoeva, N. 2002. Physiological effects of the synthetic growth regulator thidiazural on gamma irradiation stress in pea plants. J. central Eur. Agric. 3: 293-300

Vieira,G.S.; L.R.Goular; J.C. Penna; and J.J. Fernandes. 1996. Modification of morphological traits of common bean through gamma-ray irradiation: analysis of three consecutive generations. Plant Breed. Abst. 66:4945.

Wada H.;T.Koshiba; T.Matsui and M.Sato. 1998. Involvement of peroxidase in different sensitivity to gamma radiation in seedling of two Nicotiana species. Plant Science. 132:109.

Xiao, H; W. Zaho. 1994. Genes from wild rice improve yield. Nature. 384:1223-1224.

Zahed H; M. andal; D. Subodh; A.K. Biswas. 2006. Isolation of a NaCl-tolerant mutant of Chrysanthemum morifolium by gamma radiation: in vitro mutagenesis and selection by salt stress. Func. Plant Biol., 33: 91-101.

Zahed, H; A. K Mandal; S. Ratnakar; S. Datta. 2004. NaCl stress- its chromotoxic effects and antioxidant behavior in roots of Chrysanthemum morifolium Ramat Botanic Gardens & Floriculture, National Botanical Research Institute, Lucknow 226 001, India. Plant-Science. 166(1): 215-220

Zalewska-M; S. Sorvari; S. Karhu; E. Kanervo; and S. Pihakaski. 2001. In vitro formation of adventitious meristem and its significance for mutation breeding of Dendranthema grandiflora. Acta-Hort. 560: 225-228.

الملخص العربى

احداث طفرات فى نباتات الأقحوان المروية بماء مالح باستخدام أشعة جاما.

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**الزهور ونباتات الزينه وتنسيق الحدائق - كلية الزراعة - جامعة الأسكندرية

يتناول هذا البحث دراسة التأثير المنفرد لكل من أشعة جاما والملوحة وكذلك تأثيرهما المشترك على نبات من نباتات الزينة، واسع الانتشار فى جمهورية مصر العربية ، وهو نبات الأقحوان الذى يعتبر نباتا حوليا شتويا ، وذلك بغرض استنباط أجيال منه لها القدرة على مقاومة الملوحة. يتناول البحث أيضا مدى تأثر انزيم البيروكسيديز فى نبات الأقحوان بأشعة جاما والملوحة و تمت دراسة الإنزيمات فى نهايه الجيل الثانى.

وقد زرع نبات الأقحوان فى أرض رملية رويت بمياة تحتوى على تركيزات مختلفة من الملح تراوحت بين صفر، ٥٠، ١٠٠، ١٥٠، ٢٠٠ ملى مولمن كلوريد الصوديوم.

تمت هذه الدراسة خلال الفترة من ٢٠٠٤ -٢٠٠٥ ، حيث أجريت التجارب داخل صوبة مزرعة كلية الزراعة بجامعة الأسكندرية. عولجت البذور بجرعات من أشعة جاما قدرها ٥، ١٠، ١٥، ٢٠ كيلوراد. بعد إنبات البذور المشعة لنبات الأقحوان نقلت النباتات إلى أرض رملية رويت بمياه متباينة الملوحة (٥٠، ١٠٠، ١٥٠، ٢٠٠ ملى مول)، وذلك من أجل تتبع تأثير كل من أشعة جاما ، وتأثير الملوحة منفردين ، وتاثيرهما المشترك. حيث تمت دراسة الصفات التالية لكل نبات على حدة: ارتفاع النبات - عدد الأوراق - عدد الأفرع الرئيسة - المحتوى الكلوروفيللى للأوراق - الوزن الطازج والجاف للأوراق - عدد النورات - الوزن الطازج والجاف للنورات- قطر النورة - التغيرات المورفولوجية - الطفرات - نشاط انزيم بيروكسيديز

ويمكن تلخيص النتائج بأن: تأثير أشعة جاما كان معنويا على كل الصفات ، ماعدا الوزن الجاف للأزهار فى الجيلين الأول و الوزن الجاف للأزهار وعدد الأفرع الرئيسة فى الجيلين الأول والثانى. عند ٥ كيلوراد زاد الوزن الطازج للأوراق وعدد الأزهار وقطر الزهرة ووزنها الطازج فى الجيل الأول. وكان تأثير الملوحة معنويا على كل الصفات المدروسة فيما عدا الوزن الجاف للأزهار فى الجيلين الأول الثانى، وقطر الزهرة فى الجيل الثانى. ولقد كان التأثير المشترك لأشعة جاما والملوحة معنويا مع معظم الصفات، ما عدا عدد الأوراق ووزنها الجاف، وعدد الأفرع الرئيسة ووزن النورات الجاف فى الجيل الأول، ومعنويا مع عدد ووزن الأوراق الجاف و الطازج و عدد الأفرع الرئيسة. عند ٥ كيلوراد مع ٥٠ ملى مول زاد عدد النورات ووزنها الجاف وقطر النورة فى الجيل الأول. إنزيم بيروكسيديز أظهر نشاطا ملحوظا فى الجيل الثانى عند ١٠ ، مع ٥٠ ملى مول ، وعند ١٠ كيلوراد مع ١٠٠ ملى مول، و عند ١٥ كيلوراد مع ٢٠٠ ملى مول.

التوصيه:- تحتاج النتائج إلى استمرار التجارب مع معاملات متعددة بالمواد المطفرة حتى يمكن الحصول على طفرات ينتج عنها أصناف مقاومة للملوحة وذات صفات تجارية مرغوبة.