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Effect of Gamma Radiation on Some Morphological and Biochemical Characters of *Tagetes Erecta* Grown in Saline Soil.

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ABSTRACT

This work concerns with the study of the independent effect of each gamma radiation and salinity as well as their combined effect on some vegetative and flowering characters, as well as enzyme peroxidase of *Tagetes erecta*.

The study was carried out in the farm of the faculty of Agriculture, Alexandria University. Seeds were cultivated in saline soil for two successive generations (M1 and M2) during 2003 and 2004. Seeds were irradiated by gamma radiation at doses of 0, 5, 10, 15 and 20 Kr. After germination, the irradiated seeds were transferred into saline soil and peatmoss to study the combined effect of both gamma radiation and salinity on the plant. The following parameters were studied for each plant: plant height, number of leaves, number of main branches, chlorophyll content, leaves fresh and dry weight, inflorescence number, diameter, and fresh and dry weight, morphological changes, and peroxidase activity.

The results illustrated that salinity caused decrease of all studied parameters of the first generation. It was also found that the plant height decreased by increasing the doses of gamma radiations during the first and second generations. However, the number of leaves as well as their dry weight increased at 10 Kr during the second generation. While, the number of leaves as well as their fresh weight increased at 5 Kr during the first generation. With regard to the number of inflorescence and their fresh weight it increased in the second generation at the same dose (5 Kr). At 15 and 20 Kr the color of inflorescence showed clear variations between the first and the second generation. It was also found that the enzyme peroxidase displayed more activity at 10, 15 and 20 Kr during the second generation in the saline soil.

INTRODUCTION

A primary objective of agriculture is to provide the food and fiber needs of humans. This need is proportional to population. The expected population, world wide, is to be 8.2 billion by 2025 (U.N.1990). These increases in population will require an increase in agricultural production of about 40 to 50% by 2025. About two-thirds of these needed require increasing in food production in developing countries and must produced from the existing crop land (Alexandratos, 1988).

Soil salinity is a major problem in arid and semi-arid regions, where rainfall is insufficient to leach salts out of the root zone. Newly established irrigation projects, with improper planning and management practices may also add salts to soils. Salinization of millions of hectares of land continues to reduce crop productivity severely worldwide. Of the approximately 13 billion hectares total land on earth, about 1 billion are affected by salinity. More than 80 million hectares of such soils are in Africa (Tester, and Davenport, 2003). The significance of soil salinity for agricultural yields is enormous. The agricultural problem of salinity tolerance is probably best tackled by either altering farming practices or by implementing programmes to increase salt tolerance of plants, by either traditional breeding or genetic manipulation technologies. In this way, yield can be increased on salt affected soils whilst they are being remediated and encountered saline sub soils.

Tagetes erecta is a golden flower belong to the family Composite. This species is a double purpose plant .i.e used as ornamental and aromatic plant. The petals of the flowers of some varieties can be eaten. A yellow dye obtained from the flowers can be used as a saffron substitute for coloring and flavoring foods.

The responses to salinity vary not only among the different ornamental crops but also among the different organs of a plant. Depending on osmotic upset, some physiological changes occur in stomata conductance,

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transpiration, photosynthesis, chlorophyll content and root and leaf activity. Consequently, reduction of flower quality (color, size, stem thickness and length) and yield might be observed (Küçükahmetler, 2003).

Munns, (2002) mentioned that *Tagetes erecta*, grown in soil having E.C of 6.0 dSm^{-1}, vegetative growth and flower production were decreased. Moreover, Atam et al., (2002) reported that, in *Tagetes erecta*, the plants failed to establish at a salinity of 8 dS m^{-1}. Chakraborty and Sadhu (1990) reported that in Callistephus, Calendula, and Tagetes plants were grown in soil at 3 levels of salinity (0.5, 1.0 and 2.0 dSm^{-1}), and with increasing salinity, the vegetative growth decreased in all 3 cultivars. Flower diameter, weight and longevity were most reduced; the number of seeds/flower and the weight of 100 seeds decreased with increasing salinity. Jacobsen et al (2001) reported that the Amaranthus demonstrated very little ability for regulation of leaf water potential and stomatal conductivity, and the plants died at high salinity levels.

Indeed, 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 ORS (Oxygen Reactive Species) and also leads to plant damage, adaptation effectors that mediate ion homeostasis biosynthesis, toxic radical scavenging. Antioxidant resistance mechanisms may provide a strategy to enhance salt tolerance (Charpanzadeh et al., 2003). Under salinity stress peroxide activity of chirysanthemum chanetii maintained high levels (Chen et al., 2003). Also, Khan and Darell (2004) found that under salinity stress plants activate antioxidative enzymes as a part of protection mechanism. The enzymes metabolism is affected first before any visual effects of salinity stress are noted.

The effect of gamma irradiation, 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. Rahi et al. (1998) found that *Tagetes erecta*, and 3 Gladiolus cultivars were studied under saline 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 stressed soil. Zahed et al. (2006) found that a stable NaCl-tolerant mutant (R1) of *Chrysanthemum morifolium Ramat* has been developed by in vitro mutagenesis with gamma radiation (5 Gy). Enhanced salt tolerance of the R1 mutant was attributed to increased activities of reactive oxygen species scavenging enzymes, namely superoxide dismutase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase, and to reduced membrane damage. 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. Xiao and Zhao (1994) Treated young spikes of six spring wheat cultivars with 1-2 kr and were cultured in medium containing from 0.5 up to 2.5% NaCl. They concluded that 1 krad and 1% NaCl were the optimum doses.

Omar et al. (1988) studied the effect of gamma rays and NaCl on *Helianthus annuus* callus and reported that NaCl caused a significant reduction in callus fresh weight, while contents of protein soluble carbohydrates and ribonucleic acid increased at 2% NaCl level. Salam (1991) treated wheat varieties with gamma rays to obtain mutant genotype tolerant to environmental stresses (salinity and drought); and found the best dose was 7.5krad for variety saka8, while 10 krad dose was the best dose fore variety Giza 157. Both doses gave increase in yield and yield rated traits under salinity and drought streets.

Therefore, the objective of this work concerned with: (1) the effect of saline soils and different doses of gamma radiation on plant growth. (2) the possibility of inducing mutation to develop salt resistant plant. (3) investigate of the role of peroxidase enzyme activity in the ability of salinity tolerant plant.

MATERIALS AND METHODS

An open field experiment was conducted in the Agriculture research station (Abis) of faculty of agriculture, university of Alexandria Egypt. This study was carried out during 2004, 2005. Two series of experiments were conducted during this period. The first one dealt with the M_1- generation, while the second one dealt with the M_2 - generation.

1- Seeds

Seeds of *Tagetes erecta* were obtained from Floriculture and Ornamental Horticulture. Research branch, Antoniades Garden Alexandria, Egypt.

2- Radiation

Gamma–rays used in this study were generated from 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- Soil.

The soil was obtained from agriculture Research

Table 1. Main chemical and physical characteristics of the studied soil

Parameters		Parameters	
*pH	7.1	Soluble ions($meqL^{-1}$)	
*EC (dSm^{-1})	8.2	Na^+	31.2
Soil texture	Silty clay loam	K^+	15.8
% O.M	1.89	Ca^{++}	5.5
% T.N	0.89	Mg^{++}	4.6
P(Olsen) μgg^{-1}	16.3	HCO_3^-	2.5
K (Avai.) μgg^{-1}	29.28	Cl^-	17.2
		SO_4^{--}	2.4

*measured in saturated soil paste extract

station (Abis). It was collected from the upper soil layer(0-20cm). The soil was air dried, ground, passed thorough a 5 mm mesh screen, and thoroughly mixed, and analyzed for some chemical and physical characteristics according to the methods outlined by Page et al. (1982). The Main chemical characteristics of the soil are presented in Table (1)

2-Experimental Design

The lay–out of the experiment was designed to provide complete randomized blocks in factorial experiment containing three replicates.

2-1 - Preparing The seeds .

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

2-2- Irradiation Treatments

The dry seeds of *Tagetes erecta* were exposed to five different doses of gamma rays from Co-60 in Gamma–cell on March 2004. The used doses were 0, 5, 10, 15, and 20 Kr. The dose rate was 763 and 668r/sec in the first and second seasons, respectively.

2-3 Cultural

In the first season, the treated seeds, with Gamma – rays were germinated in sandy soil in seed-pans. Each seed pan, containing 70 seeds, was used as a plot within each replicate and set in the greenhouse and watered thoroughly. After 25 days, when seedling had reached approximately 5cm height, one seedling was transplanted into ceramic pot of 25 cm in diameter and 30 cm height. The pots were divided into two equal parts; the first part was packed with the studied soil, while the second one was packed with peat moss for using as a control.

Before planting, uniform rates of NPK fertilizers were added at the rate of 150 Kg fed^{-1} as superphosphate and 50 Kg fed^{-1} potassium sulfate. Nitrogen was added at the rate of 300Kg fed-1 in three equal doses (initially, 25 and 50 days) of cultivation date.

Experimental Data

The following characteristics of the cultivated plants were recorded for two successive years. Each part,of the gamma rays treated seeds, had five treatments and three replicates

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 units caporal leaf chlorophyll meter of Minolta crop.

4-Fresh and dry weight

At harvest time, plants were cut at 1 cm above the soil. The leaves were washed with 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 season.

B-Flowering parameters

1-Number of inflorscences per plant

The number of inflorscences was calculated from the average number of three plants of treatments.

2-Diameter of inflorscences

The diameter of inflorscences 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 calculated at the end of the two generations.

4-Fresh and dry weight

At harvest time, plants were cut at 1 cm above the soil. The flowers were washed with tap water and then by distilled water, air dried to determine the fresh weight, then oven dried at 60 °C for 24 hours to determine the oven-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) were 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. Grinding was continued until the tissue was well macerated and the mixture is homogenous. 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 storaged 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 (Palanichamy and Siddig, 1977).

Effect of radiation on morphological variations

All plant 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 ver 8.1, 2002.

RESULTS AND DISCUSSION

1--Effect of salinity on vegetative parameters

1-1-plant height

As shown in Table 2, the mean plant height was significantly low for plant grown in saline soil in first generation as compared to the control, meanwhile it showed no variation in the second generation.

Greenway and Munns 1980, found that the growth of cells is primarily correlated with turgor potential, and decreased turgor is the major caused of inhibition of plant growth under saline condition. The absence of variation in the plant height in the second generation could be due to the adaptation of the plant formed in the seeds of the first generation to salinity. The physiology of plant adaptation to salinity has been reported by Rhoades and Loveday (1990), and Munns., (2002).

1- 2-Number of leaves per plant.

According to the analysis of variance, the variation in the number of leaves per plant with soil salinity was significant in the first generation and the second generation.

The number of leaves was lower by 4.13% and 0.69% in saline soil related to peatmoss, in the first and second generations, respectively (Table2).

It is clear that the salinity caused marked drop in the number of leaves in the first generation as compared to slight decrease in the second generation. The great drop during the first generation indicates that salinity caused a depression in the growth rate of the plant which was translated into decrease of the number of leaves. Meanwhile, the slight decrease in the number of leaves in the second generation may be related to the adaptation of the plant to the high salinity. This result seemed to agree with Chakaraborty and Sadhu (1990), Roades and Loveday (1990) and Munns, (2002). Also, Haoula (2002) found that increasing salinity resulted in a decrease in the number of flower buds per plant and in vegetative growth.

1-3- Number of the main branches.

The mean number of the main branches in the first generation was lower by about 34% in saline soil relative to the control. In the second generation, the branches number showed more decrease than that occurred in the first generation, where it decreased by 39% in saline soil relative to the control (Table2).The results indicate that the number of main branches had decreased with salinity in first and second generations which means that salinity had pronounced effect on the number of main branches. These results are in agreement with Chakraborty and Sadhu (1990) who reported that Callistephus, Calendula and Tagetes plants grown in a fertilized alluvial clay soil at 3 levels of salinity (0.5, 1.0 and 2.0 mmhos cm^{-1}), with increasing salinity, the branching was reduced by 40% and 33.6% for Tagetes and Calendula, respectively.

1- 4-Total chlorophyll content of leaves

The mean values of the total chlorophyll content of leaves(Table 2) were low by 10% and 4.5% in saline soil in the first and second generation, respectively,

relative to the control. These results indicate that high salinity caused a significant decrease in the chlorophyll content of leaves. The chlorophyll content demonstrated more decrease with high salinity in the first generation than occurred during the second generation. This means that high salinity caused deterioration of chlorophyll synthesis in the first generation as a result of sudden salinity effect, in the second generation the effect of salinity was some how less because the plant had adapted during the second generation to salinity variation. Yamane et al. (2003) reported that swelling of thylakoids is induced at the early stage of the damage when plants are affected by salt stress and leads to reduction in chlorophyll synthesis. Huany, (1987) found that increasing E.C of the growth media induced leaf yellowing in *Tagetes erecta.* Indeed, Munns, (2002) reported that, Na^+specific damage is associated with the accumulation of Na^+ in leaf tissues and resulted in necrosis of older leaves, starting at tips and margins and working back through the leaf. On the other hand, Jacobsen et al (2001) reported that the chlorophyll content expressed on leaf area basis, increased under conditions of salinity due to a reduction in leaf tissue water content.

1-5- Leaves fresh weight

The mean values of the leaves fresh weight in the first and second generation (Table 2) were lower by 8.23% and 1.76% respectively, in saline soil relative to peat moss. This means that, salinity caused significant decreases in leaves fresh weight, particularly in the first generation more than the second generation.

The high salinity may caused a disturbance in observation of different element by the plant which lead to the observation of low molecular weight elements than the higher molecular weight elements which consequently reflected on the fresh weight on the leaves. The reduction could be attributed to toxic effects of Na+ and Cl- in the physiologically active parts of tissues, and to inefficient compartmetation for these ions in vacuoles. Yeo and Flowers (1986) reported that high salinity caused a greater reduction in relative growth rate and leaf area ratio. Under salinity stress, decrease osmotic potential and increased turgor potential were accompanied by an enhanced Na^+ and Cl^- concentrations in the leaves, and alteration of nutrient uptake. These results were in agreement with Charpazadeh et al., (2003). It has been found also that growth and yield reductions occur as result of the shortening of the lifetime of individual leaves, thus reduction of net productivity and crop yield (Munns, 2002).

1- 6-Leaves dry weight

The mean values of the leaves dry weight presented in Table2 indicated that the dry weight during the first generation was lower by 14% , and 6.63% for plants grown in saline soil relative to that in peatmoss in first and second generations, respectively. These results illustrated that the water content was also affected by soil salinity. The same trend was observed by Jacobsen et al. (2001) who found that leaf tissue water content was decreased under condition of salinity. Huany (1987) reported that increasing E.C of growth media reduced dry weight of *Tagetes erecta.*

2-Effect of salinity on flowering parameters

2-1-Number of Inflorescence

The number of inflorescence was lower by 13% and 9% for plant grown in saline soil (Table 2) relative to that grown in peatmoss in the first and second generation, respectively. These results indicate that high salinity lead to significant decreases in the number of inflorescence.

The effect of salinity on the Number of inflorescence was more pronounced with the first generation than in the second one. This means that the production of the plant decreased with increasing salinity. These results are in agreement with those of Haouala., 2002; and Sonneveld et al., 1999) who stated that the flowering yield was decreased by the addition of Na, when the plant exposed to high salinity for the first time but when the plant adapted to salinity, the production showed slight decrease. The same trend was observed by Zahed et al. (2004).

2-2 Inflorescence diameter

The inflorescence diameter decreased by 26% and 12% in saline soil relative to peatmoss in the first and second generation, respectively (Table 2). This parameter showed slight variation with high salinity in the second generation while in the first generation the variation was comparative wide. This means that high salinity had crucial role inhibiting the growth of the inflorescence which decreased of its diameter. Chakraborty and Sadhu (1990) reported that with increasing salinity, flower diameter was reduced in *C.chinensis* at 2 dSm^{-1}. The number of seeds/flower and the weight of 100 seeds decreased with increasing salinity in *C. chinensis* and *C. officinalis* but were less affected in *T. signata.*

2-3-Inflorescence fresh weigh

The mean values of the inflorescence fresh weight (Table2) for the first generation indicated that the inflorescence fresh weight was significantly lower by 5.3% when grown in saline soil relative to the peatmoss. In the second generation, the inflorescence fresh weight was significantly lower by 1.5% in saline soil relative to peatmoss.

Table 2. Effect of salinity on vegetative and flowering parameters of Tagetes erecta.

culture	Plant height (cm)	Leaves D.W g/plant	Leaves No No/plant	Chlor.	Leaves F.W g/plant	branchNo/plant	Inflo. F.W g/inflo	Inflo. Diam. cm	Inflo. No/plant	Inflo. D.W g/inflo
First generation										
Peat moss	121.86a	32.32a	345.2a	35.48a	165.92a	5.00a	55.06a	4.16a	27.36a	11.43a
soil	120.17b	28.88b	331.53b	32.17b	153.31b	3.73b	47.80b	3.3b	24.03b	10.54b
L.S.D	0.86	0.74	1.36	0.85	0.84	0.54	1.69	0.15	0.13	0.20
Second generation										
Peat moss	121.91a	30.38a	359.13a	32.40a	155.7a	4.46a	52.86a	4.294a	25.9a	11.26a
soil	122.01a	28.49b	356.60b	31.00b	153.5b	3.20b	50.20b	3.842b	23.6b	9.30b
L.S.D	1.01	1.70	1.38	0.60	1.11	0.68	1.27	0.16	0.72	0.13

The high salinity during the first generation led to a pronounced decrease in the inflorescence fresh weight.

This reduction could be attributed to toxic effects of Na^+ and Cl^- in the physiologically active parts of tissues, and to inefficient compartmetation for these ions in vacuoles (Yeo and Flowers, 1986)

2-4-Inflorescence Dry weight

In the first and second generations (Table2), the inflorescence dry weight was lower when plants grown in saline soil than in peatmoss. However, in the first generation it was lower by 8% in saline soil and the second generation by 21% in saline soil relative to thecontrol

In contrast to that mentioned for all parameters the effect of salinity on the inflorescence dry weight was higher in the second generation than in the first generation. The reduction could be attributed to the effect of high salinity which caused a disturbance in plant uptake which lead to forming low molecular weight than higher molecular weight which consequently reflected on fresh weight and dry weight (Yeo and Flowers,1986) .

3-Effect of gamma radiation on vegetative parameters

3-1-plant height

The mean values of plant height, for the first and second generations presented in Table (3), indicate wide variation with the different levels of gamma radiation.

The result shows that gamma rays inhibited the height of the plants. However, in second generation the 10kr level caused an increase of 0.2% in the plant height but this increase was not significant. Otherwise, the higher doses of gamma radiation caused more decrease (2.7%) in the second generation. This means that with the exception of the case of 10 Kr, in the second generation the increasing dose of gamma-rays caused relatively slight decrease in the plant height. The reduction in plant height is known to be caused by the reduction in the level of IAA auxin (Chandorkar and Dengler, 1987).

Radiation can caused indirect damage in living systems by the various radicals in irradiated cells, it would be reasonable to conceder that the more OH- is produced, the more radiosensitive the tissue is.(Wada et al.,1998). 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 (Ichikawa, 1981).

These results are in agreement with Banerje and Datta (2002), Diltaet al. (2003) obtained similar effect of gammarays on the vegetative parameters of *Chrysanthemum*. 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 .Gorden (1958) has emphasized the changes in amount of auxin occurring as a result of radiation, as possible factor responsible for the decrease of growth.

3-2-Number of leaves per plant

In first generation, the number of leaves demonstrated slight increase (0.6-0.8%) with increasing dose of gamma radiation up to 10 Kr (Table 3), and in the second generation, the number of leaves increased also slightly by 0.6 % at 10Kr relative to the control. It appeared that the effect of gamma radiation on the number of leaves was more pronounced in the first generation than in the second one; particularly at 20 Kr .The decrease in first generation at 20 Kr was 22.2% relative to the control, while in the second generation the decrease was 2.1% than the control. This may be explained according to the fact that the plants produced from irradiated seeds with 20 Kr in the first generation

Table 3. Effect of gama rays in yegetaire and flowering parameters of Tagetes erecta.

Gamma Levels	Plant height (cm)	Leaves D.W g/plant	Leaves No No/plant	Chlor	Leaves F.W g/plant	branchNo/plant	Inflo. F.W g/inflo	Inflo. Diam. cm	Inflo. No/plant	Inflo. D.W g/inflo
First generation										
0	123.6a	33.15a	353.83b	35.65a	156.5a	5.00a	56.33b	4.00b	25.58bc	10.75b
5	120.8c	33.86a	356.83b	35.58a	157.2a	4.5ab	58.83a	3.93c	26.33b	11.08a
10	122.2ab	31.15b	356.00a	34.83a	157.3a	5.00a	52.00c	3.53a	27.33a	10.76b
15	120.9bc	29.18c	350.0c	31.43b	151.18c	4.00bc	48.83d	4.4b	24.86cd	9.48c
20	118.9d	25.66d	275.16d	31.63b	153.3b	3.33c	41.66e	3.13c	24.36d	9.41b
L.S.D	1.35	1.16	2.15	1.34	1.32	0.85	2.01	0.23	0.78	0.21
Second generation										
0	124.2a	32.00a	359.83b	35.46a	157.4a	5.16a	57,66b	4.196b	26.20ab	11.683b
5	120.9b	30.2c	357.8bc	31.16c	155.1ab	4.00bc	60.83b	3.67c	26.7a	12.608
10	124.5a	32.51a	362.00a	33.0bc	153.4ab	4.33ab	54.66c	4.936a	25.33b	11.18c
15	120.8b	28.41c	357.50c	30.55bc	153.5bc	3.16dc	45.16d	4.088b	23.66c	9.933d
20	119.2c	24.41c	352.16d	28.3d	153.4c	2.500d	38.83e	3.441c	21.95d	9.53e
L.S.D	1.59	1.85	2.19	0.95	1.76	0.30	0.19	0.25	1.14	0.32

was highly affected by the gamma radiation while plants produced in the second generation were not affected pronouncedly by gamma radiation. This means that the variation in the number of leaves were significant in the second generation. This may be due to the changes in amount of auxin occurring as a result of radiation effect (Gorden, 1958). These results are in agreement with those reported by Banjerje and Datta (2002) and Dilta et al. (2003) on *Chrysanthemum*.

3-3-Number of the main branches.

The decrease in the number of main branches during the second generation was greater than that in the first one (Table 3). These findings are in agreement with those obtained by Bader et al. (2004) who reported that the reduction in the main branching of *Gomphorina globosa* may be due to the effect of on growth hormones such as canibal gibberellins, cytokinins and abssecic acid which play important roles in controlling activity. Similar results were obtained by Banerje and Datta, (2002) on *Chrysanthemum morifolium*. According to several studies, it is clear that gamma rays could lead to the mitotic activity of cambial cells which led to branches production increase (El Mahrouk, 2000), supply gibberellins, cytokininis, and abscisic acid which may played important roles in stimulating branches production increase (Chandokar and Dengler 1987, El Mahrouk, 2000), and increase nutrients which were not utilized in the stem elongation (chandokar and Dengler, 1987).

3-4-Total chlorophyll content of leaves

As shown in Table (3), the mean values of total chlorophyll content decreased with increasing gamma-rays in the first and second generations. However, this decrease was more pronounced in the second generation (6.9-12.1%) relative to the control, at 5 and 10Kr, respectively .While in the first generation this decrease recorded (0.2-0.3) at the same gamma rays doses.

It is known that the changes in chlorophyll are associated with the changes in chloroplasts (Preil1985). The important factors which control chloroplast differentiation are: genetic information present in plastids which contain the chloroplast DNA (Bidwell,1979), cytokinins which have been shown to control chloroplast differentiation independently of their action on cell division (Laloue,1978) and inorganic salts (magnesium, iron, copper, potassium and ammonium) which play important roles in the chlorophyll synthesis or metabolism (Bidwell,1979).

3-5-Leaves fresh and dry weight

Although, the leaves fresh weight in the first generation (Table 3) showed slight increase at 5kr and 10 kr (0.4-0.5%) it displayed decrease of about 3.4%with increasing gamma radiation. By contrast, with the second generation, the leaves fresh weight displayed decrease at all doses of tested gamma radiation. This may be due to the changes in amount of auxin occurring as a result of radiation effect (Gorden, 1958).

In the second generation the dry weight of leaves insignificantly increased at 10 Kr, while with 5, 15 and 20 Kr the dry weight decreased significantly (Table 3).

4-Effect of gamma radiation on flowering parameters

4-1-Number of inflorescence

In the first generation, the number of inflorescence attained the highest value (27.33) at 10 Kr while at 5, 15 and 20Kr it decreased. By contrast, in the second generation, the number of inflorescence decreased gradually from 26.20 to 21.95 with increasing gamma radiation (Table 3).

In comparison between the first and second season the number of inflorescence was more depressed in the second generation than in the first generation at 15 and 20 kr. It increased by 2.9 and 6.8% at 5 Kr and 10 Kr respectively in the first generation, while it increased by

1.9 % only at 5Kr. This may indicate that gamma radiation promote the production of inflorescence at 10 Kr particularly in the first generation. These results are in agreement with Banerji and Datta (2002), Zalweska et al., (2001) and Dilta et al., (2003). The decrease of number of inflorescence may be attributed to decrease in cell number and /or cell size, (Datta1987).

Bidwell (1979) mentioned that all steps in flowering process are programmed in totipotent cells of meristem. All that needed a trigger or a release that sets these cells on the way on the program for flowering. The capacity to flower is inherent. In this work, the doses of 5-10Kr stimulated the totipotent cells of meristem to change the developmental pattern from vegetative to floral and this might increase the number of inflorescence.

4-2- Inflorescence diameter

In the first generation, data in (Table 3) showed that the inflorescence diameter increased by 10% at 15Kr compared with control while increasing gamma doses decreased inflorescence diameter. In the second generation the inflorescence diameter increased gradually by 17.6% than the control at 10 kr but it decreased at higher gamma radiation levels.

These results revealed that the variation in the inflorescence diameter with the increasing gamma radiation was significant. This result was in agreement with Banerji and Datta (2002) on *Chrysanthemum morifolium*.

4-3-Inflorescence fresh and dry weight

The inflorescence fresh weight increased significantly at 5Kr by 4.4% and 5.5% in the first and second generations, respectively (Table 3). This means that 5 Kr caused promotion in the fresh weight of the inflorescence in both first and second generations which indicates that the small doses of gamma radiation may promote the fresh weight of the inflorescence. Similar results were obtained by Silveira et al., (1996) on sunflower. The inflorescence dry weight had a similar trend to that of fresh weight.

4-4- Induction of variation:

The plates in Fig.3 showed that treatment of 15Kr in the first generation (M1) induced change in inflorescence color that it turned from red to yellow while forothers, the red pigment was increased. In the second generation (M2), the dose of 20Kr formed two inflorescences in the same inflorescence and died after 10 days in the mutant flower (yellow ones). This change may be due to the effect of radiation on the genetic components of the treated plants which increased the genetic variation and caused greater frequencies of adaptive (including lethal) genotypes. These results are in agreement with Vieira at al. (1996) who found that several morphological characteristics in irradiated *Phaseolus vulgaria* with 20 and 25 kr from the M1 to M3. Zalewska et al. (2001) mentioned the effect of irradiation on Chrysanthemum morifolium at 20 Gy. All the changes which occurred in the M1 generation reoccurred in the M2 and M3 generations, while Banerji et al. (2002) found different types of morphological abnormalities in leaves and flowers. in chrysanthemum treated with gamma rays (1.0, 1.5 and 2.0 Kr),

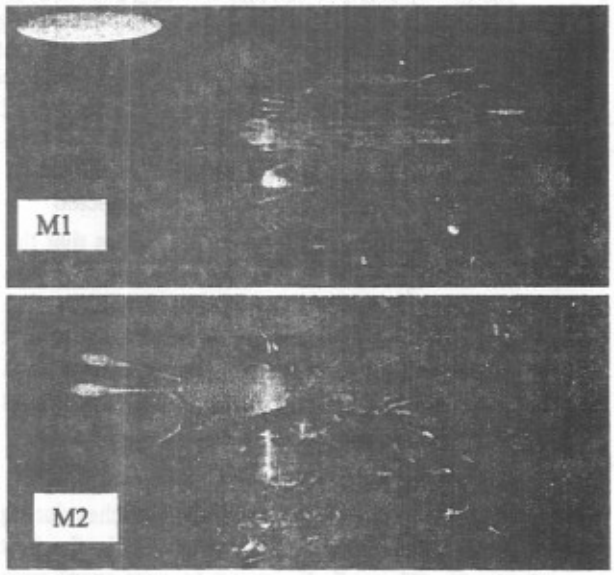

Fig 3. Variations in inflorescence shape and color

5-Combined effect of gamma radiation and salinity on Vegetative growth

5-1-Number of leaves.

Tables (4) showed that, in the first generation, the mean number of leaves grown in peat moss increased by 1.3% at 5Kr relative to control, but it decreased than the control at all other doses of gamma radiation.

5-2-Total chlorophyll content of leaves

As shown in Table (4) the chlorophyll content in plant leaves grown in peatmoss and exposed to gamma radiation displayed pronounced decrease in the second generations. Regarding both soil salinity and gamma radiation the chlorophyll content decreased also than the peatmoss in both generations.

Gamma rays may increase endogenous cytokines and nutrient components utilization which reflected on an increase in the leaf chlorophyll content. In this respect, Preil (1985) stated that changes in leaf chlorophyll were caused by genetic change in chloroplast DNA and gene and /or plastid changes.

5-3 Leaves fresh weight

The mean values of leaves fresh weight displayed high leaves fresh weight with plant grownin peatmoss with 5kr and with plants grown in saline soil with 5kr in

the first generation. However, there was clear decrease under the combined effect of soil salinity and gamma radiation in the second generation, while treatment with 10kr increased leaves fresh weight of plant grown in saline soil (Table 4). These results were agreement with Salam (1991) and Rahi et al. (1998).

6- Combined effect of gamma radiation and salinity on flowering growth

6-1.Number of Inflorescence

In the first generation, the mean number of inflorescence was increased by 16% at 10Kr and by 7% at 5Kr in peatmoss and saline soil, respectively relative to the control (Table 4).

The present findings agree with those of Bidwell (1979) who mentioned that all steps in flowering process are programmed in totipotent cells of meristem. The capacity to flowering is inherent, like the capacity to form leaves. In this work, the dose of 5Kr in saline condition stimulated the totipotent cells of meristems to change the developmental pattern from vegetative to floral and this might increase the number of inflorescence. Also, the increase in the mean number of inflorescence could be attributed to the increased number of main branches.

6-2-Inflorescence diameter

In the first and second generations, the data shown in Table (4) illustrated that the inflorescence diameter was increased by 6% and 19% at 5 and 15 Kr in peatmoss, respectively to the control.

6-3-Inflorescence Fresh weight

The mean values of the inflorescence fresh weight showed an increase with 5Kr in the first generation by 8% and 1% in peatmoss and saline soil, respectively, while it recorded an increasing of 15% in saline soil for the second generation with 5kr. This result agree with obtained by Salam (1991) who found that7.5 to 10Kr were the best gamma doses to give an increase in wheat yield under salinity stress.

6-4 Inflorescence dry weight

The mean values of the inflorescence dry weight displayed different trends of variations with soil salinity and gamma radiation in the first generation, in peat moss

Table 4. Effect of combined factors of different gamma and salinity levels of Tagetes erecta.

culture	Gama Level	Plant height (cm)	Leaves D.W g/plant	Leaves No No/plant	Chlor.	Leaves F.W g/plant	branchNo/ plant	Inflo. F.W inflo g/	Inflo. Diam. cm	Inflo. No/plant	Inflo. D.W g/inflo
First generation											
peatmoss	0	124 a	34.9	358.0a	37.3	159.4b	5.66	58.66b	4.23bc	26.8b	12.1b
	5	121 b	35.6	362.6a	37.9	162.1a	4.31	62.65a	4.49bb	26.6b	11.1c
	10	124 a	32.7	357.0b	37.0	159.6b	6.33	52.6c	3.83ad	31.1a	12.5a
	15	120bd	30.3	51.0cd	32.2	150.0d	4.65	50.0d	5.06ba	26.4b	10.1d
	20	119cd	28.0	297.3e	33.0	153.6cd	3.31	40.33f	3.18be	25.7b	10.3d
Saline soil	0	123ab	31.4	349.6d	34.0	153.3cd	4.67	54.0c	3.77cd	24.3c	9.3e
	5	120bcd	32.1	51.0cd	33.3	152.3d	4.65	55.0c	3.37bd	26.0b	11.0c
	10	119bcd	29.5	355.0b	32.6	154.8c	3.66	51.33d	3.23ce	23.5c	8.9f
	15	121b	28.0	349.0d	30.6	152.3d	3.32	47.66e	3.37cd	23.3c	8.8f
	20	118d	23.3	253.0f	30.2	153.3cd	2.65	43.00f	3.03ef	23.0e	8.3e
L.S.D		1.87	N.S	2.97	N.S	1.82	N.S	2.78	0.11	1.08	0.2 8
Second generation											
peatmoss	0	124.7	33.6	361.6	35.6a	162.8ab	5.66	62.3a	4.31b	27.8	12.2bc
	5	120.5	30.9	359.6	31.1b	159.1b	4.33	60.3a	3.93c	27.1	12.5b
	10	123.7	32.9	363.0	35.8a	149.9d	6.00	61.3a	5.54a	27.1	12.0c
	15	121.3	29.8	359.0	31.4b	151.8cd	3.60	49.3cd	3.96c	24.7	10.5e
	20	119.3	24.5	352.3	28.5cd	154.8bc	2.66	42.0ef	3.72cd	22.7	9.8f
Saline soil	0	123.7	30.4	358.0	35.8a	152.1cd	4.66	53.0b	4.08b	24.5	11.1d
	5	121.3	29.5	356.0	31.1b	151.1cd	3.66	61.3a	3.40d	26.3	12.6b
	10	125.3	32.1	361.0	30.1bc	156.8b	2.66	48.0d	4.3b	23.5	16.3a
	15	120.4	26.9	356.0	29.7c	155.3bc	2.66	41.0f	4.2b	22.6	9.3g
	20	119.2	23.4	352.0	28.1d	152.1cd	2.33	35.0g	3.1d	21.1	9.2g
L.S.D		N.S	N.S	N.S	1.31	4.25	N.S	3.66	0.34	N.S	0.45

the inflorescence dry weight increased by 3.3% than the control at 10kr (Table 4) In the second generation, the gamma radiation of 5Kr and 10Kr promoted the inflorescence dry weight by about 3.3% and 33.6% respectively, in saline soil.

Although the inflorescence dry weight decreased in the first generation under the combined effect of salinity and gamma radiation, it showed an increase in the second generation. This means that the plant of the second generation could adapt to high salinity and higher gamma radiation which resulted in high production of inflorescence. El Halim et al. (1989) recorded an increase in germination, plant growth and yield characteristics with gamma radiation between 2 and 8 Kr under salinity stress.

6-5-Peroxidase isozymes

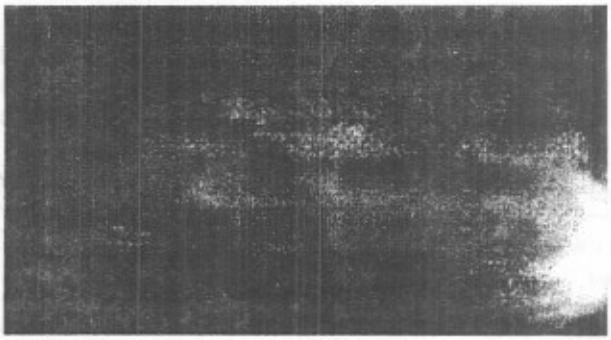

Fig 2. Peroxidase Isozymes Patterns

The cathodal peroxidase isozymes bands were designated C1, C2, and C9, while the anodal isozymes were designated A1, A2, and A4 according to their mobility from the original line. Variations for band numbers and densities were encountered for *Tagetes* under different treatments (Fig. 2). Figure (3) showed the Zymogram of the peroxidase isozymes patterns for the ten treatments of *Tagetes erecta.*

The data showed that peroxidase isozymes showed different activities in *Tagetes erecta.* The first Cathodal band (C1) was absent in two treatments (plants cultivated in peatmoss and irradiated by 20Kr and plants cultivated in saline soil and irradiated by10Kr). This may be due to stress of high doses of gamma radiation and also the combination of high salinity and radiation. The second Cathodal band (C2) was present only in three treatments (plants cultivated in peatmoss and irradiated by 5, 10Kr and plants cultivated in saline soil and irradiated by15Kr).This excess band could be referred to the role of gamma radiation which helped plants to form excess peroxidase isozymes bands which were not been in non-irradiated plants. The third Cathodal band (C3) was detected in all *Tagetes* treatments and it was heterozygous (showed as double rectangle) in plants cultivated in peatmoss and irradiated by 15Kr, and plants cultivated in saline soil and irradiated by 20, 15, and 10Kr.

From the previous zymogram, it was noted also that gamma radiation affected the density of isozyme as shown in Fig. (3), which means that the plants withstand the effect of salinity by gamma radiation. The Anodal band had no presence in all treatments.

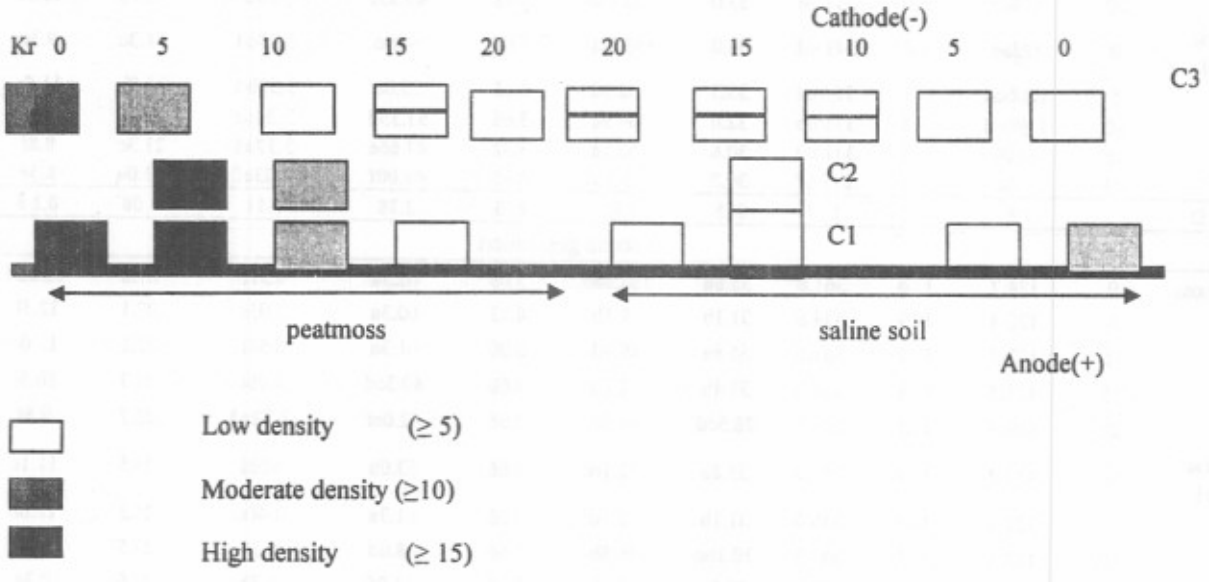

Fig. 3. Zymogram of the peroxidase isozymes patterns of Tagetes cerecta

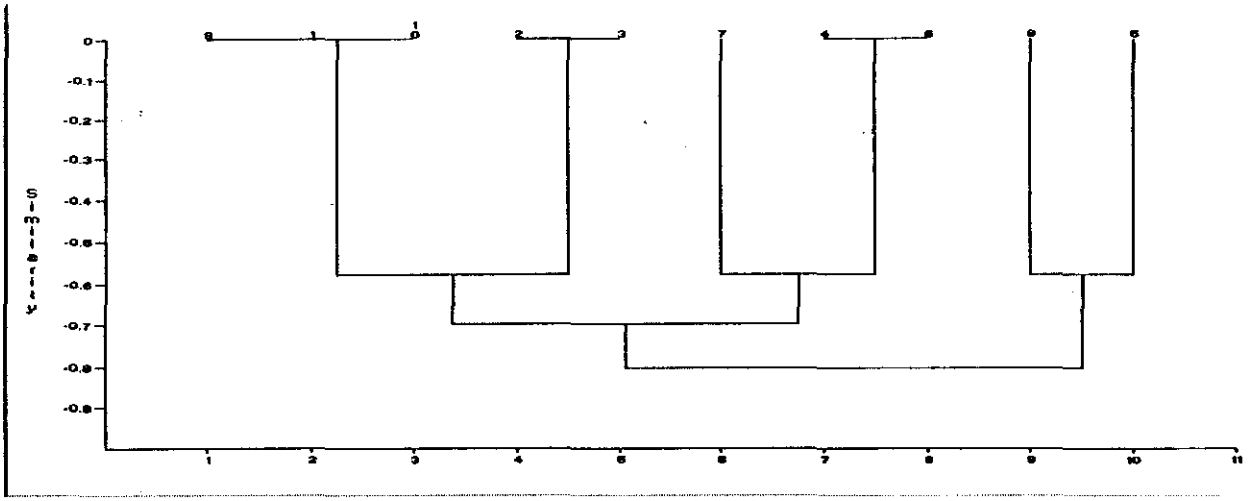

Fig. 4. Genetic relationship among Tagetes cerecta samples based on Peroxidase isozymes patterns

Such mechanism may be related to genetic changes or osmo-regulation process through ion exchanges between plant and the soil.

Chen et al. (2003) found that under salt stress, the peroxidase activity maintained high levels. Bowler et al., (1992) found that enzymes are nuclear encoded and the enzyme is present in all aerobic organisms and in all sub cellular compartments susceptible of oxidative stress. Chaparzadeh (2004) reported that in leaves of Calendula, peroxidase activity decreased by 22 and 28% at 50 and 100mM salinity respectively, such differences may be attributed to difference in the age of the plant. Sairam et al. (1998) found that, drought imposed at two different levels induced an increase in H_2O_2 accumulation and lipid peroxidation and decreased in ascorbic acid content. They noticed that the tolerant genotype had the highest antioxidant enzymes such as superoxidase dismutases, ascorbate peroxidase and catalase activity under water stress in comparison to the sensitive genotype. Zahed (2006) found that, on Chrysanthemum morifolium, enhanced salt tolerance of the R1 mutant by increasing activities of reactive oxygen species scavenging enzymes, namely superoxide dismutase. Also, Fabio et al., (2004) found that in *Vigna unguiculata* leaves exposed to a salt-induced oxidative stress, the 200 mM NaCl caused almost complete cessation of leaf relative growth rate in parallel with the transpiration rate. The restriction in leaf growth was associated with a progressive increase in membrane damage, lipid peroxidation and proline content.

6-6-Genetic relationship among Tagetes cerecta based on Peroxidase isozymes patterns

It is clear from Fig.(4) that at 10% of genetic similarity, all treatments samples were divided into two main clusters, the first contained samples of 20 Gama ray + Peatmoss and 10 Gama ray + salinity. On the other hand, at 20 % of genetic similarity, the second cluster includes two sub clusters. The first contained two groups, samples of 15 Gama ray + salinity composed the first, but 15 Gama ray + peatmoss and 20 Gama ray + peatmoss presented the second. In addition, the second sub cluster contained two groups. Firstly, composed of 5 Gama ray + peatmoss and 10 Gama ray + peatmoss. Finally, the second included Gama ray + peatmoss, 5 Gama ray + salinity and Gama ray + salinity samples.

A way from our expect, it was shown that all the treated samples differed in their genetic similarity as a result of gama ray and different levels of salinity. Also, it is clear from this pattern that the two treatments of 0 Kr + peatmoss and 5 Kr + salinity were similar in their peroxidase isozymes patterns, i.e. according to the peroxidase activity pattern it could be grouped these two treatments in one group which means that the 5Kr dose under saline condition was similar to control. Finally it could be concluded that the plants withstand the effect of salinity by gamma radiation.

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الملخص العربي

تأثير أشعة جاما على بعض الصفات المورفولوجية والبيوكيميائية لنبات القطيفة النامى فى أرض ملحية

فاطمة كمال شريف ، مصطفى رسلان مصطفى ، فاطمة زين السماك

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 القطيفة الذى يعتبر نباتا حوليا صيفيا، وذلك بغرض استنباط أجيال منه لها القدرة على مقاومة الملوحة. يتناول البحث أيضا تأثر انزيم البيروكسيديز بأشعة جاما والملوحة.

تمت هذه الدراسة خلال الفترة من ٢٠٠٣ -٢٠٠٤، حيث أجريت التجارب بمزرعة كلية الزراعة بجامعة الأسكندرية، زرع نبات القطيفة فى أرض ملحية موسمين متتالين (M_1, M_2) عوملت بذور النبات بجرعات من أشعة جاما قدرها ٥، ١٠، ١٥، ٢٠ كيلوراد. بعد إنبات البذور المشعة لنبات القطيفة نقلت النبتات إلى أرض ملحية وأخرى غير ملحية (بيتموس)لدراسة تأثير أشعة جاما منفردة على النباتات التى نمت فى كلتا الحالتين، وكذلك دراسة التأثير المشترك لأشعة جاما والملوحة. ركزالبحث على دراسة الصفات التالية لكل نبات على حدة: ارتفاع النبات - عدد الأوراق - عدد الأفرع الرئيسة - المحتوى الكلوروفيللى للأوراق - الوزن الرطب الجاف للأوراق - عدد النورات - الوزن الرطب الجاف للنورات- قطر النورة - التغيرات المورفولوجية - الطفرات - تركيز انزيم بيروكسيديز فى نبات القطيفة

يمكن تلخيص النتائج فيما يلى: تأثير الملوحة على ارتفاع النبات بدا غير معنوى فى الجيل الثانى بينما كان تأثير أشعة جاما معنويا على كل الصفات للجيلين الأول والثانى.

و لم يكن التأثير المشترك لأشعة جاما والملوحة على بعض الصفات معنويا فى الجيل الأول، مثل عدد الأفرع الرئيسة، الكلوروفيل، الوزن الجاف للأزهار، و فى الجيل الثانى لم يكن معنوياعلى عدد النورات، عدد الأفرع الرئيسة، عدد الأوراق، الوزن الجاف للأوراق،ارتفاع النبات. كما قل ارتفاع النبات مع زيادة جرعات الإشعاع فى الجيلين الأول والثانى. وقد ازداد عدد الأوراق ووزنها الجاف فى الجيل الثانى عند ١٠ كيلوراد من أشعة جاما. وعند ٥ كيلوراد زاد عدد الأوراق ووزنها الرطب، وعدد النورات ووزنها الجاف فى الجيل الأول، بينما زاد عدد النورات ووزنها الرطب فى الجيل الثانى.

وقد أدت الملوحة نقصا فى قياسات كل الصفات المدروسة فى الجيل الأول، عند ١٥،٢٠ كيلوراد تم الحصول علىتغير فى لون النورة shape abnormalities فى الجيل الاول و فى الجيل الثانى. وقد أظهر إنزيم بيروكسيديز نشاطا ملحوظا عند ١٠، ١٥ ، ٢٠ كيلوراد فى الأرض الملحية وخاصة فى الجيل الثانى.