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EFFECT OF GAMMA IRRADIATION ON INCREASING SALINITY TOLERANCE OF MICROPROPAGATED BANANA PLANTS

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ABSTRACT

This investigation was carried out on micropropagated plants of banana cv. Williams, to study the effect of sea salt (0, 2000, 4000, 6000 and 8000 ppm), and gamma irradiation (0, 10, 20, 30, 40, 50 and 60 Gy) either alone or in combinations on increasing salinity tolerance. Data were calculated *in vitro* as well as under greenhouse conditions.The obtained results indicated that the gradual increase in sea salt levels was negatively correlated with all growth parameters (survival percentage, shoots and pseudostem height, leaf and root numbers as well as shoot fresh and dry weights), anatomical parameters of leaf (leaf blade and midrib thickness, number and diameter of air cavities, number of midrib vascular bundles), and root (root and vascular cylinder diameter, cortex width and number of vessels) and photosynthetic pigments. Moreover, sea salt application was positively correlated with total free amino acids, proline, total soluble phenols,sugars(reducing and non-reducing) as well as Na and Cl concentrations, meanwhile, N, P, K, Ca and Mg concentrations as well as K/Na ratio decreased under salt stress conditions and all plants were dead when sea salt was applied at 8000 ppm. Exposure to gamma

irradiation (10 and 20 Gy) either alone or combined with sea salt application had stimulative effects on growth characters, anatomical parameters, organic components as well as minerals. Meanwhile, higher doses (30 and 40 Gy) adversely affected all the tested parameters. Photosynthetic pigments, however,were negatively correlated with all doses under study and the (LD_{50}) was observed beyond 50 Gy.

Key words: *banana,dna fingerprinting, gamma irradiation, micropropagation,salt stress, sea salts.*

1. INTRODUCTION

Banana is a staple food crop for the people living in tropical and subtropical countries (Lee *et al.*, 1997). Environmental stresses in arid and semi-arid areas, mainly salt limit growth and productivity of most banana species (Shin *et al.*, 2000). The excess of salt in the soil or in irrigation water is one of the biggest problems in agriculture since almost all cultivated plants are sensitive to it (Jóse *et al.*, 2000). Moreover, a low tolerance to excess salt in the soil or water is a major limitation to growing crops or fruits (Stefano, 2001). In this concern, Israeli *et al.* (1986) studied the effect of sodium salt on growth and productivity of banana and found that increasing of salt concentration in the irrigation water led to a marked decrease in the growth and productivity of field grown plants. Several methods of selection for enhanced salt tolerant genotypes have been developed (Tal, 1993). Approaches based on biochemical, molecular and physiological studies of tissue culture can provide more reliable means for the development of salt-tolerant plants (Winicov and Bastola, 1997). *In vitro* plant tissue culture has been proposed as a useful, quick and economical tool to evaluate salt tolerance (Jośe *et al.*, 2000) as a system for testing and selection for salt tolerance (Cano *et al.*, 1998) and plant material selected from salt stressed cultures can be used to establish plantations in saline soils (Shin *et al.*, 2000). Plant tissue culture techniques provide a promising and feasible approach to develop salt tolerant plants. This is because of the totipotency of plant cells which contain a complete genome, thus, are at least potentially capable to becoming entire plants (Murray *et al.*, 1984). In addition

in vitro mutation induction has been used to improve vegetatively propagated banana (El-Agmawy and Afza, 2000).Genetic modification of crop plants to improve their salt tolerance is a possible way for increasing their productivity especially for regions of the world where arable lands must be extended to marginal area and sometimes irrigated with saline water (Dorion *et al.*, 1999). Mutants induced in response to environmental stress appeared as a promising way to detect genes involved in normal and stressed-plant behaviour (Nicole, 1996).

This investigation was conducted to study the potential of gamma irradiation (as a mutagen) in inducing mutant plants of banana cv. Williams, as well as to study the potential of tissue culture technique in producing banana plants (either normals or mutants) tolerant to salinity stress.

2. MATERIALS AND METHODS

2.1. Experiment design

This work was carried out in the Biotechnology,Bioengineering and Plant Analysis Laboratories, Fac. of Agric., Univ. of Cairo, and Middle East Regional Radioisotope Center for the Arab Countries as well as the Anatomy Laboratory, Fac. of Science, Ain Shams Univ., Egypt, during the period from 2000-2003.

Banana cv. Williams growing in the experimental field of the Agriculture Research Center, at Kanater El-Khairia was utilized as the mother plant material for obtaining the explant.Small suckers of about 50-70 cm in length were carefully cut from mother plants growing in the field and taken immediately to the lab.The older leaves and extraneous corm tissues were carefully removed with a stainless steel knif. Shoot tip explants were prepared by removing the outer leaf primordia and excising of shoot tip containing the apical meristem and 2-4 leaf primordia.The isolated shoot tip explants were washed in running tap water for 20 min., soaked in antioxidant (citric/ascorbic acids at 100/150 mg l^{-1}) solution for 20 min, then soaked under aseptic conditions in 80% clorox (5.25% sodium hypochlorite NaOCl) for 20 min., Tween 20 (0.1%) was used as surfactant.The shoot tip explants were then rinsed twice for 5 min., each in sterile distilled water to emove all traces of clorox. The

meristem tips with at least 1-2 leaf primordia were excised and used as explant material.

The following experiment was conducted with Murashige and Skoog (MS) basal medium (1962) .The pH of the prepared medium was adjusted at 5.7±0.1 prior to addition of agar at 7 g l^{-1}. The medium was distributed into the culture jars (325 ml) where each jar contained 45 ml of the medium. The jars were immediately capped with polypropylene closer, then were autoclaved at 121°C at 15 lbs/inch for 20 min..

The MS basal medium supplemented with 3 mg l^{-1} BA + 30 g l^{-1} sucrose + 7 g l^{-1} agar was used as an initiation medium for culturing sterilized shoot tip explants. Shoot tip explants were incubated at day and night temperature of 27± 2°C. Light was provided by fluorescent lamps giving intensity of 1500 Lux for 16 hours per day.

Established shoot tip explants were transferred and cultured individually on shoot multiplication MS basal medium supplemented with 5 mg l^{-1} BA + 30 g l^{-1} sucrose + 7 g l^{-1} agar. The explants were repeatedly subcultured 3 times at 4 week intervals till obtaining cluster explants, each one containing 2-4 developed buds. Cluster explants were gamma irradiated at doses of 0, 10, 20, 30, 40, 50 and 60 Gy (lethal dose LD50 was observed beyond 40 Gy) . The dose rate was 2.99 rad/sec. from gamma irradiation cell with a ^{60}Co source in Middle East Regional Radioisotope Center for the Arab Countries.

Irradiated and un-irradiated explants were then transferred and cultured on a multiplication basal MS medium supplemented with 5 mg l^{-1} BA + 30 g l^{-1} sucrose + 6 g l^{-1} agar. Subculturing was done at 4 week intervals for at least three vegetative regeneration (M1V3). Explants were treated with sea salt (Sigma) at levels of 0, 2000, 4000 and 6000ppm. Subculturing was done 4 times at 4 week intervals into corresponding multiplication fresh medium. Adventitious growing shoots of each treatment were separated *in vitro* and transferred to rooting ½ MS basal medium supplemented with1mg l^{-1}IBA+1mgl^{-1}NAA+ 30 g l^{-1} sucrose + 6 g l^{-1} agar.The same levels of sea salts were added to the culture medium. In all *in vitro* experiments, each treatment consists of 3 replicates; each replicate consists of 10 jars, with 3 shoots for each jar.

The morphological parameters(survival percentage, shoot height, leaf and root numbers per plantlet, fresh and dry weights of the shoots) and chemical composition (photosynthetic pigments, organic components and minerals) were determined at the end of this period (4 weeks after culturing on rooting media).

Rooted plantlets were washed with tap water three times to remove all traces of agar then immersed in a fungicide vitafax (0.1% for 3 min.) and cultured individually in black plastic pots (8 cm) containing a mixture of peatmoss and sand 1:1 (w/w); covered with white transparent plastic sheets (which were punched up 3 cm from two sides) under greenhouse conditions: light intensity of about 1500 Lux for 16 hours per day was provided by white fluorescent lamps, the temperature of about 28±2°C and the humidity was adjusted to 85-90% by adding water at three hours intervals through the mist (for half hour) during the nursery stage (15-21 days after transplanting). The white transparent plastic sheets were completely removed at the end of this stage. After one month, the acclimatized plants were transplanted to black plastic bags (15 × 25 cm) containing a mixture of clay loamy soil, peatmoss and sand at a ratio of 1:1:1 by weight. Plants were irrigated at three day intervals with tap water.

Rooted plantlets were prepared and transferred to the greenhouse conditons in the same way of the first experiment. Survived plants were irrigated with sea salt solution at levels of 0, 2000 and 4000 ppm at three day intervals. Control plants were irrigated with tap water throughout the whole growth stages. The plants were grown at the greenhouse under the same experimental conditions for 3 months. In all *ex vitro* experiments, each treatment consisted of 3 replicates with 10 plants for each replicate (one plant for pot). The morphological parameters and chemical composition data as well as leaf anatomy (leaf blade and midrib thickness, number and diameter of air cavities and number of midrib vascular bundles) and root anatomy (root and vascular cylinder diameter, cortex width and number of vessels) were estimated at the end of this period (three months after acclimatization).

2.2.Isolation of genomic DNA and RAPD analysis

DNA fingerprinting was done at the end of M1V1. DNA was isolated using CTAB method of Proebski *et al.* (1997). For RAPD analysis,PCR amplification was performed in 0.01 ml reaction mixture

containing 20 ng template DNA, 0.5 unit Taq polymerase, 200 µM each of dATP, dCTP, dGTP, dTTP, 10 p mol random primer (10 mer) and appropriate amplification buffer. The mixture was assembled on ice, overlaid with a drop of mineral oil. Amplification was preformed for 45 cycles at 92°C for 3 min. and then 45 cycles at 92°C for 30 sec., 35°C for 60 sec. and 72°C for 2 min. (for denaturation, annealing and extension, respectively).Reaction was finally incubated at 72°C for 10 min. and further 10 min. at 62°C.The amplification products were analyzed by electrophoresis in 2% agarose in TAE buffer, stained with 0.2 µg ml^{-1} ethidium bromide and photographed under UV light.

2.3. Chemical analysis

Nornai (1982) method was employed to determine the photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) using dimethyl formamide as a solvent.

The ethanol extracts of shoots were used to determine reducing and total sugars, total free amino acids and total soluble phenols. Reducing and non-reducing sugars were determined by using phosphomolybdic acid reagent as described in A.O.A.C.(1975). Total free amino acids were determined by using ninhydrin reagent according to Moore and Stein (1954).

Free proline concentration was measured colorimetrically in the exraction of fresh shoot using ninhydrin reagent according to Bates *et al.* (1973). The colorimetric method of Folin-Denis as described by Swain and Hillis (1959) was employed for determination the total soluble phenols.

The determination of N, P, K, Ca, Na, Mg and Cl were carried out on the shoot ground dry material.Dry sample was digested by using sulphoric and perchloric acids according to Piper (1947). Nitrogen was determined using the micro kjeldahl apparatus of Parnas-Wagner as described by Van-Schouwenburg and Walinga (1978). Phosphorus was estimated colorimetrically by using chlorostannous reduced molybdophosphoric blue color method according to Chapman and Parker (1961). Sodium, magnesium, potassium and calcium were determined by using atomic absorption spectrophotometer. Chloride was determined in the dry leaves by using titration method with silver nitrate as recommended by Brown and Jackson (1955) .

2.4. Anatomical studies

Leaves and roots were fixed and killed in FAA solution (40% formalin, 100% glacial acetic acid and 70% ethanol at 5:5:90 v/v/v). The sections were examined and photographed using Johanson(1940) method.

2.5. Statistical analysis

Randomized complete block design for two factor experiments was used. The mean comparisons were made using Duncan's multiple range test at 5% significant level (Duncan, 1955).

3. RESULTS AND DISCUSSION

3.1. DNA Fingerprint

The application of RAPD analysis was used for the identification of banana cv. Williams and the detection of differences between the control and the irradiated banana with different doses of gamma irradiation. DNAs were isolated from the control and different treatments. Two random primers were used as illustrated in Table (1).

Table (1): List of 10-mer random primers, their nucleotide sequence and amplification results with the control and the different treatments.

Primer name	Sequence $5' \rightarrow 3'$	Gamma irrad. doses (Gy)						
		0.0	10	20	30	40	50	60
		No. of bands						
OPA-15	AAGGGAGACA	5	4	4	8	7	6	5
Roth-P11	GGAAGCCAAC	7	7	7	7	7	7	4

Primer OPA-15: The results of primer OPA-15 are illustrated in Figure (1). It gave amplification products in the control and all treatments. The molecular size of the Polymerize Chain Reaction (PCR) products ranged from 450 bp to 1900 bp. It gave a maximum of

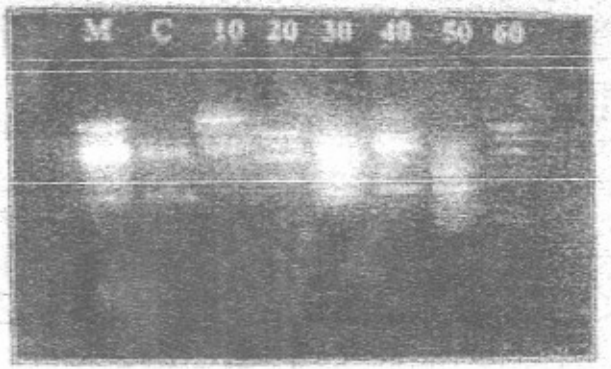

Figure (1):RAPD-profile using OPA-15 primers of banana cv. Williams treated with different doses of gamma irradiation.

C: control M: marker

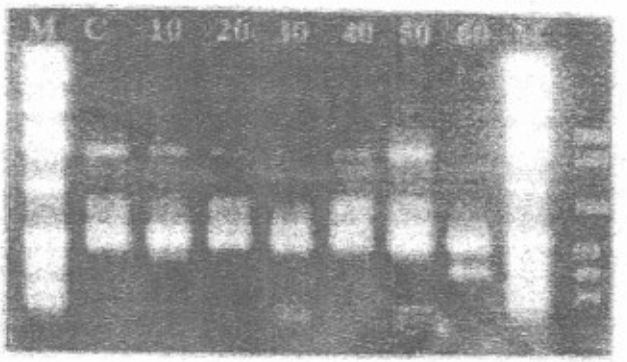

Figure (2):RAPD-profile using Roth-p11 primer of banana cv.Williams treated with different doses of gamma irradiation.

C: control M: marker

eight amplification products with different molecular sizes which were not necessarily present in the control and the treatments. It exhibited that there are differences between the control and gamma treatments and also between the different treatments.

Primer Roth-P11: The results of primer Roth-P11 are shown in Figure (2). It exhibited a maximum of seven amplification products which ranged between 400 bp to 1600 bp. There was difference between the treatments from 10 Gy to 50 Gy and the 60 Gy treatment.As compared with the chemical mutagen, the physical mutagen (ionizing and non-ionizing radiation), may cause chromosomal changes rather than gene mutation (Novak, 1991). These changes include chromosomal breaks, inversions, duplications, translocations and point mutations (Britt, 1996). It is worth mentioning that, induced mutation techniques are based on random mutation, and the proportion of chimerism was progressively reduced through the propagation cycles (Roux *et al.*, 1999). RAPD markers of banana, however, revealed the presence of polymorphic bands with at least one set of primers, enabling the possible early detection of mutations *in vitro* (Walther *et al.*, 1997).Moreover,Vuylsteke *et al.* (1998) reported that RAPD technique holds great promise for banana genome analysis.

3.2. Growth characters

Generally, it is clear from the results in Table (2) that the gradual increases in salinity levels resulted in gradual and significant decreases in survival percentage (%), shoots and pseudostem height, leaf and root numbers as well as shoot fresh and dry weights either applied alone or in combination with different gamma irradiation doses. In this respect, Mass and Nieman (1978) stated that high salinity levels retarded all plant growth processes such as enlargement and cell division. On the other side, Greenway (1963) attributed the reduction in shoot height caused by salinity treatments to the accumulation of soluble ions or the drastic changes in cellular ionic relationships, or to that the reduction in RNA, DNA and protein synthesis under saline conditions. Another explanation is that the difficulty for salt stressed plants to withdraw water from the soil even in moist soils (Nassar *et al.*,1999). When the soil water potential,however, drops below a critical value as a result of salt stress

Table (2): Effect of gamma irradiation doses and sea salt levels on growth characters of banana cv. Williams grown *in vitro* (4 weeks after culturing on rooting media) and *ex vitro* (3 months after acclimatization).

In vitro plants

Growth characters	Survival percentage (%)						Shoots height					
Sea salt levels (ppm)	Gamma irrad. doses (Gy)					Mean (A)	Gamma irrad. doses (Gy)					Mean (A)
	0	10	20	30	40		0	10	20	30	40	
0	100	95.55	83.33	65.56	47.78	**78.44**	7.13	9.12	8.65	6.53	5.93	**7.51**
2000	64.44	70.07	57.41	40.06	28.13	**52.02**	6.20	8.73	8.47	6.07	5.40	**6.97**
4000	45.56	53.08	37.59	26.22	10.11	**36.31**	4.80	7.22	6.60	4.20	3.47	**5.26**
6000	31.11	40.34	31.48	18.94	10.62	**26.50**	4.33	5.87	5.67	4.00	3.27	**4.63**
Mean (B)	**60.28**	**64.76**	**52.45**	**37.69**	**26.41**		**5.62**	**7.74**	**7.40**	**5.20**	**4.52**	
L.SD. 5%	A= 2.77		B=2.48		A*B=5.53		A=0.45		B=0.40		A*B=0.90	
	No. of leaves/plantlet						No. of roots/plantlet					
0	8.33	8.76	7.91	6.87	5.40	**7.65**	6.53	7.87	8.20	6.20	6.33	**7.43**
2000	7.47	8.93	8.60	6.20	5.87	**7.41**	5.27	7.80	7.33	5.20	5.07	**6.13**
4000	5.53	7.93	6.87	5.40	5.27	**6.20**	5.33	7.40	6.73	5.47	5.00	**5.99**
6000	4.60	5.73	5.67	3.47	3.33	**4.56**	3.27	5.27	4.07	3.13	3.13	**3.89**
Mean (B)	**6.48**	**7.84**	**7.26**	**5.49**	**5.22**		**5.60**	**7.09**	**6.73**	**5.00**	**4.88**	
L.SD. 5%	A=0.54		B=0.48		A*B=1.08		A=0.53		B=0.47		A*B=1.05	
	Shoots fresh weight (g)						Shoots dry weight (g)					
0	9.89	9.37	9.13	7.21	6.18	**8.16**	0.46	0.48	0.47	0.33	0.29	**0.41**
2000	6.18	8.25	7.84	5.68	5.49	**6.67**	0.38	0.42	0.40	0.27	0.25	**0.34**
4000	4.02	6.31	5.78	4.52	3.88	**4.90**	0.27	0.36	0.30	0.23	0.21	**0.27**
6000	3.17	5.34	4.44	3.52	3.62	**4.04**	0.21	0.29	0.25	0.18	0.16	**0.22**
Mean (B)	**5.57**	**7.32**	**6.80**	**5.26**	**4.79**		**0.33**	**0.39**	**0.36**	**0.25**	**0.23**	
L.SD. 5%	A=0.62		B=0.55		A*B=1.23		A=0.047		B=0.042		A*B=0.090	

Acclimatized plants (*ex vitro*)

	Survival percentage %						Pseudostem height (cm)					
0	100	83.33	78.67	63.33	40.00	**73.07**	16.82	16.23	15.86	14.60	12.78	**15.26**
2000	53.33	50.00	44.58	31.66	17.33	**39.38**	14.25	15.21	13.84	12.22	12.61	**13.63**
4000	23.33	27.77	23.60	12.67	8.00	**19.06**	11.93	13.34	12.72	10.21	9.51	**11.54**
Mean (B)	**58.89**	**53.70**	**48.95**	**35.89**	**21.78**		**14.33**	**14.93**	**14.14**	**12.34**	**11.63**	
L.SD. 5%	A= 5.07		B= 3.93		A*B= 8.79		A=0.77		B=0.60		A*B= 1.33	
	No. of leaves/plant						No. of roots/plant					
0	7.22	7.13	6.45	5.83	5.16	**6.36**	9.27	10.40	10.33	8.53	8.33	**9.37**
2000	5.77	6.39	6.21	4.61	4.21	**5.44**	7.73	9.73	8.87	7.67	6.00	**8.00**
4000	4.62	4.72	4.49	4.17	3.54	**4.31**	6.67	7.60	7.40	6.47	5.80	**6.79**
Mean (B)	**5.87**	**6.08**	**5.72**	**4.87**	**4.30**		**7.89**	**9.24**	**8.87**	**7.56**	**6.71**	
L.SD. 5%	A= 0.44		B=0.34		A*B= 0.75		A= 0.71		B=0.55		A*B=1.23	
	Shoots fresh weight (g)						Shoots dry weight (g)					
0	46.13	46.74	45.36	39.50	37.44	**43.03**	5.62	6.24	6.11	4.71	4.32	**5.40**
2000	40.52	42.61	42.17	34.20	34.81	**38.86**	3.94	4.79	5.16	3.72	3.53	**4.23**
4000	32.43	35.54	34.61	29.60	28.56	**32.15**	3.14	3.84	3.62	3.15	2.74	**3.30**
Mean (B)	**39.69**	**41.63**	**40.71**	**34.43**	**33.60**		**4.23**	**4.96**	**4.96**	**3.86**	**3.53**	
L.SD. 5%	A=1.00		B=0.77		A*B=1.72		A= 0.50		B= 0.38		A*B= 0.85	

a water deficit in leaves will develop. This will cause a decrease in leaf water potential, a change in turgor pressure and perhaps an increase in stomatal resistance, reducing the loss of water vapour. This stress situation is associated with plant growth reduction, *i.e.*, the reduction in leaf development rate (Beese and Moshrefi, 1985). Moreover, Huang *et al.* (1995) stated that, the increase in leaf senescence and leaf deformation caused by salt-stress condition could be attributed to the decline in leaf N-content,or to the increase in ethylene content (Slocum *et al.*, 1984). Such increment in ethylene levels accelerates leaf senescence and leaf abscission in plants. This senescence is associated with some physiological changes, *i.e.* loss of chlorophyll, proteins and nucleic acids; a decline in membrane integrity and disruption of cell homeostasis (Evans and Malmberg, 1989). Moreover, Cheesman (1988) reported that salt limits the carbon availability for biomass production, by either, the diversion of metabolic carbon to the storage pool or, the additional energy required for plant cells (*i.e.* for osmotic synthesis and ion extrusion). The reduction of fresh weight of plants subjected to saline conditions, however, might be due to the reduction of uridine diphosphate glucose (UDPG) pool along with ATP. These reductions were accompanied by more than 3-fold increase in hexoses. Reduced assimilation of photosynthesis is the possible consequence of reduced UDPG, UTP and ATP pools.Nieman *et al.* (1988) also mentioned that, under saline conditions plants suffer temporarily from wilting since most plants can recover by their ability to osmotic adjustment. Consequently, sodic toxicity is considered the main cause of inferior plant growth under stress conditions.Moreover, Zidan *et al.* (1990) mentioned that the inhibition of plant growth under saline condition, however, may be explained by the decrease in cell production rate or to the greater hardening of cell wall (Neumann *et al.*, 1994).In this respect, Lather (1980) and Simpson (1981) manifested that salinity affects many metabolic and growth aspects mainly due to sodic toxicity where Na competes with K on active metabolic sites leading to depressed growth expressed as dry matter production.However, the presence of soluble salts in the nutrient medium can affect plant growth in two ways.First, salts can depress the water potential of the nutrient medium and hence restrict the water uptake by plant roots and second,the high levels of specific ions can be toxic and induce physiological disorders. Moreover,

Gunes *et al.* (1996) cited that the reduction of plant growth under saline conditions may be attributed to either the severe accumulation of ions (specially Na and Cl) in the plant tissues, or, osmotic reduction in water availability which was supported by stomatal resistance values of plants. This reduction in plant growth may also be due to the imbalance of organic and inorganic constituents in plants. In addition, Schulze (1986) cited that, carbon acquisition and allocation are involved in induced salinity. Both stomatal and non-stomatal responses to salt stress are responsible in this connection. Gas exchange severely reduced growth when associated with high Na and Cl accumulation. Stomatal limitations were manifested by the decrease in intercellular CO_2.

Data in Table (2) reveal that the gradual increase in gamma irradiation doses resulted in gradual and significant changes in the parameters in all of the studied growth characters.On the other side, under each level of salinity, the interaction with lower doses of irradiation (10 and 20 Gy) significantly increased all growth characters compared with non-irradiated plants and salt stressed only. Meanwhile, the interaction with the higher doses (30 and 40 Gy) significantly decreased it. The most effective mutagen of physical mutagenesis is the ionizing radiations that penetrate deeper into the tissue and can induce changes at the gene, chromosome and genome levels, including, chromosomal breaks, inversions, duplications, translocations and point mutations (Britt, 1996).In this respect, LD_{50} that caused a 50% reduction of vegetative growth of banana cvs. at the first vegetative cycle after treatment (VM1) was 25 Gy (De-Guzman *et al.*, 1982) and 30-40 Gy (Neto *et al.*, 1989). The survival percentage, however, was 0.0 % at the high doses (60 and 70 Gy) of irradiation (Novak *et al.*, 1986 and Kulkarni *et al.*, 1997). Mutation induction for plant breeding and improving has been used for introducing many useful traits affecting plant size, blooming time, fruit ripening and colour, self compatibility and resistance to pathogens and abiotic stress (Spina *et al.*, 1991; Janick and Moore, 1996; Masuda *et al.*, 1997 and Sanada and Amano, 1998). The frequency of the variants was the highest when gamma irradiation (as physical mutagen) was at 40 Gy (Domingues *et al.*, 1994), but the best exposure dose, in this respect, was 20 Gy (Smith *et al.*, 1995). Moreover, De-Guzman *et al.* (1982) cited that growth stimulation of micropropagated banana may be

observed by low doses of gamma irradiation at 1.0 Krad. The growth rate was expressed as an increase in fresh weight and rate of shoot formation. The doses up to 2.5 Krad, however, slightly stimulated shoot regeneration. Furthermore, the higher dose at 60 Gy was lethal (Novak *et al.*, 1986).In this context, radiosensitivity of the micropropagated banana plants was assessed by root number, root length, shoot length, and leaf number. Increasing gamma irradiation exposure dose decreased all the growth parameters (Karmarkar *et al.*, 2001). In addition, retardation of shoot growth, leaf deformation and chlorophyll streaking was observed by the gradual increase in gamma irradiation doses (Espino *et al.*, 1986). Many phenotypic variants, however, were observed by increasing exposure dose either in growth, leaf deformation, pigmentation and texture (Mak *et al.*, 1995). The harmful effect of higher exposure doses may be attributed to the ploidy number. In this respect, Novak *et al.* (1991) reported that the diploid clone (AA) was the most sensitive to gamma irradiation, while the tetraploid (AAAA) showed the lowest level of damage among the tested clones. Plantains triploid clones (AAB, ABB) of *Musa accminata (A)* × *Musa balbisiana (B)* has radiosensitivity between AA and AAAA clones.

3.3. Chemical composition

3.3.1.Photosynthetic pigments

Regardless of the different used gamma irradiation doses and sea salt levels in the present study, data presented in Table (3) reveal that, all treatments decreased the photosynthetic pigment concentrations either applied alone or in combinations. In this respect, concerning the effect of gamma irradiation, Sprey *et al.* (1972) found that low doses of gamma irradiation induced changes in thylakoid and chlorophyll content. In addition, higher doses inhibited chloroplast development and caused changes in the synthesis of nucleic acids in the plastids. Moreover, Espino *et al.* (1986) and Silayoi *et al.* (1995) noticed chlorophyll streaking, chlorosis and necrosis in banana plants treated with gamma irradiation. Moreover, as regards to salinity effects, the decline in growth observed in many plants subjected to excess salinity is often associated with a decrease in photosynthetic capacity, since photosystem II (PS II) is believed to play a key role in the response of

Table (3): Effect of gamma irradiation doses and sea salt levels on chemical composition (mg/g f.w.) of banana cv. Williams grown *in vitro* (4 weeks after culturing on rooting media) and *ex vitro* (3 months after acclimatization).

In vitro plants												
Chemical composition	Chlorophyll a						Chlorophyll b					
Sea salt levels (ppm)	Gamma irrad. doses (Gy)					Mean (A)	Gamma irrad. doses (Gy)					Mean (A)
	0	10	20	30	40		0	10	20	30	40	
0	1.39	1.37	1.25	1.25	1.16	1.28	0.65	0.58	0.58	0.49	0.42	0.54
2000	1.25	1.30	1.19	1.12	1.01	1.17	0.51	0.51	0.46	0.40	0.35	0.45
4000	1.09	1.11	0.98	0.90	0.89	0.99	0.43	0.39	0.31	0.29	0.29	0.34
6000	0.79	0.80	0.79	0.69	0.61	0.74	0.35	0.31	0.22	0.22	0.18	0.26
Mean (B)	1.13	1.14	1.05	0.99	0.92		0.48	0.45	0.39	0.35	0.31	
L.SD. 5%	A=0.18		B= 0.16		A*B=0.35		A=0.095		B= 0.085		A*B=0.189	
	Carotenoids						Reducing sugars					
0	0.57	0.48	0.41	0.36	0.34	0.43	3.15	3.80	4.01	4.30	3.98	3.85
2000	0.43	0.40	0.31	0.22	0.25	0.32	4.06	4.15	4.31	4.42	4.21	4.23
4000	0.33	0.27	0.22	0.19	0.21	0.24	4.71	4.98	5.23	5.25	4.89	5.01
6000	0.26	0.21	0.14	0.14	0.11	0.17	5.10	5.20	5.50	5.61	5.28	5.34
Mean (B)	0.40	0.34	0.27	0.23	0.23		4.26	4.53	4.76	4.90	4.59	
L.SD. 5%	A= 0.090		B= 0.080		A*B=0.180		A= 0.18		B=0.16		A*B=0.36	
	Non-reducing sugars						Total free amino acids					
0	9.49	9.91	10.59	11.16	9.11	10.0	3.34	4.15	4.36	3.84	3.57	3.85
2000	10.21	10.66	10.92	11.33	10.33	10.6	3.85	4.53	4.76	4.10	3.96	4.24
4000	10.57	11.59	11.69	11.40	11.00	11.25	4.53	5.10	5.37	4.86	4.64	4.90
6000	11.20	11.60	12.04	11.72	11.43	11.60	4.93	5.73	6.29	5.28	5.06	5.46
Mean (B)	10.37	10.94	11.31	11.40	10.47		4.16	4.88	5.19	4.52	4.31	
L.SD. 5%	A= 0.27		B= 0.24		A*B=0.54		A=0.28		B=0.25		A*B=0.57	
	Proline						Total soluble phenols					
0	1.18	1.47	1.71	1.20	1.04	1.32	1.95	2.33	2.78	3.37	3.68	2.82
2000	1.62	1.96	2.09	1.72	1.63	1.80	2.46	2.69	2.92	3.79	4.07	3.19
4000	2.12	2.62	2.86	2.35	2.18	2.43	2.95	2.95	3.37	4.12	4.50	3.58
6000	1.89	2.21	2.47	1.96	1.74	2.05	3.39	3.58	3.96	4.46	4.88	4.05
Mean (B)	1.70	2.06	2.28	1.81	1.65		2.69	2.89	3.26	3.93	4.28	
L.SD. 5%	A=0.20		B=0.18		A*B=0.41		A=0.31		B= 0.28		A*B=0.62	
Acclimatized plants (*ex vitro*)												
	Chlorophyll a						Chlorophyll b					
0	1.79	1.69	1.51	1.43	1.41	1.57	0.84	0.81	0.69	0.58	0.56	0.70
2000	1.54	1.55	1.39	1.27	1.25	1.40	0.70	0.72	0.57	0.45	0.36	0.56
4000	1.29	1.24	1.11	1.04	0.96	1.13	0.54	0.49	0.38	0.31	0.31	0.41
Mean (B)	1.54	1.49	1.34	1.25	1.21		0.69	0.67	0.55	0.45	0.41	
L.SD. 5%	A=0.22		B=0.17		A*B= 0.39		A=0.17		B= 0.13		A*B=0.30	
	Carotenoids						Reducing sugars					
0	0.72	0.72	0.59	0.49	0.46	0.60	3.49	4.11	4.44	5.00	3.93	4.19
2000	0.58	0.49	0.45	0.31	0.28	0.42	4.21	4.50	4.86	5.20	4.41	4.64
4000	0.41	0.37	0.31	0.24	0.19	0.30	5.04	5.46	5.67	5.07	5.16	5.48
Mean (B)	0.57	0.53	0.45	0.35	0.31		4.24	4.69	4.99	5.42	4.50	
L.SD. 5%	A= 0.15		B=0.12		A*B=0.26		A= 0.41		B=0.31		A*B=0.70	
	Non-reducing sugars						Total free amino acids					
0	9.78	10.31	11.00	11.18	10.63	10.68	3.98	4.32	4.76	4.20	3.95	4.24
2000	11.07	11.39	11.45	11.58	11.10	11.32	4.45	4.80	5.00	4.94	4.40	4.72
4000	11.22	11.28	11.91	12.08	11.71	11.64	4.16	5.87	6.37	5.60	5.26	5.45
Mean (B)	10.69	11.16	11.45	11.61	11.15		4.20	5.00	5.38	4.91	4.54	
L.SD. 5%	A=0.42		B= 0.32		A*B=0.72		A=0.39		B= 0.30		A*B=0.67	
	Proline						Total soluble phenols					
0	1.59	1.98	2.07	1.80	1.44	1.78	2.37	2.76	3.11	3.65	3.88	3.15
2000	2.15	2.74	2.97	2.38	2.17	2.48	2.92	3.10	3.55	4.08	4.44	3.62
4000	2.77	3.26	3.43	2.94	2.66	3.01	3.67	3.76	4.19	4.68	5.10	4.26
Mean (B)	2.17	2.66	2.82	2.37	2.09		2.95	3.21	3.62	4.14	4.47	
L.SD. 5%	A=0.18		B=0.14		A*B=0.30		A=0.40		B= 0.31		A*B=0.70	

leaf photosynthesis (Baker, 1991). Furthermore, the decline in photosynthesis caused by salinity is due to reduced photosynthetic surface area available for CO_2 assimilation, *i.e.* the salinity effect on leaf extension (Papp *et al.*, 1983).In this respect Garcia and Morard (1979) and Helal and Mengel (1981) concluded that salinity affects the rate of photosynthesis and that associated with changes in pigment composition.

3.3.2. Organic components

Data in Table 3 reveal that ,the gradual increase in sea salt application or exposure to gamma irradiation either alone or in combinations resulted in gradual increases in all the studied simple organic components(reducing and non reducing sugars, free amino acids ,total soluble phenols and proline), with some exceptions at the high doses of gamma irradiations.

Concerning sugar concentration, the data in Table (3) indicate that the increase in reducing and non-reducing sugar concentrations reached its peak when the highest level of sea salt was combined with gamma irradiation at 30 Gy (*in vitro*) and 20Gy (*ex vitro*). In this respect, Aledesuquy *et al.* (1998) noticed biochemical changes in somaclones of tobacco tolerant to NaCl;such as accumulation of reducing sugars which increase with increasing salinity level. Hanafy Ahmed *et al.*(2002 a) suggested that reducing sugars and other solutes contributed more to the solute potential and therefore maintained turgor leading to tolerance.The results of sugars were also explained by that cells exposed to saline stress tend to redirect carbon flow to osmoregulation (osmotic adjustment) by accumulation of a variety of common solutes, including sugars, amino acids, organic acids and ions and other metabolically protective osmolites leading to water retention.

Treating the irradiated plants with sea salt solutions significantly increased free amino acids and proline concentrations compared with the non-irradiated plants as well as those treated only with the different levels of salinity. The superior in this respect was to the low doses of irradiation (10 and 20 Gy).In this respect, Strogonov (1970) reported that under saline conditions the accumulations of non-toxic substances such as sucrose, amino acids, organic acids, pigments, nucleic acids and protein are considered to be protective adaptation

and that the survival of plants under saline conditions depends upon the regulation of the metabolic processes and the quantitative ratio between the protective and the toxic metabolic intermediates. In addition, proline may play its role as a compatible cytoplasmic solute, of low molecular weight existing in the vacuole state (Dov-Posternak, 1987 and Hanafy Ahmed *et al.*, 2002 b) and its accumulation might be due to stimulation of proline biosynthesis by an increase of proline-5-carboxylate reductase activity and the reduction in proline dehydrogenase activity during salt stress (Delauey and Verma, 1993) or to the alteration in the protein turnover (Das *et al.*, 1990).

Data in Table (3) reveal that the maximum accumulation of total soluble phenols (4.88 and 5.10 mg g^{-1} F.W . *in vitro* and *ex vitro*, respectively) was due to the combination between the highest levels from both salts and irradiation. Phenolic compounds may have indirect effect on the physiological process through more non-specific effects on intermediately metabolism, *i.e.* phenolic compounds are capable to inhibit ATP synthesis in the mitochondria (Stenlid, 1970).Regardless of the irradiation doses under the present study condition, data also show that irradiated plant accumulate higher concentrations of phenols under each level of salinity compared to those non-irradiated. It is worth mentioning that gamma irradiation at high doses led to physiological changes in the activity of the enzymes responsible of browning (Pendharkar and Madhusudanan, 1978). Moreover, the exposure to gamma irradiation at different doses might induce the activity of phenylalanine ammonia lyase (PAL) which led to the formation of phenolic compounds starting from cinnamic acids which successively hydroxylated enzymatically to caffeic acids and finally yields chlorogenic acid (Thomas *et al.*, 1977).

3.3.3. Minerals

Generally, the data in Table (4) reveal that there are decreases in N, P, K, Ca, and Mg concentrations as well as K/Na ratio due to the gradual increase in salinity level either applied alone or in combination with the different irradiation doses. Meanwhile, Na and Cl concentrations were increased due to the same treatments. The reduction in nitrogen concentration observed from the present result might be explained by the reduction in water absorption and / or the decrease in root permeability (Forta and Tucker , 1978) . Another

Table (4): Effect of gamma irradiation doses and sea salt levels on minerals (mg/g d.w.) of banana cv. Williams grown *in vitro* (4 weeks after culturing on rooting media) and *ex vitro* (3 months after acclimatization).

In vitro plants												
Minerals	Nitrogen						Phosphorus					
Sea salt levels (ppm)	Gamma irrad. doses (Gy)					Mean (A)	Gamma irrad. doses (Gy)					Mean (A)
	0	10	20	30	40		0	10	20	30	40	
0	28.93	37.71	40.51	33.41	29.87	34.09	2.35	2.51	2.65	2.40	2.22	2.43
2000	21.28	30.61	35.84	26.99	23.99	27.74	2.16	2.25	2.36	2.13	1.97	2.17
4000	17.62	24.55	29.77	21.65	18.76	22.51	1.87	1.97	2.15	1.85	1.74	1.92
6000	14.93	20.91	24.62	17.45	14.65	18.51	1.69	1.75	1.87	1.61	1.55	1.69
Mean (B)	20.69	28.44	32.68	24.92	21.82		2.02	2.12	2.26	2.00	1.87	
L.SD. 5%	A=3.40		B= 3.04		A*B=6.78		A=0.20		B= 0.18		A*B=0.40	
	Potassium						Calcium					
0	37.10	40.78	44.84	37.46	33.88	38.81	13.15	15.77	16.29	12.44	10.73	13.68
2000	29.76	34.33	40.77	31.69	25.77	32.46	11.12	12.86	13.19	10.26	8.28	11.14
4000	24.20	28.30	33.67	25.79	21.03	26.60	8.40	9.71	10.20	8.02	7.74	8.81
6000	18.98	22.95	27.02	20.12	18.90	21.59	7.30	8.19	8.42	7.67	7.29	7.77
Mean (B)	27.51	31.59	36.57	28.76	24.89		9.99	11.63	12.02	9.60	8.51	
L.SD. 5%	A=3.78		B=3.38		A*B=7.54		A=0.80		B= 0.72		A*B=1.61	
	Sodium						Magnesium					
0	1.21	1.52	1.59	1.77	1.88	1.59	3.00	3.33	3.75	2.74	2.53	3.07
2000	1.60	1.84	1.93	2.16	2.11	1.93	2.65	3.11	3.37	2.46	2.11	2.74
4000	1.89	2.19	2.13	2.38	2.47	2.21	2.19	2.84	3.06	2.08	1.72	2.38
6000	2.28	2.61	2.54	2.76	2.85	2.61	1.80	2.51	2.75	1.73	1.63	2.08
Mean (B)	1.74	2.04	2.05	2.27	2.33		2.41	2.95	3.23	2.25	2.00	
L.SD. 5%	A= 0.25		B= 0.22		A*B=0.49		A=0.34		B= 0.30		A*B= 0.68	
	Chloride						K/Na					
0	1.71	1.87	1.85	1.87	1.94	1.85	30.66	26.83	28.20	21.16	18.02	24.97
2000	1.96	2.05	2.11	2.05	2.17	2.07	18.60	18.66	21.12	14.67	12.21	17.05
4000	2.31	2.43	2.49	2.49	2.54	2.45	12.80	12.92	15.81	10.84	8.51	12.18
6000	2.63	2.71	2.72	2.86	2.85	2.75	8.33	8.79	10.64	7.29	6.63	8.33
Mean (B)	2.15	2.26	2.29	2.32	2.37		17.59	16.80	18.94	13.49	11.34	
L.SD. 5%	A= 0.21		B= 0.19		A*B=0.41		A=3.80		B= 3.40		A*B= 7.60	
Acclimatized plants (*ex vitro*)												
	Nitrogen						Phosphorus					
0	39.29	46.57	51.41	43.21	41.62	44.42	2.50	2.66	2.81	2.50	2.34	2.56
2000	30.89	39.29	45.36	36.87	33.51	37.18	2.24	2.35	2.49	2.05	2.00	2.23
4000	21.93	31.39	38.92	27.72	22.59	28.51	1.97	2.26	2.43	1.92	1.92	2.10
Mean (B)	30.70	39.08	45.23	35.93	32.57		2.24	2.42	2.58	2.16	2.09	
L.SD. 5%	A= 5.09		B= 3.94		A*B=8.81		A=0.32		B= 0.25		A*B= 0.55	
	Potassium						Calcium					
0	43.45	46.12	52.03	45.94	42.09	46.33	15.15	17.51	17.22	13.54	12.24	15.13
2000	35.57	40.63	44.17	35.75	31.36	37.50	12.52	13.82	15.15	11.59	11.19	12.85
4000	29.27	33.51	39.90	28.72	24.18	31.12	10.75	12.15	12.61	10.62	9.17	11.06
Mean (B)	36.10	40.75	45.37	36.80	32.54		12.81	14.49	14.99	11.92	10.87	
L.SD. 5%	A= 5.33		B= 4.13		A*B=9.24		A=1.20		B=0.93		A*B=2.08	
	Sodium						Magnesium					
0	1.56	1.87	1.92	2.11	2.19	1.93	3.21	3.74	4.10	3.13	2.94	3.42
2000	1.91	2.05	2.00	2.34	2.46	2.15	2.93	3.20	3.51	2.66	2.66	2.99
4000	2.29	2.55	2.61	2.89	2.94	2.66	2.54	3.11	3.28	2.46	2.21	2.72
Mean (B)	1.92	2.16	2.18	2.45	2.53		2.89	3.35	3.63	2.75	2.60	
L.SD. 5%	A= 0.38		B=0.29		A*B= 0.66		A=0.54		B=0.42		A*B=0.93	
	Chloride						K/Na					
0	1.89	2.00	2.07	2.05	2.00	2.00	27.85	25.73	25.10	21.37	19.22	24.33
2000	2.17	2.28	2.20	2.35	2.54	2.31	18.62	19.82	22.08	15.27	12.75	17.71
4000	2.51	2.69	2.74	2.79	2.87	2.72	12.78	13.14	15.29	9.94	8.22	11.87
Mean (B)	2.19	2.32	2.33	2.40	2.47		19.75	19.56	21.49	15.66	13.40	
L.SD. 5%	A= 0.25		B=0.19		A*B=0.43		A= 4.77		B= 3.69		A*B= 8.30	

explanation is that the competitive effect between the ions in chloride salinized soils produced unfavourable conditions for the uptake of minerals by the plant roots (Solovyov, 1969). Chloride salts (NaCl, $CaCl_2$, KCl) might be implicated indirectly with nitrogen reduction due to the role played by chloride ions (Hanafy Ahmed *et al.*, 2002 b) which cause a decrease in nitrate accumulation since its uptake is antagonistic to nitrate (Hanafy Ahmed, 1996). Such nutrient imbalance might result in a reduction in P concentration (Dhankar *et al.*, 1981). Phosphorus as a vital element involved in metabolic processes such as formation of energy enzymes (ATPase), nucleic acids, phospholipids, coenzymes, NAD, NADP and the main function of phosphate is the formation of pyrophysphate bonds which allow energy transfer (Mengel and Kirkby, 1979). The decrease in K concentration due to the increase of salinity levels was also observed by Hanafy Ahmed *et al.* (2002 b) who suggested that the increasing in sodium concentrations in the soil might be implicated indirectly with potassium reduction in wheat plants due to the role played by sodium ions. This interference caused by Na on the uptake of K is known to inhibit many enzyme activities . Furthermore, the internal K content in salt stressed cells might be a major factor which changed in the presence of NaCl and probably played a key role in the determination of the growth capacity (Jindal *et al.*, 1979; Clemens *et al.*, 1983; and Picchioni *et al.*, 1991). The increase in Na concentration was generally associated with a reduction in K and Ca ions (Greenway *et al.*, 1981). The negative correlation between Na and K and between Na and Ca points clearly to the antagonism between these ions. These antagonisms could partially reduce K and Ca content (Hu and Cramer, 1993). Moreover, this negative relationship between these ions do not reduce only the leaf area and dry matter production in the plant but also decrease the concentration and the uptake of the major elements.Hence, it has a double negative influence (Hanafy Ahmed *et al.*, 2002 b).The high Na level in the external solution can displace membrane-associated Ca from the plasmalemma. This leads to an increase in membrane leakiness resulting in a higher efflux of K ions (Cramer *et al.*, 1985 and Liang *et al.*, 1996). Moreover, magnesium ion is strongly competitive with Ca ion and the binding sites on the root plasma membrane appear to have less affinity for the highly hydrated Mg^{+2} than for Ca^{+2} (Marschner, 1995). In salt stressed plants,

changes in membrane properties, as composition of lipids, ATPase activity and conformation of enzyme proteins cause disturbance in the selectivity of ion uptake as well as in the distribution of the ions in the cell among particular organelles (Maynard and David, 1987).The specific effect of Ca/Na ratio was recorded by Greenway and Munns (1980).Growth of plants was negatively influenced by the external Ca/Na ratio.In Macademica seedlings when K/Na ratio was 2.5, adverse effect of salinity could be expected. K/Na ratio, 1.5 is corresponding to 50% reduction in plant growth (Hue and Mc-Call, 1989).

On the other hand, data in Table (4) indicate that, under each level of salinity, the interaction with the lower doses of irradiation (10 and 20 Gy) significantly increased mineral concentrations compared with higher doses (30 and 40 Gy) . In this respect, Eleiwa and Rabie (1994) reported that N concentration in the irradiated plants depend on the irradiation exposure doses, *i.e.*, low doses of irradiation (less than 4 Krad) markedly increased N concentration in sorghum plants, while, higher doses of irradiation (more than 4 Krad) decreased it. In addition, El-Shihy *et al.* (1994 a and b) found that P, K and Ca concentrations were negatively affected in *Vicia faba* plants due to the interaction of gamma irradiation at different doses and different salinity levels. Meanwhile, Na concentration was positively correlated with the combination of sea salt and gamma irradiation treatments. Similarly, He and Yu (1995) pointed out that mutant callus derived from gamma irradiation treated immature embryos of rice cv. Shuangfeng contained more Na concentration than the original genotype.

3.4. Leaf and root anatomy

The interaction of gamma irradiation at 10 Gy and different levels of sea salt markedly stimulate leaf growth expressed by increasing in thickness of leaf blade and midrib, number and diameter of air cavities, number of midrib vascular bundles, and root growth expressed by increasing in cortex width, roots and vascular cylinder diameter as well as the number of vessels (Table,5 and Figs.,3, 4, 5 and 6) compared with non-irradiated stressed plants. Similarly, De-Guzman *et al.* (1982) observed that low dose of irradiation (1.0 Krad) markedly stimulate all the growth parameters in banana plants expressed by increasing in fresh weight , shoot formation and leaf

Table (5): Effect of gamma irradiation at 10Gy and sea salt (ppm) on the structure of leaves and roots of micropropagated plants of banana cv. Williams grown *ex vitro* (3 months after acclimatization).

Plant organ	Leaves					Roots			
Anatomical parameters Gamma irrad. (Gy) + salt levels (ppm)	Midrib thickness µm	Leaf blade thickness µm	No. of air cavities	Diameter of air cavities (µm)	No. of vascular bundles	Root diameter (µm)	Cortex width (µm)	Vascular cylinder diameter (µm)	No. of vessels
Control	1160	420	5	330	13	1840	640	600	13
10 Gy + 0 ppm	1070	370	6	250	15	1840	630	620	15
200 ppm	800	350	3	210	11	1400	420	440	9
400 ppm	510	220	2	140	5	900	320	300	6
10 Gy + 2000 ppm	820	310	4	290	13	1460	460	540	13
10 Gy + 4000 ppm	580	260	2	180	5	1020	360	360	10

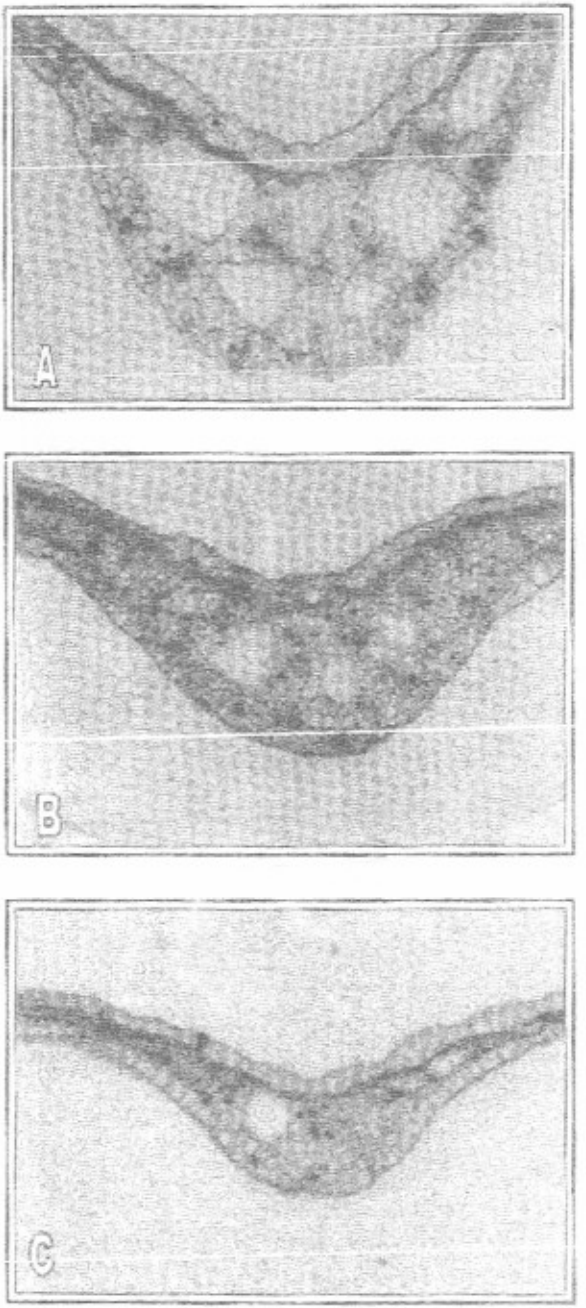

Fig.(3): Effect of sea salt levels on leaf structure of micripropagated plants of banana cv. Williams grown *ex vitro* (3 months after acclimatization)(30X).

A:0 ppm B:2000 ppm C:4000 ppm

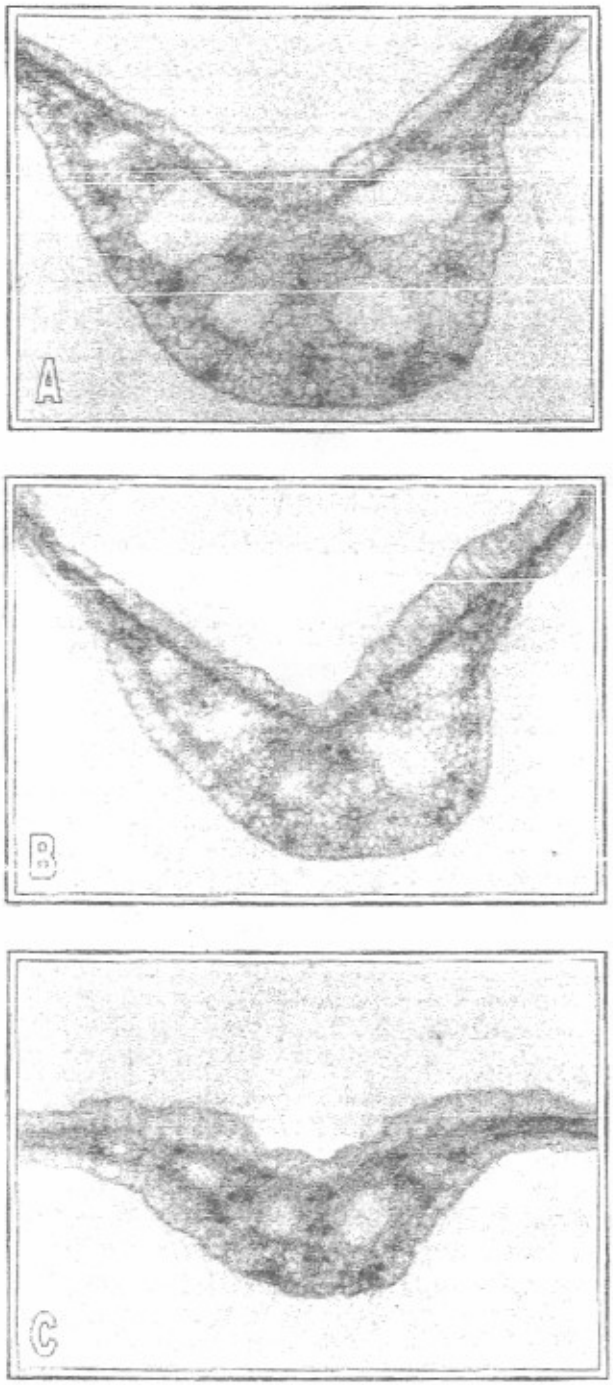

Fig.(4): The interaction effect of gamma irradiation at 10Gy and sea salt levels on leaf structure of micropropagated plants of banana cv. Williams grown ex vitro (3 months after acclimatization)(30X).

A: 10Gy +0ppm B:10Gy + 2000ppm C:10Gy+4000ppm

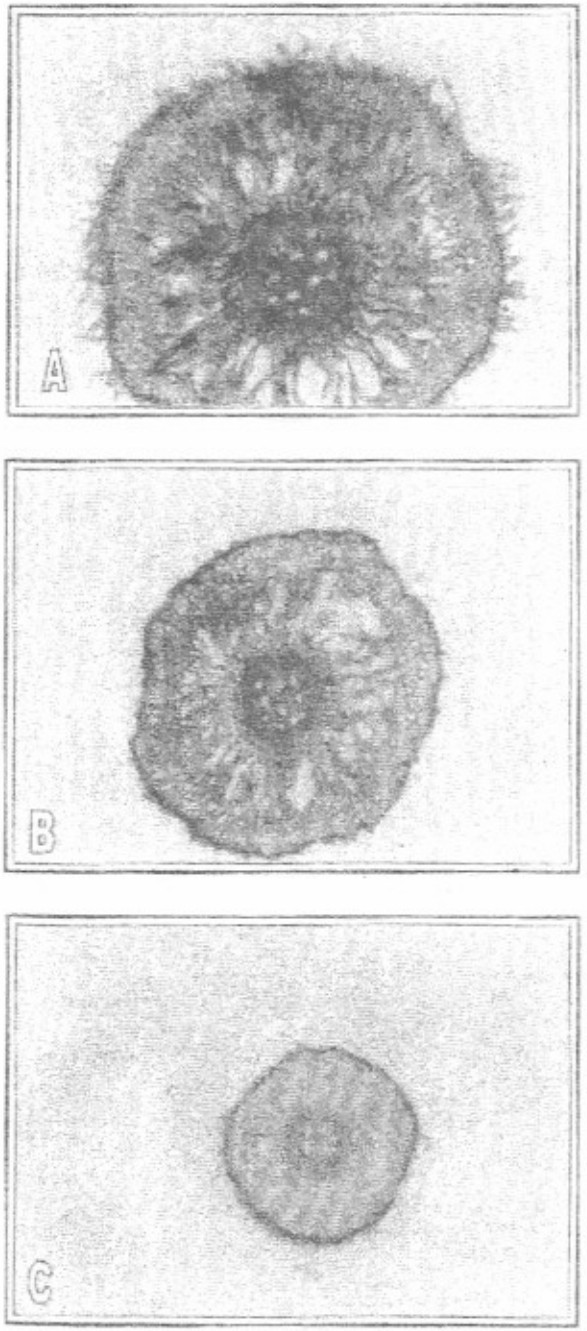

Fig.(5): Effect of sea salt levels on root structure of micropropagated plants of banana cv Williams grown *ex vitro* (3 months after acclimatization)(30X).

A:0 ppm B:2000ppm C:4000ppm

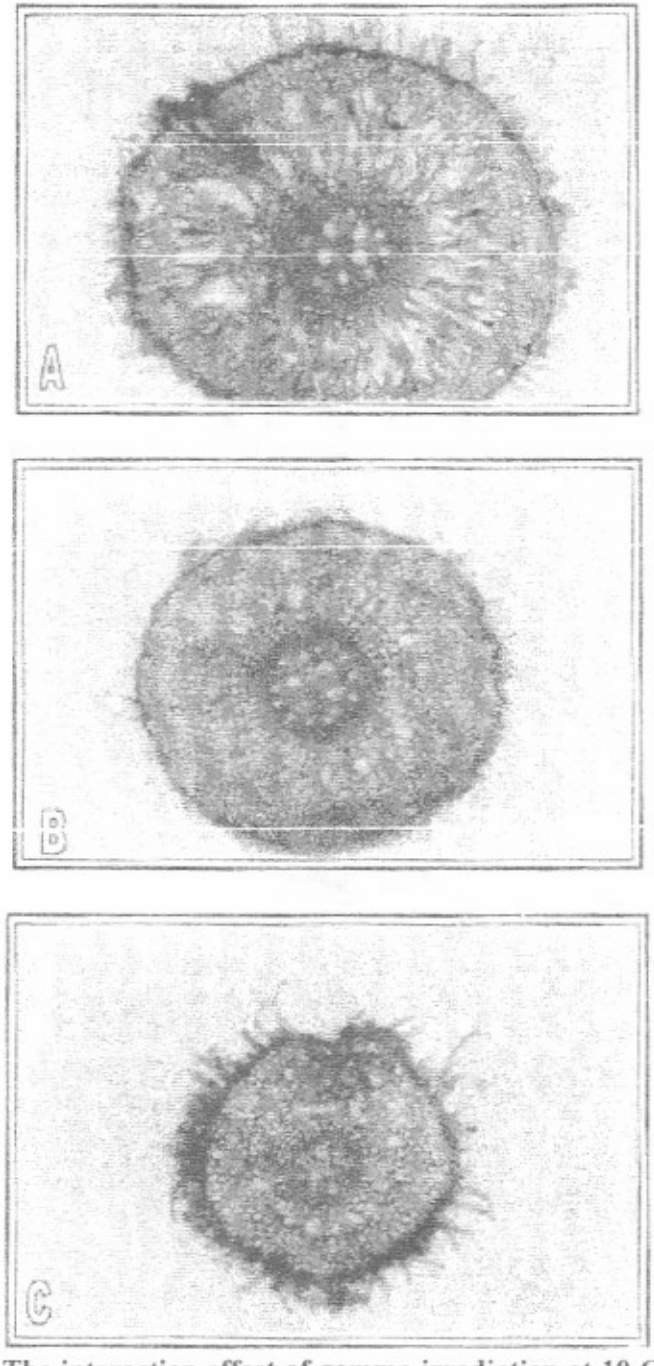

Fig.(6): The interaction effect of gamma irradiation at 10 Gy and sea salt levels on root structure of micropropagated plants of banana cv.Williams grown *ex vitro*(3 months after acclimatization)(30X).

A: 10Gy+0 ppm B: 10Gy+2000ppm C:10Gy+4000ppm

production. In addition , Smith *et al.,* (1995) found that the optimal dose for micropropagated banana was 20 Gy. On the other hand, higher exposure doses (30-45Gy) caused 50% reduction in survival percentage, all plants were dead, however, beyond 60 Gy (Novak *et al.*, 1986).

Generally, it might be suggested that, the presence of excess amount of soluble salts in the nutrient medium adversely affects the growth and development of shoots and roots of banana plant, and this adverse effect induced by salinity stress can be overcome, to some extent, by using the low doses of gamma irradiation. However, further work will be needed to support this suggestion under field conditions.

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تأثير أشعة جاما على زيادة تحمل نبات الموز المكاثر معملياً للاجهاد الملحي

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ملخص

أجريت هذه الدراسة على نبات الموز صنف ويليامز بهدف دراسة تأثير أملاح البحربتركيزات (٢٠٠٠و ٤٠٠٠و ٦٠٠٠و ٨٠٠٠ جزء في المليون) و كذا أشعة جاما بجرعات(٠و ١٠و ٢٠و ٣٠و ٤٠و ٥٠ و ٦٠ جراي) سواء بمفردها أو بالتفاعل مع المعاملات المختلفة للملوحةعلى تحمل الإجهاد الملحي . سجلت النتائج معملياً و في الصوب. أظهرت النتائج أن زيادة تركيز أملاح البحر ارتبط سلبياً مع جميع صفات النمو (النسبة المئوية لاستمرارية البقاء حية – طول الأفرع و السوق الكاذبة – عدد الأوراق و الجذور – الوزن الطازج و الجاف للمجموع الخضري)، صفات التركيب التشريحي للورقة (سمك الورقة و العرق الوسطي – عدد و قطر التجاويف الهوائية – عدد الحزم الوعائية) و للجذر (قطر الجذور و الأسطوانة الوعائية – سمك القشرة و عدد الاوعية) و كذلك الصبغات النباتية. إضافة إلى ذلك فإن المعاملة بأملاح البحر ارتبطت إيجابياً مع تركيزات كل من الأحماض الأمينية الحرة – البرولين – الفينولات الذائبة الكلية – السكريات المختزلة وغير المختزلة و كذا تركيز الصوديوم و الكلوريد. من ناحية أخري إنخفض تركيز النيتروجين و الفوسفور و البوتاسيوم و الكالسيوم و الماغنسيوم و كذلك نسبة البوتاسيوم / الصوديوم تحت ظروف الإجهاد الملحي و كان التركيز ٨٠٠٠ جزء في المليون مميتا لجميع النباتات.

وقد أثر التعرض لأشعة جاما (١٠ و ٢٠ جراي) بشكل مفرد أو بالتفاعل مع معاملات الملوحة تأثيرا إيجابياً على خصائص النمو و المقاييس التشريحية و المركبات العضوية و العناصر المعدنية بينما ارتبطت الجرعات المرتفعة (٣٠ و ٤٠ جراي) سلبياً مع جميع الصفات المذكورة. و قد انخفض تركيز الصبغات النباتية تحت تأثير جميع الجرعات المستخدمة و كانت الجرعات المميتة للنبات بدءاً من ٥٠ جراي.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٥٦)العدد الأول (يناير ٢٠٠٥):٥٥-٨٨.