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EFFECT OF GAMMA IRRADIATION ON INCREASING WATER STRESS TOLERANCE OF MICROPROPAGATED BANANA PLANTS

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ABSTRACT

This investigation was carried out on micropropagated banana plants cv. Williams, to study the effect of PEG (0, 3, 6, 9, 12 and 15%) and gamma irradiation (0, 10, 20, 30, 40, 50 and 60 Gy) either alone or in combinations. Data were calculated *in vitro* as well as under greenhouse conditions.The obtained results indicated that the gradual increase in PEG levels was negatively correlated with all growth parameters (survival percentage, shoot 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. On the other hand, PEG application was positively correlated with total free amino acids, proline, total soluble phenols, sugars(reducing, non-reducing and total) as well as Na and Cl concentrations, meanwhile, N, P, K, Ca and Mg concentrations were decreased under water stress conditions. Exposure to gamma irradiation (10 and 20 Gy) alone or prior to PEG application had stimulative effects on growth characters , anatomical parameters,

organic components as well as mineral concentrations. Meanwhile, higher doses (30 and 40 Gy) were adversely affected in all the tested parameters. Photosynthetic pigments, however, were negatively correlated with all doses under study and the lethal dose (LD_{50}) was observed beyond 50 Gy.

Key words: *banana, dna fingerprinting, , gamma irradiation, micropropagation, peg, water stress .*

1. INTRODUCTION

Bananas and plantains are important staple food crops for people living in tropical and subtropical countries (Lee *et al.*, 1997). Edible cultivars are inter-specific hybrids of the two wild diploid species (2n =2x=22), *Musa accuminata* (AA) and *Musa balbisiana* (BB) (Simmonds and Shepherd, 1955 and Rowe, 1984). The interspecific hybrids (cultivars) have been grouped according to the contribution to the ploidy of the two wild species. *Musa accuminata* providing the "A" genome and *M. balbisiana* providing the "B" genome. The resulting hybrids are designated (AA, AB, BB, AAA, ABB, BBBB and AAAA) (Stover and Simmonds, 1987). The cultivar Williams, however, is belonging to AAA genomic group (2n= 3x=33) (Okole and Schulz, 1996). Environmental stress in arid and semi-arid areas, mainly drought limits growth and productivity of most species (Shin *et al.*, 2000). In addition, a large percentage of the world crops is exposed to chronic or sporadic periods of drought (Boyer, 1982) even in the absence of drought the limiting amounts of rainfall are primary determinants of crop selection and yield (Ludlow and Muchow, 1990). Thus, water deficit being one of the most common environmental limitation of crop productivity through alternation in metabolism and gene expression, causes a diverse set of physiological, morphological and developmental changes (Anders *et al.*, 1996). Recent advances in biotechnology for crop improvement have a great impact on banana and plantain cultivation. Micropropagation of shoot tip *in vitro* is one of the most common applications of biotechnology in agriculture (Dole, 1990) and it has been proposed as a useful, quick and economical to evaluate stress (Jośe *et al.*, 2000) as a better system for testing and selecting for stress tolerance (Cano *et al.*, 1998) mainly

drought to establish plantations in dry soils (Shin *et al.*, 2000).Moreover, mutants obtained in response to environmental stress appeared as a promising way to detect genes involved in normal and stressed-plant behaviour (Nicole, 1996). Drought resistance at any early stage of growth would be correlated with resistance at older stages in the field. On the other hand, *in vitro* plants must develop ways to prevent water loss to survive after transfer to low humidity under *ex vitro* conditions (Brainerd *et al.*, 1981). In this concern, Dix (1993) mentioned that *in vitro* selected plantlet resistant to polyethylene glycol (PEG) induced water stress was described as "osmotically adapted" in various species. PEG lowers the water potential but does not enter the cell wall thus imposing a stress similar to that by desiccation (Sala *et al.*, 1990).

This investigation was conducted to study the potential of gamma irradiation (as a mutagen) in inducing micropropagated banana plants more tolerant to drought using tissue culture technique .

2.MATERIALS AND METHODS

2.1. Experiment design

This work was carried out in the Biotechnology, Bioengincering and the Plant Analysis Laboratories, Fac. of Agric., Univ. of Cairo, and the 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 Agriculture Research Center, at Kanater El-Khairia was utilized as the mother plant material for obtaining the explants. Small suckers of about 50-70 cm in length were carefully cut from the 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 knife. 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 hypoclorite 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 remove 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 the 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 3mg l^{-1} BA +30 g l^{-1} sucrose + 7 g l^{-1} agar was used as 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 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 the 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 7 times at 4 week intervals into corresponding multiplication fresh media. Adventitious growing shoots were then separated *in vitro* and transferred to rooting ½ MS basal medium supplemented with 1 mg l^{-1} IBA + 1 mg l^{-1} NAA + 30 g l^{-1} sucrose + 6 g l^{-1} agar. Rooted plantlets were then transferred to MS basal liquid medium supplemented with 1 mg l^{-1} IBA + 1 mg l^{-1} NAA + 30 g l^{-1} sucrose.Polyethylene glycol (PEG ,molecule weight 8000) was added at the levels of 0, 3, 6, 9, 12 and 15 % .Filter papers (Whatman,No.1 90 mm) were designed in (M) letter shape as a bridge and inserted into the jars to be in contact with the medium in order to support plantlets. Osmotical potential (O.P.) of

the medium was measured before culture and 4 weeks after culturing, using the electric osmometer (whereas 1000 mhos mol = 2.24 MPa).In all *in vitro* experiments each treatment consisted of 3 replicates, each replicate consisted of 10 jars where each one contained only one plantlet.

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 calculated 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 polyethylene 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 was about 28±2°C and the humidity was adjusted to 85-90% by adding water at three hour intervals through the mist (for half an hour) during the nursery stage (15-21 days after transplanting). The white transparent polyethylene 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 conditions at the same manner of the first experiment. Survived plants were irrigated with polyethylene glycol (PEG) (MW 8000) water solution at concentrations of 3, 6, 9 and 12% at three day intervals. Control plants were irrigated with tap water throughout the whole growth stages. The plants were incubated at the greenhouse conditions for three months. In all pot experiments each treatment consisted of 3 replicates with 10 plants for each replicate (one plant for a 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 calculated 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 total 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 extraction fresh shoots 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 the determination of the total soluble phenols.

The determinations of N, P, K, Ca, Na, Mg and Cl were carried out on the shoots ground dry material.Dry sample was digested

by using sulphuric and perchloric acids according to Piper (1947). Nitrogen was determined using the micro kejeldahl 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 concentration was determined in the dry leaves by using titration method with silver nitrate as recommended by Brown and Jackson(1955) .

2.4. Anatomical studies

Specimens of 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 prepared using Johanson(1940) method.

2.5. Statistical analysis

Randomized complete block design for two factors 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 Fingerprints

Data in Table (1) reveal the application of RAPD analysis 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						
Roth-P15	AACGCGTCGG	6	6	6	6	6	6	8
Roth-P19	GGGAAGGACA	10	10	10	10	10	10	10

Primer Roth-P15:The results of primer Roth-P15 are shown in Figure (1). It gave a maximum of eight amplification products which ranged between 300 bp to 1650 bp. The only difference was recorded with 10 Gy to 50 Gy and the 60 Gy dose.

Primer Roth-P19:The results of primer Roth-P19 are shown in Figure (2). It gave a maximum of ten amplification products at the molecular sizes which ranged between 378 bp to 1400 bp. It gave DNA product with the control and all treatments. All bands were polymorphic between the control and the treatments.There was no difference between treatments.As compared with 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, duplication, translocation and point mutation (Britt, 1996). To avoid the negative side affects of radiation, Roux (1998) prefered the use of relatively low doses. 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).

3.2. Osmotic potential

PEG (for water stress) ,an inert, non-ionic, large chain polymer (HO CH_2-(CH_2-O-CH_2) × -CH_2O) has been widely used to maintain experimental media at predetermined ψw values (Allan *et al.*, 1981). It lowers the water potential but does not enter the cell wall thus imposing a stress similar to that by desiccation (Sala *et al.*, 1990).Data in Table (2) reveal that the gradual increase in PEG level resulted in a gradual increase in the osmotic potential of the liquid media either before or after culture (Table, 2).Similarly, Naidu *et al.* (1987) observed significant reduction in leaf water potential (ψw), osmotic potential (ψs), turgor potential (ψp) and relative water content (R.W.C.) in response to water stress treatments, however, the osmotic pressure values reflect the water status in the plant (Batanony *et al.*, 1991). The decrease in osmotic potential resulting from accumulation of solutes is a pronounced response to water stress in many plants. This process is known as osmotic adjustment, by lowering the osmotic potential in plant tissues subjected to water stress conditions (Shin *et al.*,

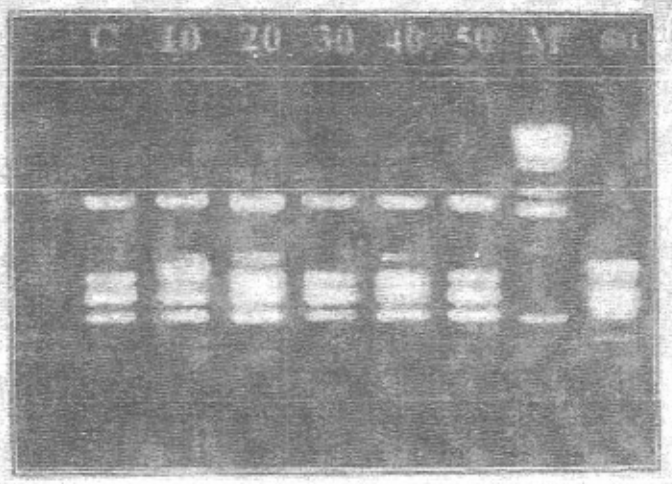

Figure (1): RAPD-profile using Roth-P15 primer of banana cv. Williams treated with different doses of gamma irradiation.
C: control, M: marker

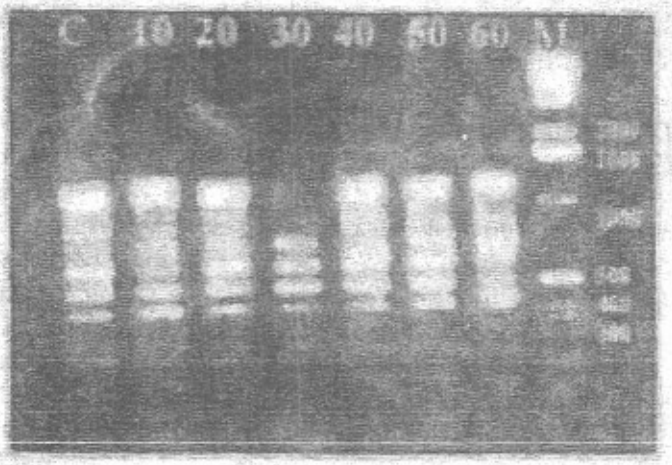

Figure (2): RAPD-profile using Roth-P19 primer of banana cv. Williams treated with different doses of gamma irradiation.
C: control, M: marker

Table (2): Effect of polyethylene glycol (PEG) levels on osmotic potential (mosmol) of the liquid medium before and after culture of rooted plantlets of banana cv. Williams.

Osmotic potential PEG. levels (%)	Before culture	After culture
0	197.75	122.75
3	229.25	147.50
6	266.00	230.00
9	351.25	339.75
12	465.00	562.25
15	577.50	610.00
L.S.D. 5%	**19.75**	**28.15**

2000). Meanwhile, the increase in solute concentration is expected as a result of dehydration and decreasing cell volume, osmotic adjustment which refers specifically to a net increase in solute concentrations due to metabolic process triggered by stress. Osmotic adjustment generated a more negative leaf water potential, thereby, helping to maintain water movement into the leaf, consequently, leaf turgor. Solutes that accumulate slowly during osmotic adjustment are relatively small, less than 1.0 MPa (William, 1995).

3.3.Growth characters

Data calculated in Table (3) show that the gradual increase in gamma irradiation doses or PEG levels either alone or in combination resulted in a gradual and significant decrease in survival percentage (%)with different PEG levels. The reduction could cause indirect damage in the living system by way of the various radical in radiated cells. It is worth to consider that the more OH produced the most radiosensitive. The tissues differ in its sensitivity to radiation, because of the difference in the number of OH^- generated by irradiation (Wada *et al.*, 1998). Mutations either spontaneous or induced have the same effects in inducing variations. Phillips *et al.* (1994) and Brar and Jain (1998) reported that these variations include point mutation , somatic

cross over, sister chromatid exchange, somatic gene rearrangement, changes in organelle DNA, insertion or excision of transposable elements, DNA amplification, DNA methylation, epigenetic variation and segregation of pre-existing chimeral tissue. These combinations have been recommended to upgrade cultivars of vegetative nature by micropropagated plants for induced mutation and improve crop plants were exposed to gamma irradiation (as physical mutagen) at low doses of about 25 Gy (De-Guzman *et al.*, 1982) and 40 Gy (Tulmann *et al.*, 1990; Domingues *et al.*, 1994; Mak *et al.*, 1995 and Smith *et al.*, 1995). The exposure doses 60 and 70 Gy, however, were completely lethal (Novak *et al.*, 1986 and Kulkarni *et al.*, 1997).

Concerning the PEG effects, Nieman *et al.* (1988) reported that the decrease in survival percentage under stress-conditions may be due to the energy spent to maintain turgor pressure at the expense of growth or the decrease in the availability of water to plants (Gunes *et al.*, 1996). Moreover, the disturbance in water uptake results in severely reduction in plants growth and maintenance (Greenway and Munns, 1980). However, when the soil water potential drops below a critical value, a water deficit in leaves will develop, this will associate with the depression in plant growth rate expressed by plant maintenance (Beese and Moshrefi, 1985).

Data in Table (3) also show that the gradual reduction in shoot and pseudostem heights, leaf and root numbers as well as shoot fresh and dry weights was due to the gradual increase in PEG levels either alone or in combination with different irradiation doses .In this respect, Tesu *et al.* (1980) postulated that the three stages of cell growth were adversely affected by stress condition, particularly, elongation and differentiation. These reductions, however, in cell division and enlargement may be attributed to the toxic effects of ions on metabolism or from adverse water relations (Hawker and Walker, 1978).Peroxidase isosymes are involved in the cell wall structure and expansion, it could be considered as a key factor in cell response and adaptation to water stress. In this respect, Sancho *et al.* (1996) reported that biotic stresses induce biochemical changes such as the activity of peroxidase as a group of enzymes affected by stress conditions. On the other hand , Ullah *et al.* (1993) demonstrated that the reduction in shoot growth might be due to the decrease in transpiration and photosynthesis under stress conditions . The reduction in leaf and

Table (3): Effect of gamma irradiation doses and PEG 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).

Growth characters	*In vitro* plants											
	Survival percentage (%)						Shoot height					
PEG levels (%)	Gamma irrad. doses (Gy)					Mean (A)	Gamma irrad. doses (Gy)					Mean (A)
	0	10	20	30	40		0	10	20	30	40	
0	100	91.55	83.33	65.56	47.78	**78.44**	7.13	9.12	8.85	6.53	5.93	**7.51**
3	87.78	90.24	74.07	56.82	39.81	**69.74**	7.33	8.80	8.60	6.47	5.87	**7.42**
6	82.22	81.75	69.44	53.17	38.22	**64.96**	7.20	8.73	7.93	6.33	5.80	**7.20**
9	78.89	80.38	62.04	49.54	34.51	**61.13**	6.73	7.80	6.87	6.00	4.98	**6.48**
12	73.33	74.32	58.33	45.89	31.32	**56.64**	6.60	6.40	6.27	5.67	4.40	**5.87**
15	32.00	29.09	22.59	15.89	11.73	**22.26**	5.53	5.47	5.67	4.27	4.27	**5.02**
Mean (B)	**75.70**	**75.27**	**61.63**	**47.81**	**33.89**		**6.75**	**7.68**	**7.49**	**5.88**	**5.21**	
L.SD. 5%	A=2.71		B= 2.96		A*B=6.63		A= 0.49		B= 0.53		A*B=1.19	
	No. of leaves/plantlet						No. of roots/plantlet					
0	8.33	8.76	7.91	6.87	6.40	**7.65**	8.53	7.87	8.20	6.20	6.33	**7.43**
3	8.27	9.07	8.80	6.73	6.33	**7.84**	7.87	8.00	7.80	6.13	6.00	**7.16**
6	7.67	9.00	7.27	6.47	5.87	**7.26**	7.93	7.93	7.53	6.07	6.00	**7.09**
9	7.40	8.53	7.20	5.93	5.73	**6.96**	6.80	7.40	6.93	5.80	5.47	**6.48**
12	6.60	7.67	6.73	5.53	4.49	**6.20**	6.67	6.73	6.60	5.13	4.86	**6.00**
15	6.20	6.20	6.07	4.40	4.47	**5.47**	5.11	5.00	4.13	4.27	4.41	**4.58**
Mean (B)	**7.41**	**8.21**	**7.33**	**5.99**	**5.54**		**7.15**	**7.15**	**6.86**	**5.60**	**5.51**	
L.SD. 5%	A=0.41		B= 0.45		A*B=1.00		A= 0.35		B=0.38		A*B= 0.86	
	Shoot fresh weight (g)						Shoot dry weight (g)					
0	8.89	9.37	9.13	7.21	6.18	**8.16**	0.46	0.48	0.47	0.33	0.29	**0.41**
3	7.92	9.24	8.62	7.56	6.04	**7.88**	0.44	0.50	0.48	0.39	0.31	**0.42**
6	7.63	8.41	7.72	7.30	5.86	**7.38**	0.44	0.48	0.44	0.39	0.31	**0.41**
9	6.55	7.81	7.40	6.67	5.58	**6.80**	0.38	0.45	0.43	0.35	0.31	**0.38**
12	5.94	7.45	6.70	4.54	4.25	**5.78**	0.34	0.46	0.40	0.24	0.23	**0.33**
15	4.98	5.12	4.55	4.21	4.11	**4.59**	0.23	0.31	0.29	0.20	0.19	**0.24**
Mean (B)	**6.98**	**7.90**	**7.35**	**6.25**	**5.34**		**0.38**	**0.44**	**0.42**	**0.32**	**0.27**	
L.SD. 5%	A= 0.54		B= 0.59		A*B=1.31		A=0.051		B= 0.056		A*B=0.124	
	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**
3	80.00	72.22	65.55	50.66	30.67	**59.82**	16.82	16.18	15.62	14.45	12.82	**15.18**
6	73.33	66.66	55.07	45.44	28.00	**53.90**	15.54	16.02	15.33	13.71	12.11	**14.54**
9	66.67	61.10	49.56	40.11	24.00	**48.29**	14.76	15.08	14.56	12.86	11.51	**13.75**
12	53.33	52.77	41.95	31.66	17.33	**39.41**	12.89	12.50	12.84	11.18	11.25	**12.13**
Mean (B)	**74.67**	**67.22**	**58.16**	**46.44**	**28.00**		15.37	15.20	**14.84**	**13.36**	**12.09**	
L.SD. 5%	A=2.43		B= 2.43		A*B=5.47		A=0.59		B= 0.59		A*B=1.32	
	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**
3	6.76	6.93	6.92	5.86	5.53	**6.40**	9.06	10.13	9.40	8.27	8.00	**8.97**
6	6.25	6.81	5.84	5.21	4.65	**5.75**	8.73	10.00	8.73	8.07	7.60	**8.63**
9	5.65	6.11	5.34	4.63	4.73	**5.29**	6.87	8.73	7.80	6.47	6.27	**7.23**
12	5.28	5.84	5.11	4.15	4.35	**4.95**	6.67	7.87	7.40	5.20	5.13	**6.45**
Mean (B)	**6.23**	**6.56**	**5.93**	**5.14**	**4.88**		**8.12**	**9.43**	**8.73**	**7.31**	**7.07**	
L.SD. 5%	A=0.44		B=0.44		A*B=0.97		A=0.63		B=0.63		A*B=1.41	
	Shoot fresh weight (g)						Shoot 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**
3	45.76	46.84	45.79	40.11	37.41	**43.18**	5.81	6.12	5.76	4.63	4.51	**5.37**
6	42.70	43.16	44.62	39.15	36.52	**41.23**	4.29	5.11	5.52	4.05	3.75	**4.55**
9	37.52	40.35	38.16	35.21	33.61	**36.97**	3.75	4.61	5.11	3.25	3.39	**4.02**
12	33.67	33.62	35.15	26.22	26.81	**31.09**	3.49	3.84	4.53	3.11	3.21	**3.64**
Mean (B)	**41.16**	**42.14**	**41.82**	**36.04**	**34.36**		**4.59**	**5.18**	**5.41**	**3.95**	**3.84**	
L.SD. 5%	A=1.03		B= 1.03		A*B=2.30		A= 0.29		B=0.29		A*B=0.66	

root numbers might be due to the imbalances in phytohormone levels under stress conditions which may affect the biosynthesis or the destruction of plant hormones, *i.e.,* the increase in ethylene concentration (Slocum *et al.*, 1984) and ABA accumulation (Shakirova and Bezrukova, 1998) or the reduction in endogenous IAA levels (Dunlap and Binzel, 1996). In addition, the ability of plant itself to absorb water may be affected under stress conditions or directly affected the plant biochemical process due to toxicity. The depression in shoot fresh weight under stress conditions may be attributed to the nutrient deficiency mediated by roots, *i.e.,* loss of K due to membrane depolarization and loss of Ca from plasmalemma due to displacement by Na ions (Cramer *et al.*, 1991), consequently, alteration in nutrient uptake due to the antagonism with essential elements (Shibli, 1993). The retardant in plant growth may be also explained by that great portion of energy will be used for water stress tolerance rather than for growth and biomass production of the organism. In addition, high metabolic activity is necessary for transformation of ions from the aerial parts to the roots (Cornillon and Palloix, 1995). ATP is an important regulator of cell metabolism and UDPG could often be limiting factor for cell wall synthesis and growth (Takeuchi and Amino, 1984). In this respect, Nieman *et al.* (1988) ascertained that, water stress conditions reduced the synthesis of uridine nucleotides or the production of uridine triphosphate (UTP) and uridine diphosphate glucose (UDPG).

On the other hand, it is clear from the results in Table (3) that, under each level of PEG, the interaction with lower doses of irradiation (10 and 20 Gy) significantly increased all growth characters compared with non- irradiated plants and treated only with different PEG levels. The combinations of *in vitro* technology and treating with mutagens (either physical or chemical) have been recommended for micropropagated plants to induce mutations and improving crop plants (Annonymous, 1987) such as plant size and resistance to pathogen and abiotic stresses (Donini and Sonnino, 1998). The exposure dose of 20 Gy was the optimal selected dose to make the technique on a large scale (Smith *et al.*, 1995). In addition, Devi and Nayar (1995) manifested that, exposure of micropropagated banana plants to 1, 1.5, 2.5 and 3 Krad of ^{60}Co gamma irradiation resulted in a gradual decrease in sucker size and bunch length. Moreover, De-Guzman *et al.* (1982) mentioned that lower doses of

irradiation at 10 Krad markedly stimulated the growth and the cultures were vigorous at 2.5 Krad and showed more shoot formation and more leaf production. On the other hand, many phenotypic variants in growth, leaf formation, pigmentation and texture were observed in the nurseries when gamma irradiation was used at 38 Gy (Mak *et al.*, 1995). The harmful effect of high exposure doses may be attributed to the indirect damage of the radiation on the living system by way of various radicals in radiated cells (Wada *et al.*, 1998).Similarly, Mak *et al.* (1995) observed a reduction in the average number of shoots produced per explant by increasing gamma irradiation exposure dose. Generally, shoot proliferation and the general vigor of plantlets was poor under mutagen treatment. The optimal dose, in this respect, was 20 Gy (Smith *et al.*, 1995) and 10 Gy (Silayoi *et al.*, 1995). Multiplication seldom occurred, however, beyond 50 Gy and 70 Gy was 100% lethal (Kulkarni *et al.*, 1997). The suppressive effect of high exposure doses may be due to the ploidy number and the differences between genomic groups (Novak *et al.*, 1990). In this context, Kulkarni *et al.* (1997) *in vitro* multiplied six cultivars of banana belonging to four genomic groups [(AAA), (AAB), (ABB) and (BB)]. They observed that hybrid genomes were more vulnerable compared with those with a single genome type.

3.4. Chemical composition

3.4.1. Photosynthetic pigments

Data presented in Table(4) exhibit the gradual and significant decrease in photosynthetic pigment concentrations as a result of the gradual increase in PEG levels and /or gamma irradiation doses either alone or in combinations. In this respect, Mak *et al.* (1995) and Toruan *et al.* (1995) pointed out that the increase in gamma irradiation exposure doses led to phenotypic variants in banana plants. These variants were determined by pigmentation, texture, total chlorophyll deficiency and growth habit. The frequency of these variants was the highest at dose 40 Gy in comparison with non-irradiated control plants (Domingues *et al.*, 1994).The depressive effect of water stress conditions on the absorption of some ions involved in the chloroplast formation such as Mg and Fe could be expected as a reason for chlorophyll suppression in leaves, and/or an increase in growth inhibitors such as ethylene or abscisic acid production which enhanced

Table (4): Effect of gamma irradiation doses and PEG levels on photosynthetic pigments(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												
Photosynthetic pigments	Chlorophyll a						Chlorophyll b					
PEG levels (%)	Gamma irrad. doses (Gy)					Mean	Gamma irrad. doses (Gy)					Mean
	0	10	20	30	40	(A)	0	10	20	30	40	(A)
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**
3	1.34	1.37	1.21	1.23	1.18	**1.27**	0.66	0.53	0.55	0.44	0.42	**0.52**
6	1.21	1.20	1.11	1.04	0.94	**1.10**	0.51	0.44	0.38	0.38	0.35	**0.41**
9	1.14	1.09	0.96	0.87	0.89	**0.99**	0.45	0.39	0.31	0.28	0.29	**0.34**
12	0.97	0.95	0.77	0.68	0.64	**0.80**	0.37	0.38	0.28	0.24	0.23	**0.30**
15	0.86	0.90	0.69	0.61	0.52	**0.72**	0.30	0.30	0.21	0.24	0.21	**0.25**
Mean (B)	**1.15**	**1.15**	**1.00**	**0.95**	**0.89**		**0.49**	**0.44**	**0.38**	**0.34**	**0.32**	
L.SD. 5%	A=0.15		B= 0.16		A*B=0.36		A=0.066		B= 0.073		A*B= 0.160	
	Carotenoids						Total chlorophylls					
0	0.57	0.48	0.41	0.36	0.34	**0.43**	2.04	1.95	1.83	1.74	1.58	**1.83**
3	0.55	0.48	0.40	0.31	0.31	**0.41**	2.00	1.90	1.76	1.67	1.60	**1.79**
6	0.42	0.42	0.36	0.29	0.25	**0.35**	1.72	1.64	1.49	1.42	1.29	**1.51**
9	0.36	0.37	0.32	0.29	0.22	**0.31**	1.59	1.48	1.27	1.15	1.18	**1.33**
12	0.31	0.30	0.24	0.24	0.22	**0.26**	1.34	1.33	1.05	0.92	0.87	**1.10**
15	0.27	0.30	0.20	0.18	0.18	**0.23**	1.16	1.20	0.90	0.85	0.73	**0.97**
Mean (B)	**0.41**	**0.39**	**0.32**	**0.28**	**0.25**		**1.64**	**1.58**	**1.38**	**1.29**	**1.21**	
L.SD. 5%	A=0.049		B=0.053		A*B=0.115		A=0.17		B=0.19		A*B=0.42	
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**
3	1.70	1.58	1.51	1.41	1.37	**1.51**	0.79	0.74	0.62	0.51	0.49	**0.63**
6	1.55	1.56	1.39	1.30	1.28	**1.42**	0.58	0.61	0.62	0.46	0.40	**0.53**
9	1.39	1.34	1.25	1.17	1.11	**1.25**	0.51	0.54	0.50	0.39	0.33	**0.45**
12	1.18	1.20	1.06	0.94	0.83	**1.04**	0.46	0.41	0.37	0.34	0.33	**0.38**
Mean (B)	**1.52**	**1.47**	**1.34**	**1.25**	**1.20**		**0.64**	**0.62**	**0.56**	**0.46**	**0.42**	
L.SD. 5%	A= 0.13		B= 0.13		A*B= 0.30		A= 0.104		B=0.104		A*B=0.243	
	Carotenoids						Total chlorophylls					
0	0.72	0.72	0.59	0.49	0.46	**0.60**	2.63	2.50	2.20	2.01	1.97	**2.26**
3	0.70	0.66	0.54	0.42	0.40	**0.54**	2.49	2.32	2.13	1.92	1.86	**2.14**
6	0.47	0.52	0.52	0.42	0.38	**0.46**	2.13	2.17	2.01	1.76	1.68	**1.95**
9	0.42	0.39	0.37	0.31	0.29	**0.36**	1.90	1.88	1.75	1.56	1.44	**1.71**
12	0.37	0.38	0.29	0.29	0.25	**0.32**	1.64	1.61	1.43	1.28	1.16	**1.42**
Mean (B)	**0.54**	**0.53**	**0.46**	**0.39**	**0.36**		**2.16**	**2.10**	**1.90**	**1.71**	**1.62**	
L.SD. 5%	A=0.088		B= 0.088		A*B=0.198		A=0.20		B=0.20		A*B=0.46	

senescence under stress conditions. In addition, un-available uptake of specific ions by the plants, and the accumulation of some ions in the leaves are widely assumed to result in the inhibition of photosynthesis. However, biosynthesis of chlorophyll and subsequently CO_2 fixation were inhibited under water stress conditions (Robinson *et al.*, 1983).

3.4.2. Organic components

It is clear from the results in Table (5) that the gradual and significant increases in reducing, non-reducing , and total sugars, free amino acids and proline as well as phenol concentrations were recorded as a result of the increase in PEG levels either alone or in combination with the different irradiation doses. Moreover, it is clear from the results that significant increases in sugar concentrations were detected when the different levels of PEG were combined with gamma irradiation at 10, 20 and 30 Gy, . In this respect, Handa *et al.* (1983) and Carpita *et al.* (1990) explained the cell adaptation to PEG stress by additional accumulation of sugars, amino acids and other metabolically protective osmolites. In this respect, many investigators suggested that starch and polysaccharides are converted to simple sugars to maintain more negative water potential values inside the plant, the sugars as osmolytes enable the plants to keep better water relations under water stress conditions (Simpson, 1981). Similar findings were observed by Abd El-Halem *et al.* (1989) who confirmed that the maximum accumulation of sugars was observed when wheat grains were gamma irradiated within a range of 2 Krad to 8 Krad.

Furthermore, the data in Table(5) indicate that all gamma irradiation doses increased free amino acids and phenol concentrations either alone or when combined with the different PEG levels. Similarly, Singh *et al.* (1993) found that the activities of polyphenol oxidase enzyme and phenolic compound concentrations were positively correlated with the increase of gamma irradiation exposure doses. The browning reaction is a defense mechanism and it is a result of quinine formation by oxidation of phenolic compounds due to the activity of polyphenol (Rohodes and Wooltorton, 1975). In this respect, Hanafy Ahmed (1991, 1996 and 1997) noticed that the increasing in sugars, total free amino acids and total soluble phenol concentrations when plants subjected to stress conditions could be explained on the assumption that such plants might have less

Table (5): Effect of gamma irradiation doses and PEG levels on organic components(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**

Organic components	Reducing sugars						Non-reducing sugars					
PEG levels (%)	Gamma irrad. doses (Gy)					Mean (A)	Gamma irrad. doses (Gy)					Mean (A)
	0	10	20	30	40		0	10	20	30	40	
0	3.15	3.80	4.01	4.30	3.98	**3.85**	9.49	9.91	10.59	11.16	9.11	10.05
3	3.58	3.95	4.22	4.49	3.69	**3.99**	9.66	10.24	10.55	11.36	10.08	10.38
6	3.95	4.45	4.69	4.90	4.21	**4.44**	10.10	10.12	10.46	11.38	10.34	10.48
9	4.56	4.66	5.05	5.28	4.58	**4.83**	10.42	10.46	10.85	11.57	10.79	10.82
12	4.67	5.11	5.31	5.61	4.88	**5.12**	10.46	10.65	10.93	11.61	10.90	10.91
15	5.20	5.39	5.64	6.00	5.29	**5.50**	11.07	11.15	11.24	11.56	10.94	11.19
Mean (B)	**4.18**	**4.56**	**4.82**	**5.10**	**4.44**		**10.20**	**10.42**	**10.77**	**11.44**	**10.36**	
L.SD. 5%	A=0.38		B=0.42		A*B=0.94		A=0.55		B= 0.58		A*B=1.29	
	Total sugars						Total free amino acids					
0	12.64	13.71	14.60	15.46	13.09	**13.90**	3.34	4.15	4.36	3.84	3.57	**3.85**
3	13.24	14.19	14.77	15.85	13.77	**14.36**	3.44	4.44	4.66	4.20	3.87	**4.12**
6	14.05	14.57	15.15	16.28	14.55	**14.92**	3.97	4.96	5.13	4.62	4.17	**4.57**
9	14.98	15.12	15.90	16.85	15.37	**15.65**	4.45	5.27	5.66	5.12	4.64	**5.03**
12	15.13	15.76	16.24	17.22	15.78	**16.0**	4.95	5.84	6.10	5.57	5.11	**5.51**
15	16.27	16.51	16.88	17.56	16.23	**16.0**	5.38	6.15	6.53	5.96	5.65	**5.93**
Mean (B)	**14.38**	**14.98**	**15.59**	**16.54**	**14.80**		**4.25**	**5.13**	**5.41**	**4.88**	**4.50**	
L.SD. 5%	A=0.63		B=0.69		A*B=1.55		A=0.25		B=0.28		A*B=0.61	
	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**
3	1.39	1.72	1.87	1.52	1.32	**1.56**	2.18	2.68	2.95	3.73	3.95	**3.10**
6	1.71	2.00	2.25	1.90	1.65	**1.92**	2.77	2.97	3.25	3.87	4.24	**3.42**
9	2.15	2.52	2.70	2.31	2.18	**2.37**	3.17	3.38	3.68	4.13	4.71	**3.81**
12	2.31	2.73	2.95	2.54	2.39	**2.58**	3.63	3.89	4.05	4.66	5.08	**4.26**
15	1.87	2.11	2.36	1.97	1.79	**2.02**	3.97	4.22	4.53	5.12	5.38	**4.64**
Mean (B)	**1.77**	**2.10**	**2.31**	**1.91**	**1.73**		**2.94**	**3.24**	**3.54**	**4.15**	**4.51**	
L.SD. 5%	A= 0.15		B=0.16		A*B=0.36		A= 0.38		B= 0.41		A*B=0.92	

Acclimatized plants (*ex vitro*)

	Reducing sugars						Non-reducing sugars					
0	3.49	4.11	4.44	5.00	3.93	**4.19**	9.78	10.81	11.00	11.18	10.63	10.68
3	3.95	4.38	4.88	5.28	4.18	**4.53**	10.57	10.94	11.04	11.46	10.83	10.97
6	4.20	4.79	5.08	5.68	4.18	**4.79**	10.67	11.08	11.19	11.69	10.40	11.01
9	4.89	5.21	5.48	6.02	4.96	**5.31**	10.71	11.15	11.25	11.84	10.75	11.14
12	5.11	5.90	6.04	6.00	5.27	**5.66**	11.14	11.16	11.50	12.27	11.30	11.47
Mean (B)	**4.33**	**4.88**	**5.18**	**5.60**	**4.50**		**10.57**	**11.13**	**11.20**	**11.69**	**10.78**	
L.SD. 5%	A=0.51		B=0.51		A*B=1.15		A= 0.39		B= 0.39		A*B=0.87	
	Total sugars						Total free amino acids					
0	13.27	14.92	15.44	16.18	14.56	**14.87**	3.98	4.32	4.76	4.20	3.95	**4.24**
3	14.52	15.32	15.92	16.74	15.01	**15.50**	4.32	4.78	5.12	4.65	4.49	**4.67**
6	14.87	15.87	16.27	17.37	14.58	**15.79**	4.88	5.36	5.70	5.13	4.98	**5.21**
9	15.60	16.36	16.73	17.86	15.71	**16.45**	5.55	5.55	5.93	5.13	5.79	**5.79**
12	16.25	17.06	17.54	18.27	16.57	**17.14**	5.87	6.31	6.89	6.15	5.94	**6.23**
Mean (B)	**14.90**	**16.01**	**16.38**	**17.28**	**15.28**		**4.92**	**5.26**	**5.68**	**5.25**	**5.03**	
L.SD. 5%	A= 0.41		B=0.41		A*B=0.92		A=0.44		B=0.44		A*B=1.00	
	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**
3	1.85	2.17	2.41	1.95	1.88	**2.05**	2.66	3.12	3.50	3.91	4.09	**3.47**
6	2.19	2.73	2.96	2.51	2.24	**2.53**	3.21	3.53	3.92	4.38	4.35	**3.88**
9	2.57	3.10	3.36	2.84	2.51	**2.88**	3.75	3.95	4.42	4.88	5.16	**4.43**
12	2.88	3.38	3.84	3.06	2.81	**3.19**	4.27	4.27	4.88	5.34	5.64	**4.91**
Mean (B)	**2.22**	**2.67**	**2.93**	**2.43**	**2.18**		**3.25**	**3.52**	**3.98**	**4.43**	**4.66**	
L.SD. 5%	A= 0.30		B=0.30		A*B=0.67		A= 0.46		B= 0.46		A*B= 1.03	

efficiency to condensate simple organic compounds into more complex ones. In addition,the authors assumed that the higher level of total soluble phenols and total free amino acid concentrations might be due to the increase in the metabolic activity to synthesise shikimic acid. Moreover, Anderson *et al.* (1995) mentioned that, the accumulation of amino acids under drought stress condition was due to the synthesis of organic acids and not from the hydrolysis of proteins or from pre-existing of amino acids. It has been suggested that organic acids (citric, malic ... etc.) served as a C source in the synthesis of amino acids, *i.e,*. proline *via* glutamate dehydrogenase. One amino acid that appears to be sensitive to stress is proline (William, 1995).

Data also show that all gamma irradiation doses increase proline concentrations either treated alone or when combined with the different PEG levels. Proline is considered as a cytoplasm-protective osmolyte necessary for adaptation to stress. Because of its osmotic properties, it is able to contribute directly to the retention of water and hence, drought resistance. Proline accumulation under water stress is caused by (1) stimulated synthesis, (2) inhibited oxidation, probably due to effects on mitochondria and impaired protein synthesis (Miller *et al.*, 1971; Stewart *et al.*, 1977 and Sells and Koeppe, 1980). Cells subjected to water (osmotic) stress by exposure to hyperosmotic concentrations of PEG responded with an initial loss of turgor and rapid accumulation of proline. As proline accumulation continued however, turgor gradually recovered (Handa *et al.*, 1986).New class of genes, however, called "osm" (osmotic tolerance) genes that protect against osmotic stress and may work in similar manner in plants, bacteria and animals, now attracted the attention of physiologists, through their action following salinity. The over produced proline may find an explanation. Osm genes govern the production of a class of molecules such as betaine and proline that protect the cells and its constituents against dehydration "osmoprotectants",(Rudulier *et al.*, 1984).

3.4.3. Minerals

It has been illustrated from the data presented in Table (6) that the significant decreases in N, P, K, Ca and Mg concentrations were a result of the gradual increase in PEG levels either alone or in combination with the different irradiation doses. Meanwhile, Na and Cl

Table (6): Effect of gamma irradiation doses and PEG levels on the 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					
PEG levels (%)	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	2.36	2.51	2.65	2.40	2.22	2.43
3	28.47	35.75	37.71	30.43	28.09	32.09	2.30	2.42	2.56	2.31	2.15	2.35
6	25.57	31.36	36.68	28.37	26.88	29.77	2.16	2.30	2.41	2.08	1.95	2.18
9	21.75	26.79	31.64	23.80	23.71	25.54	1.96	2.07	2.22	1.91	1.80	1.93
12	16.80	22.49	26.60	20.25	18.67	20.96	1.78	1.90	2.00	1.68	1.63	1.80
15	12.51	18.57	22.68	15.31	13.35	16.48	1.55	1.70	1.84	1.45	1.43	1.59
Mean (B)	22.34	28.78	32.64	25.26	23.43		2.02	2.15	2.28	1.97	1.86	
L.SD. 5%	A=2.46		B=2.70		A*B=6.03		A= 0.14		B=0.15		A*B=0.33	

	Potassium						Calcium					
0	37.10	40.78	44.84	37.46	32.88	38.81	13.15	15.77	16.29	12.44	10.71	13.68
3	35.06	39.75	41.98	35.38	31.64	36.88	12.31	15.61	15.05	11.54	9.28	12.76
6	31.90	34.45	37.29	32.70	27.24	32.72	10.54	12.90	13.19	9.51	8.16	10.86
9	26.19	31.57	32.49	26.94	24.46	28.73	9.78	11.79	12.16	8.77	8.02	10.10
12	22.81	27.34	29.85	22.72	19.73	24.49	9.22	10.10	10.87	8.85	7.61	9.33
15	19.56	23.18	26.31	20.42	16.71	21.24	7.58	8.38	8.86	7.62	7.26	7.98
Mean (B)	28.87	32.84	35.46	29.60	25.61		10.43	12.42	12.74	9.82	8.51	
L.SD. 5%	A=2.87		B=3.15		A*B=7.03		A=0.73		B= 0.80		A*B=1.79	

	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
3	1.21	1.58	1.63	1.79	1.92	1.63	2.86	3.34	3.71	2.54	2.30	2.96
6	1.59	1.95	1.90	2.11	2.18	1.95	2.51	2.92	3.30	2.23	2.11	2.61
9	1.94	2.31	2.37	2.44	2.49	2.31	2.11	2.64	2.92	2.00	1.86	2.31
12	2.15	2.56	2.64	2.81	2.80	2.59	1.91	2.47	2.60	1.89	1.66	2.15
15	2.52	2.83	2.83	2.94	3.06	2.84	1.96	2.19	2.14	1.72	1.51	1.90
Mean (B)	1.77	2.14	2.16	2.31	2.39		2.42	2.81	3.11	2.18	1.99	
L.SD. 5%	A=0.19		B= 0.21		A*B=0.48		A=0.23		B=0.25		A*B=0.56	

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
3	35.93	43.59	47.51	39.48	37.61	40.82	2.39	2.48	2.69	2.36	2.25	2.43
6	31.64	38.64	41.72	35.56	31.73	35.86	2.21	2.34	2.47	2.20	2.08	2.26
9	24.92	33.69	35.75	29.49	26.69	30.11	1.96	2.11	2.26	1.87	1.74	1.99
12	17.55	26.88	29.68	21.65	17.64	22.68	1.71	1.87	2.05	1.64	1.60	1.77
Mean (B)	29.87	37.87	41.21	33.88	31.06		2.15	2.29	2.46	2.11	2.00	
L.SD. 5%	A=3.50		B=3.50		A*B=7.87		A=0.21		B=0.21		A*B=0.47	

	Potassium						Calcium					
0	43.45	48.12	52.03	45.94	42.09	46.33	15.15	17.51	17.22	13.54	12.24	15.13
3	40.82	45.84	49.15	41.64	36.84	42.86	14.63	15.88	16.17	12.76	12.09	14.31
6	36.89	39.56	44.68	36.66	31.61	37.88	12.67	14.39	14.90	12.83	11.28	13.21
9	31.93	34.48	38.85	32.56	30.32	33.61	11.87	13.59	14.19	10.85	9.43	12.05
12	25.90	28.31	33.66	27.55	21.29	27.34	9.92	12.63	13.45	9.66	8.21	10.77
Mean (B)	35.80	39.26	43.67	36.87	32.41		12.85	14.86	15.19	11.93	10.65	
L.SD. 5%	A=3.94		B=3.94		A*B= 8.86		A=0.87		B= 0.87		A*B=1.96	

	Sodium						Magnesium					
0	1.56	1.87	1.92	2.11	2.19	1.93	3.21	3.74	4.10	3.15	2.94	3.42
3	1.64	1.95	1.95	2.20	2.20	1.99	3.21	3.62	3.84	2.97	2.80	3.28
6	1.96	2.25	2.19	2.34	2.30	2.21	2.81	3.05	3.24	2.80	2.65	2.91
9	2.28	2.59	2.52	2.85	2.81	2.61	2.54	2.94	3.10	2.27	2.18	2.61
12	2.63	2.92	2.97	3.11	3.21	2.97	2.24	2.54	2.81	2.02	1.76	2.27
Mean (B)	2.01	2.32	2.31	2.52	2.54		2.80	3.18	3.42	2.63	2.47	
L.SD. 5%	A= 0.17		B=0.17		A*B=0.38		A=0.23		B=0.23		A*B=0.51	

Chloride

	In vitro plants						Acclimatized plants (*ex vitro*)					
0	1.71	1.87	1.85	1.87	1.94	1.85	1.89	2.00	2.07	2.05	2.00	2.00
3	1.78	1.90	1.94	1.92	1.94	1.90	1.89	2.11	2.22	2.20	2.18	2.12
6	1.96	2.13	2.17	2.27	2.31	2.17	2.13	2.25	2.22	2.45	2.40	2.29
9	2.31	2.54	2.61	2.72	2.79	2.59	2.37	2.61	2.68	2.74	2.79	2.64
12	2.73	2.91	2.90	2.96	3.05	2.91	2.54	2.84	2.85	3.06	3.11	2.88
15	2.91	3.11	3.04	3.19	3.21	3.09						
Mean (B)	2.23	2.41	2.42	2.49	2.54		2.16	2.36	2.41	2.60	2.50	
L.SD. 5%	A=0.20		B=0.22		A*B=0.49		A= 0.22		B= 0.22		A*B=0.50	

concentrations were increased due to these treatments. This decrement in N concentration might be explained by that all NH_4^+ was converted to amino acid compounds before loading in the xylem vessels under water stress conditions (Anderson and Brodbeck, 1989). This stress caused damages for the mechanisms controlling intracellular P concentration (Mass and Nieman, 1978). Some plants, such as halophytes can develop a specific mechanism for the preferential uptake of K under stress conditions. These plants have a very developed absorption system (Marschner, 1995). The reduction in N, P and K concentration might elucidate the primary role of accumulation N-containing compounds reported for numerous environmental stress in NH_4 detoxication (Robe, 1990). The diminish in N concentration might be impaired by N uptake, due to completion relationship between NO_3^- and Cl^- (Greenway and Munns, 1980 and Hanafy Ahmed,1996).

On the other hand, it is clear from the results in Table (6) that, under each level of PEG, the interaction with the lower doses of irradiation (10 and 20 Gy) significantly increased N, P, K Ca and Mg concentrations compared with the higher doses (30 and 40 Gy). Similarly, Eleiwa and Rabie (1994) mentioned that N, P and K concentration was increased as a result of gamma irradiation treatments at lower doses but decreased when gamma irradiation was used at higher doses. In addition, He and Yu (1995) confirmed that mutant callus derived from irradiated immature embryos of rice cv. Shuagfeng contained higher amounts of K in comparison with the original genotypes. However, El-Hanafy (1994) cited that most tested doses of gamma irradiation caused an increase in N and a decrease in K concentrations, while P concentration varied according to the exposure dose under study. Moreover,Chauhan and Khosha (1992) noticed similar changes in calcium concentration in the mutant seedlings of *Robinia pseudoacacia* due to gamma irradiation treatments.

Similarly, Gregely *et al.* (1980) found that Ca concentration was decreased significantly due to PEG application in water stressed-apple seedlings. In halophytes, plants have a very developed absorption system and a developed mechanism for the preferential uptake of K^+ from mixture rich in Na (Marschner, 1995). Magnesium concentration in the chloroplasts may influence photosynthesis during water stress

Table (7): Effect of gamma irradiation at 10 Gy and PEG levels on the structure of leaves and roots of micropagated plants of banana cv. Williams grown *ex vitro* (3 months after acclimatization).

Plant organ	Leaves					Roots			
Anatomical parameters Gamma irrad. doses + PEG levels	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% PEG	1070	370	6	250	15	1840	630	620	15
3% PEG	840	340	4	230	10	1720	640	600	14
6% PEG	700	290	4	150	6	1300	500	410	12
9% PEG	500	190	2	190	4	1600	380	420	8
12% PEG	340	200	-	-	3	800	240	340	7
10 Gy + 3% PEG	1070	330	4	230	14	1831	660	580	11
10 Gy + 6% PEG	730	380	4	240	10	1300	500	440	7
10 Gy + 9% PEG	640	250	3	200	7	1200	370	430	8
10 Gy + 12%PEG	500	220	2	100	4	840	260	350	7

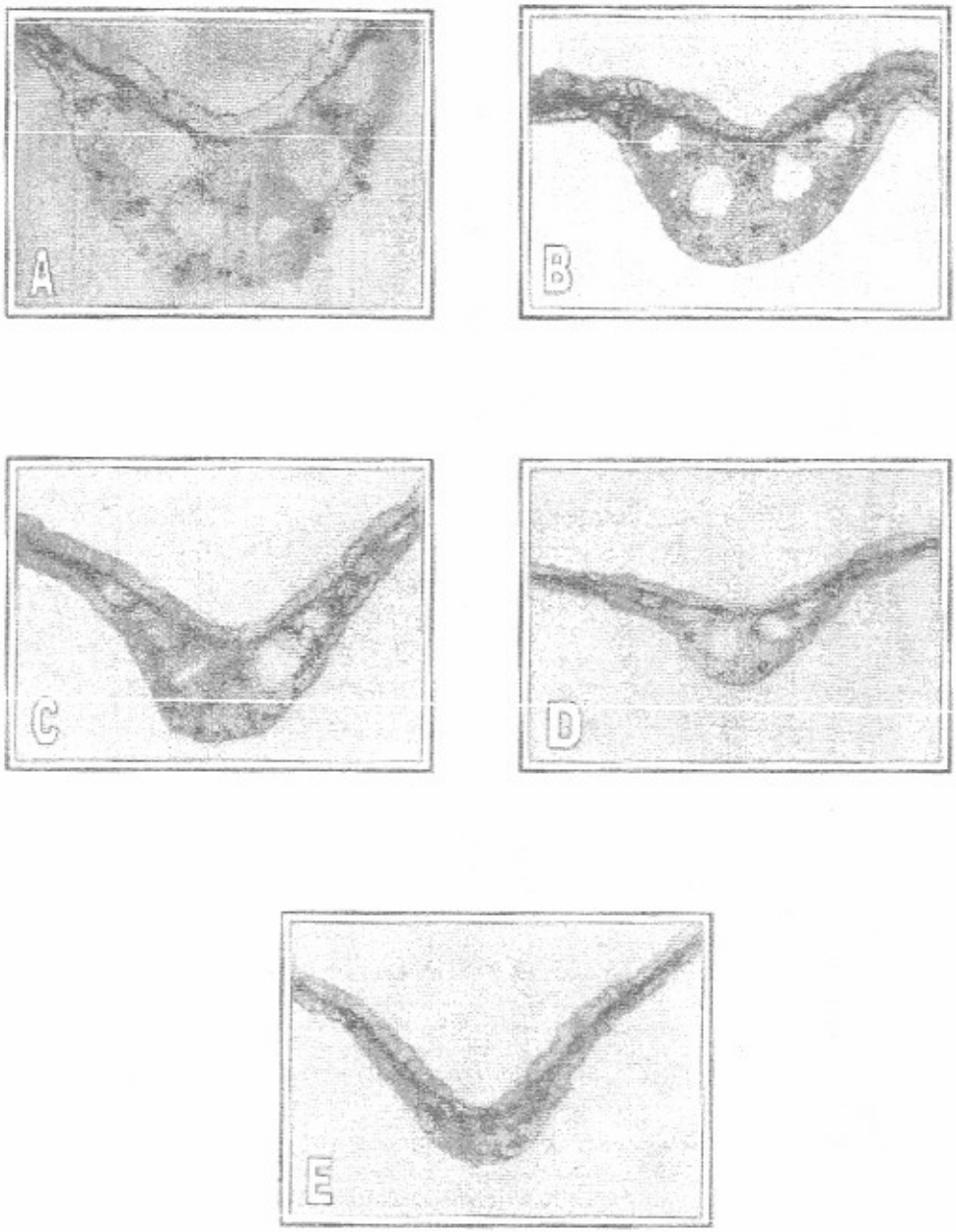

Fig.(3): Effect of PEG levels on leaf structure of micropropagated plants of banana cv. Williams grown *ex vitro* (3 months after acclimatization)(20X).

A:0% B:3% C:6% D:9% E:12%

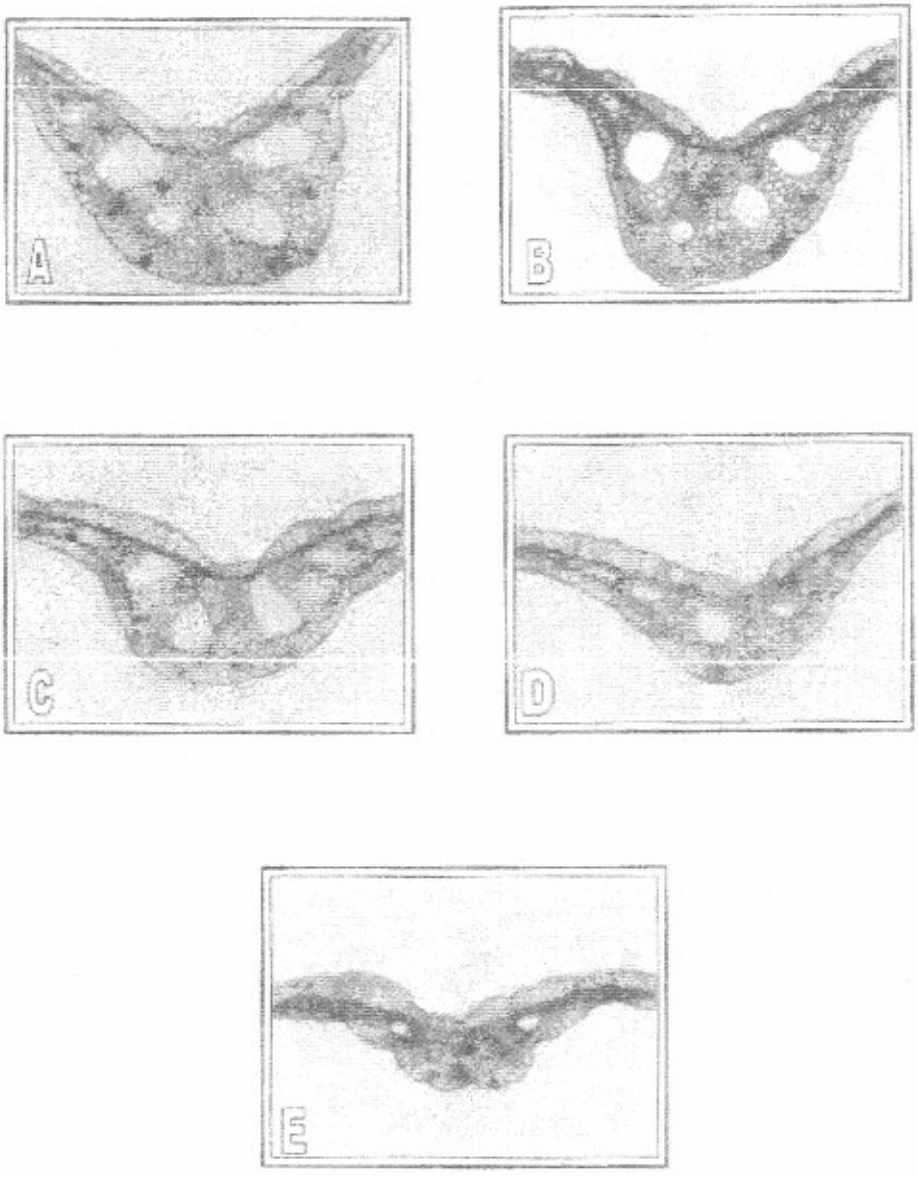

Fig (4): The interaction effect of gamma irradiation at 10 Gy and PEG levels on leaf structure of microptopagated plants of banana cv. Williams grown *ex vitro* (3 months after acclimatization)(20X).

A:10Gy+0% **B:10Gy+3%** **C:10Gy+6%**
D: 10Gy+ 9% **E:1 0Gy + 12%**

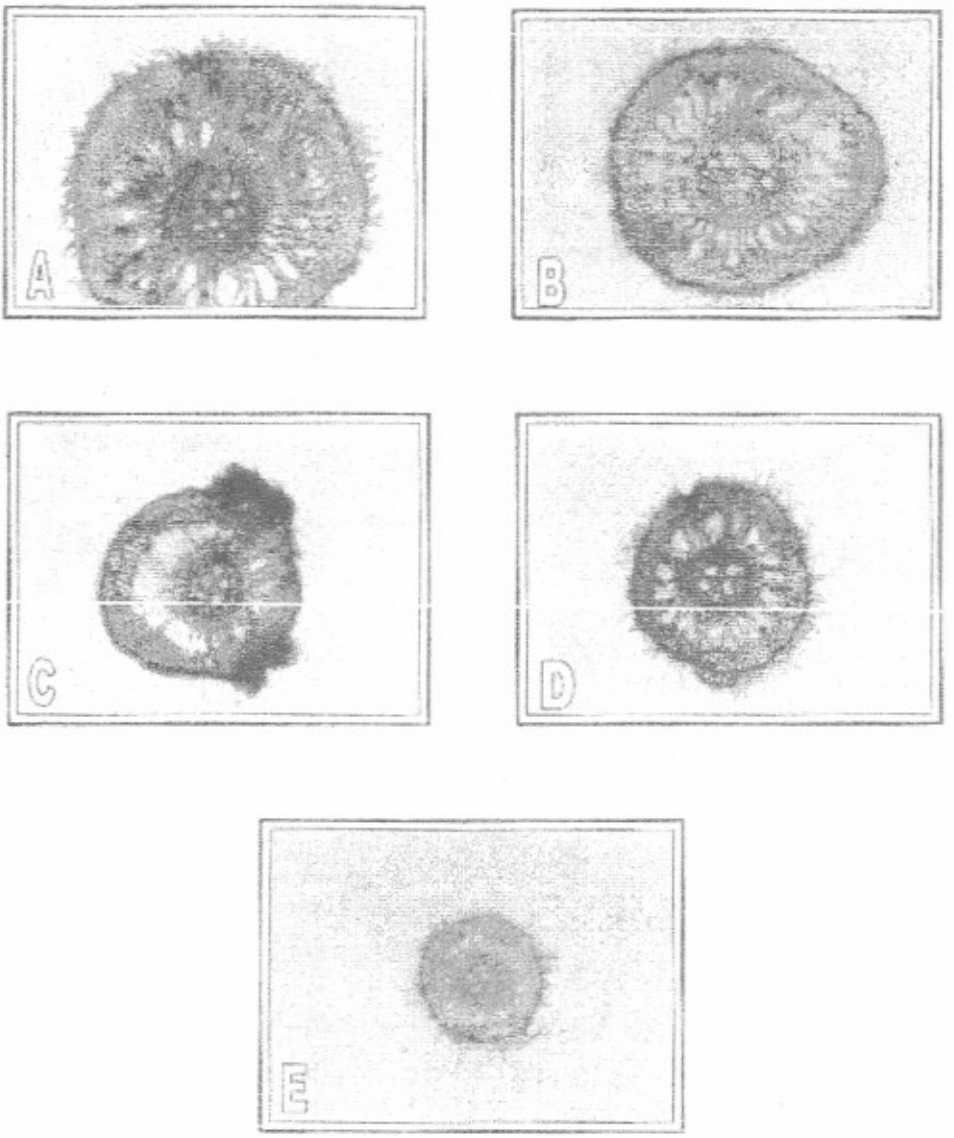

Fig.(5): Effect of PEG levels on root structure of micropropagated plants of banana cv. Williams grown *ex vitro* (3 months after acclimatization)(20X).

A:0 B:3% C:6% D:9% E:12%

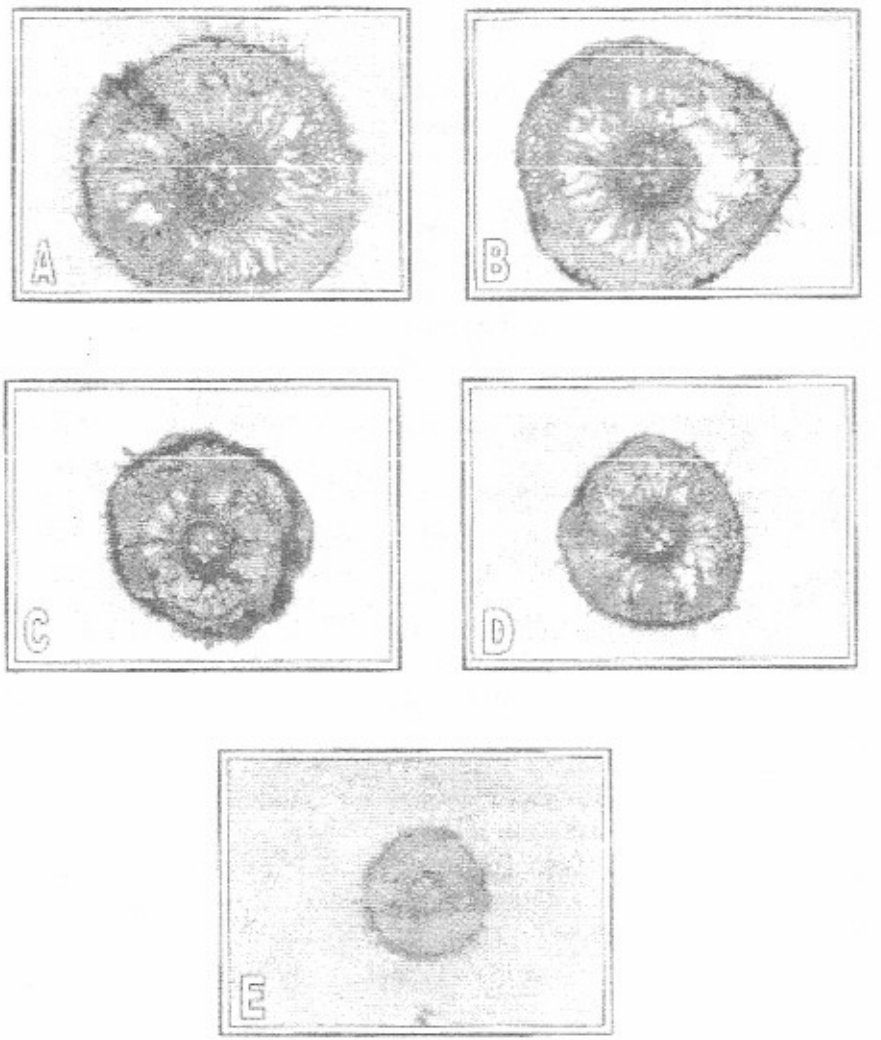

Fig.(6): The interaction effect of gamma irradiation at 10Gy and PEG levels on root structure of micropropagated plants of banana cv Williams grown *ex vitro* (3 mobths after acclimatization)(20X).

A:10Gy+0% **B:10Gy+3%** **C:10Gy+6%**
D:10Gy+ 9% **E:10Gy+12%**

through its role in coupling electron transport to ATP production. The plants with the lower Mg^{+2} concentrations maintained higher photosynthetic rates as leaves became hydrated. 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). The internal Mg concentration, however, was important only in the presence of high external Ca concentration (Ben-Hayyim *et al.*, 1987).

Regardless of the treatments under the present study, the data also show that the significant increase in sodium concentration was due to the gradual increase in gamma irradiation doses as well as PEG levels either alone or in combinations. Under water stress conditions, Na influx across the plasmalemma to the vacuole may play a major role in permitting turgor maintenance. Some crops show marked beneficial effects of Na especially if the K supply is limiting. These crops take up large amount of Na^{+} which contributes to the osmotic potential of the leaves and increase resistance to water stress (Drycot, 1972). The damage effect of Na, however, may be attributed to that Na is capable to disturbing the fine structure of the plant cell causing swelling of chloroplast which may result in chlorosis and necrosis (Hect-Buchhoiz *et al.*, 1974).

Concerning chloride concentration, the interaction with different irradiation doses was statistically equal under each level of PEG. The significant increase in Cl concentration however, was due to the gradual increase in PEG level either applied alone or in combination with different irradiation doses. The obtained results are in agreement with those observed by Barakat *et al.* (1982) , Santos *et al.* (1994) and El-Hammady *et al.* (1995).

3.5. Leaf and root anatomy

The interaction of gamma irradiation at 10 Gy and different levels of PEG markedly stimulate leaf growth expressed by increasing in thickness of the leaf blade and midrib, the number and diameter of air cavities, the number of vascular bundles, and root growth expressed by increasing in cortex width,diameter of roots and vascular cylinder as well as the number of vessels (Table, 7 and Figs., 3, 4, 5 and 6) compared with non-irradiated plants and those treated only with PEG.

In this respect, Devi and Nayar (1995) reported that higher doses of irradiation (2.5 and 3 Krad) caused reduction in all growth characters of micropropagated banana, and the LD_{50} was approximately about 38 Gy that caused reduction of growth to 50% compared with control (Mak *et al.*, 1995). Generally, growth stimulation was observed at 10 Gy (Silayoi *et al.*, 1995) or 20 Gy (Smith *et al.*, 1995).

Data in Table (7) also indicate that all the anatomical parameters of banana plants were greatly affected by the gradual increase in PEG levels. The suppression was primarily due to osmotic effects, though shoot water content decreased slightly. The first physiological reaction to the increased stress conditions was the reduced entry of water into the roots, this tends to inhibit meristematic activity and elongation of the roots (Hayward and Spurr, 1944).

Moreover, Strogonov (1974) mentioned that, stress conditions induced a reduction in the diameter of xylem vessels. Such reduction, might affect the flow of water and minerals from the roots to the shoots. Generally , it is important here to mention that the gradual increase in PEG levels was negatively correlated with all growth characters , anatomical parameters of the leaf and the root , mineral concentrations (N,P,K,Ca and Mg) ,while a reverse trend was detected by simple organic components (sugars, free amino acids and soluble phenols) as well as Na and Cl .Furthermore, it can be suggested that , exposure to gamma irradiation at the lower doses (10 or 20 Gy) either alone or combined with PEG application had stimulative effects on most of the studied growth characters, anatomical parameters, simple organic components as well as mineral comcetrations.However, the higher doses of gamma irradiation (30 or 40 Gy) adversely affected all the tested parameters.

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

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ملخص

أجريت هذه الدراسة على نبات الموز صنف ويليامز المكاثر معمليا بهدف دراسة تأثير البولي إيثيلين جليكول (٠، ٣، ٦، ٩، ١٢، ١٥%) و كذا أشعة جاما (٠، ١٠، ٢٠، ٣٠، ٤٠، ٥٠، ٦٠ جراي) سواء بمفردها أو بالتفاعل مع المعاملات المختلفة على الإجهاد المائى و قد سجلت النتائج معمليا و في الصوب. أظهرت النتائج أن زيادة تركيز البولي إيثيلين جليكول ارتبط سلبياً مع جميع صفات النمو (النسبة المئوية لإستمرارية 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 الجذر (قطر الجذور و الأسطوانة الوعائية - سمك القشرة و عدد الاوعية) و كذلك الصبغات النباتية. إضافة إلى ذلك فإن المعاملة بالبولي إيثيلين جليكول ارتبطت إيجابياً مع تراكيز كل من الأحماض الأمينية الحرة - البرولين - الفينولات الذائبة الكلية - السكريات المختزلة و غير المختزلة و الكلية و كذا تركيز الصوديوم و الكلوريد. من ناحية أخري فقد انخفض تركيز النيتروجين و الفوسفور و البوتاسيوم و الكالسيوم و الماغنسيوم تحت ظروف الإجهاد المائي.

إرتبط التعرض لأشعة جاما (١٠ و ٢٠ جراي) بشكل مفرد أو بالتفاعل مع معاملات الجفاف إيجابياً بخصائص النمو و القياسات 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.

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