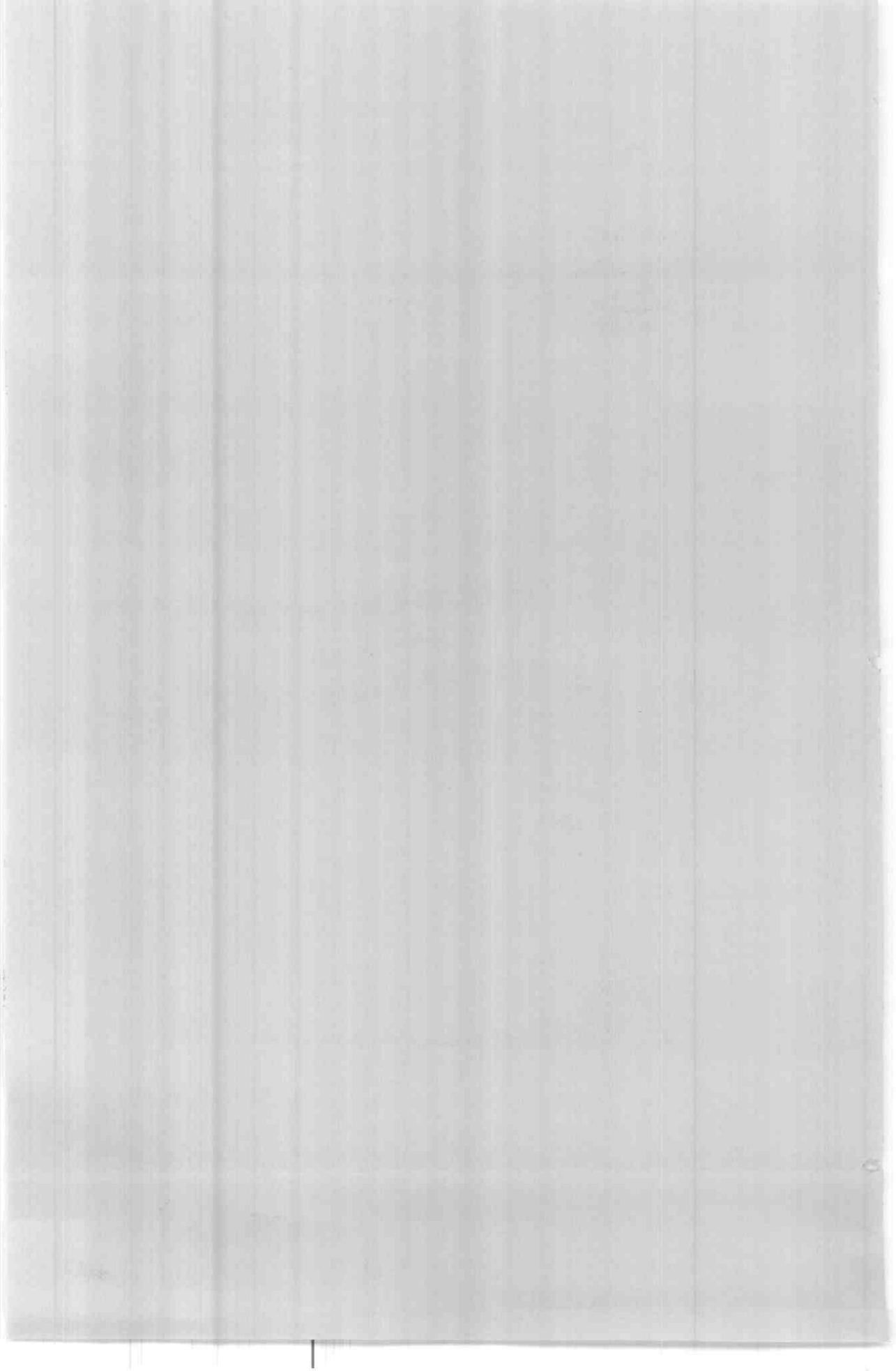
RESULTS AND DISCUSSION



IV. RESULTS AND DISCUSSION

4.I. Marianna (2624) plum rootstock:

4.I.1. Establishment stage:

4.I.1.a. Effect of medium and explant types:

Data of **Table (1-A)** reflect the effect of different medium types and explant types on necrosis, browning, explant development and greening parameters. It is clear from **Table (1-A)** that Murashige & Skoog medium was significantly superior than either Gamborg or Nitsch and Nitsch media in reducing necrosis and browning while increased both explant development and greening parameters. Meanwhile, Nitsch and Nitsch medium followed MS in improving all parameters under study Moreover, **Table (1-B)** and **Photo (1)** shows that shoot tip significantly surpassed one-node cuttings in improving all studied parameters under study *i.e.*, necrosis, browning, explant development and greening.

Regarding the effect of interaction between medium type and explant type, it is noticed from **Table (1-C)** that culturing of shoot tip explant on MS medium induced significant reduction in necrosis and browning parameters as compared with the other interactions. However, the significant differences among different interactions were nil when explant development and greening parameters were considered.

The above results assured the superiority of both Murashige and Skoog medium and shoot tip explant on the others. These results confirm the findings of **Marino (1983); Bassi (1984)** and **Pietrapalo and Reisch (1984).** They recommended Murashige and Skoog medium for culturing the shoot tip explant of plum plants.

Table (1): Effect of medium type and explant type on development of Marianna plum rootstock explant.

Table (1-A): Effect of medium type.

Parameters / Medium type	Necrosis	Browning	Explant development	Greening
Murashige & Skoog	1.51 C	1.23 C	3.65 A	3.50 A
Gamborg	3.77 A	4.10 A	1.22 C	1.32 C
Nitsch and Nitsch	2.95 B	3.35 B	2.07 B	1.72 B

Means of medium type followed by the same letter within each column are not significantly different from each other at 1% level.

Table (1-B): Effect of explant type.

Parameters / Explant type	Necrosis	Browning	Explant development	Greening
Shoot tip	2.45 B	2.58 B	2.54 A	2.54 A
One-node cutting	3.03 A	3.20 A	2.08 B	1.81 B

Means of explant type followed by the same letter within each column are not significantly different from each other at 1% level.

Table (1-C): Effect of the interaction between medium type and explant type of plum.

Parameters	Necrosis		Browning		Explant development		Greening	
Explant type / Medium type	Shoot tip	One node cutting	Shoot tip	One node cutting	Shoot tip	One node cutting	Shoot tip	One node cutting
Murashige & Skoog	1.12 F	1.90 E	1.13 D	1.33 D	4.03 A	3.27 A	3.93 A	3.07 A
Gamborg	3.43 B	4.10 A	3.60 B	4.60 A	1.33 A	1.10 A	1.60 A	1.03 A
Nitsch and Nitsch	2.80 D	3.10 C	3.03 C	3.67 B	2.27 A	1.86 A	2.10 A	1.33 A

Means of the interaction between medium type and explant type followed by the same letter within each category are not significantly different from each other at 1% level.

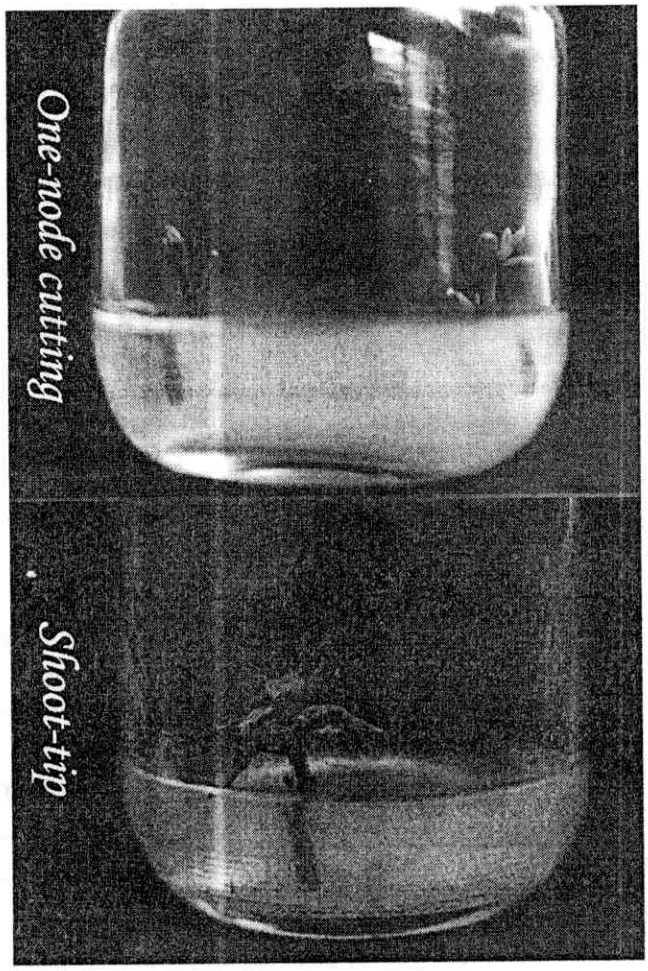


Photo (1): **Effect of explant type on development of Marianna plum rootstock explants.**

4.I.1.b. Effect of anti-oxidant treatments:

Table (2) and **Fig. (1)** deals with the effect of different anti-oxidant treatments on necrosis, browning, explant development and greening parameters. It is obvious that anti-oxidant solution as a pretreatment either alone or in combinations resulted in significant decrease of both necrosis and browning parameters as compared with the other treatments. Meanwhile, explant development and greening were significantly maximized as the explant was pretreated with anti-oxidant solution and cultured on medium containing PVP.

The above results reflected the importance of combined treatment of anti-oxidant solution and PVP in improving all parameters under study. These results are in general agreement with **Bayomy (1998)** on pome fruits and **Hassan (2004)** on apple rootstocks. They revealed that combined treatment of anti-oxidant solution and PVP were effective in reducing phenolic compounds.

Table (2): Effect of antioxidant treatment on development of Marianna plum rootstock explant.

Parameters / Treatment	Necrosis	Browning	Explant development	Greening
Control	2.97 B	2.80 B	2.17 E	2.37 F
Antioxidant	1.13 F	1.00 F	3.23 B	3.10 C
P.V.P	1.87 DE	1.47 E	3.07 BC	3.33 B
Activated charcoal	3.33 A	3.17 A	2.10 E	2.07 G
Antioxidant + P.V.P	1.03 F	1.07 F	4.33 A	4.07 A
Antioxidant + A.C.	2.13 D	2.23 C	2.47 D	2.60 E
P.V.P. + A.C	2.50 C	2.73 B	2.63 D	2.87 D
Antioxidant + P.V.P. + A.C	1.60 E	1.80 D	2.93 C	3.03 CD

Means of antioxidant treatments followed by the same letter within each column are not significantly different from each other at 1% level.

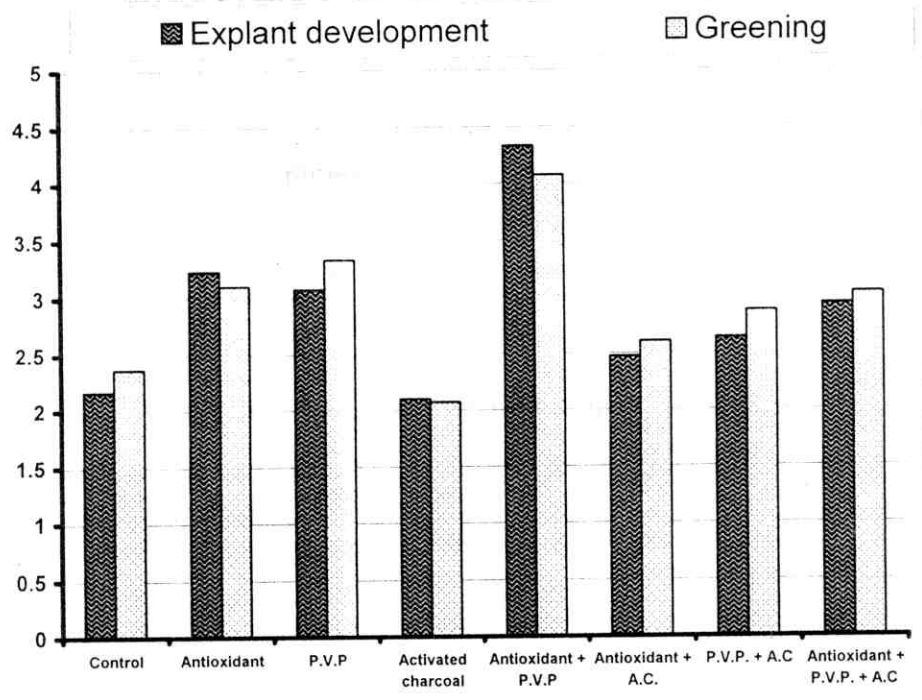


Fig. (1): Effect of antioxidant treatment on development and greening of plum explants.

4.I.1.c. Effect of cold pretreatment:

The data outlines in the **Table (3)** describe the effect of different cold pre-treatment periods on necrosis, browning explant development and greening parameters of plum explant. It reflected that keeping of the explant for 3 days in refrigerator (5°C) enhanced a significant improvement of the explant which reduced necrosis and browning while increased explant development and greening parameters in relation to the other periods and the control.

The above results demonstrate that cold pretreatment of the explant for 3 days encouraged the best responses of the explant. This may be due to the ability of the cold treatment to reduce free phenolic

compounds through converting it to combined phenolic compound which resulted in reducing their toxicity and in turn improved the tissue culture parameters. These results are in harmony with the findings of **Moriguchi *et al.* (1991)** on *Pyrus communis;* **Perez *et al.* (1999)** on apricot; **Channuntapipat *et al.* (2000)** on almond; **Ainsley *et al.* (2001)** on almond and **Gardi *et al.* (2001)** on micropropagated apple rootstocks, blackberry, kiwifruit cultivars and olive cultivars. They preferred pre-treating the explants with cold treatment.

Table (3): Effect of cold pretreatment on development of Marianna plum rootstock explant.

Parameters / Period (day)	Necrosis	Browning	Explant development	Greening
Control	2.83 A	2.73 A	1.33 D	1.43 D
1	1.47 C	1.63 C	1.76 C	1.80 C
3	1.10 D	1.24 D	3.73 A	3.73 A
5	1.43 C	1.43 CD	2.67 B	2.20 B
7	1.87 B	2.03 B	1.50 CD	1.20 D

Means of cold pretreatment followed by the same letter within each column are not significantly different from each other at 1% level.

4.I.1.d. Effect of additives:

Table (4) and **Fig.(2)** reveals the effect of different additives to the culture medium on the necrosis, browning, explant development and greening parameters. It is noticed that supplementing the culture medium with adenine sulphate significantly reduced necrosis and browning while significantly improved greening parameter in relation to the other additives. Moreover, adding of yeast extract to the culture medium succeeded significantly in reducing necrosis and maximized explant development in comparison with different additives under study.

The aforementioned results clarify that either addition of adenine sulphate or yeast extract was more effective in improving both greening and growth respectively. These results go in line with the findings of **Zaied (1997)**. She mentioned that addition of 80 mg/L

of adenine sulphate to MS medium resulted in improving of explant development criteria of stone fruit plants.

Table (4): Effect of different additives on development of Marianna plum rootstock explant.

Parameters / Additive	Necrosis	Browning	Explant development	Greening
Glutamine	2.60 B	2.73 AB	2.43 C	2.07 C
Asparagine	3.73 A	3.10 A	1.50 D	2.07 C
Adenine sulphate	1.27 C	1.53 C	3.40 B	4.03 A
Yeast extract	1.07 C	2.37 B	4.23 A	3.63 B

Means of different additives followed by the same letter within each column are not significantly different from each other at 1% level.

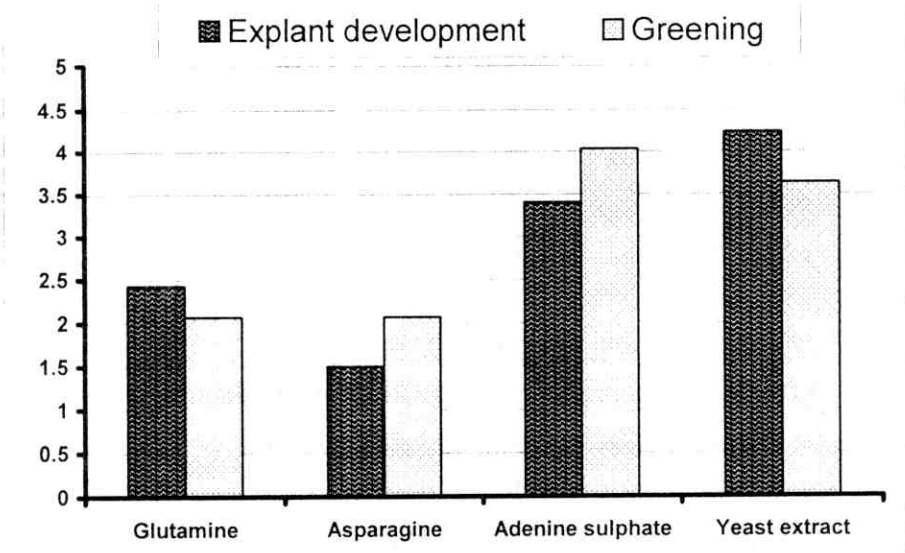


Fig. (2): Effect of different additives on development and greening of plum explants.

4.I.2. Induction of mutations and variations:-

4.I.2.a. Effect on Tissue culture parameters:

Data tabulated in **Table (5)**, **Fig. (3)** and **Photo (2)** explain the effect of different mutagens (irradiation and chemical mutagens) on tissue culture parameters (necrosis, callus production, proliferation, growth and greening). It appeared that subjecting the explant to gamma rays with 0.5 Kr dose was significantly effective in reducing necrosis and improving proliferation and greening parameters in comparison with the other mutagens at different levels. Similar results were obtained in growth when 0.5 and 1.0 Kr doses were used in relation to the others. However, callus production was significantly maximized when sodium azide mutagen was supplemented to the culture medium at 0.1 g/L concentration as compared with the other treatments.

The aforementioned results verify that gamma rays at very low dose improved all tissue culture parameters.

These results are somewhat in agreement with the findings of **Predieri *et al.* (1986)** on pear; **Predieri and Malavasi (1989)** on apple rootstocks. They reported that low doses of gamma rays maximized number of shoots and enhanced regeneration.

4. I.2.b. Effect on Phenotypic variabilities:

Table (6) reflects the effect of different mutagens at different levels on percentage of phenotypic variable plantlets. It appear that increasing sodium azide concentration up to 0.5 g/L resulted in increasing phenotypic variabilities up to 50% while the same concentration of colchicine induced only 20% variable plantlets. However, irradiation by gamma rays at doses 1.0, 2.0 and 3.0 Kr resulted in producing 70, 90 and 30% variable plantlets, respectively.

Table (5): Effect of different mutagens on necrosis, callus production, proliferation growth and greening of Marianna plum rootstock explant.

Parameters / Treatments		Necrosis	Callus production	Growth	Proliferation	Greening
Control		1.23 G	1.20 D	3.83 AB	2.37 B	2.70 C
0.1 SA	g/L	1.80 F	2.20 A	2.63 D	2.60 B	2.13 D
0.5 SA		4.33 C	1.27 CD	1.17 F	1.33 D	1.20 F
1.0 SA		5.00 A	1.00 E	1.00 F	1.00 E	1.00 G
0.1 Col.	g/L	1.90 F	1.77 B	3.07 C	2.40 B	2.57 C
0.5 Col.		4.27 C	1.23 CD	1.40 F	1.37 D	1.33 F
1.0 Col.		5.00 A	1.00 E	1.00 F	1.00 E	1.00 G
0.5 Kr		1.20 G	1.60 B	4.17 A	3.03 A	3.67 A
1.0 Kr		2.67 E	1.23 CD	3.53 B	3.23 A	3.40 B
2.0 Kr		3.47 D	1.40 C	2.13 E	1.80 C	1.87 E
3.0 Kr		4.63 B	1.17 DE	1.37 F	1.20 DE	1.30 F
4.0 Kr		5.0 A	1.00 E	1.00 F	1.00 E	1.00 G

Means of different mutagens followed by the same letter within each column are not significantly different from each other at 1% level.

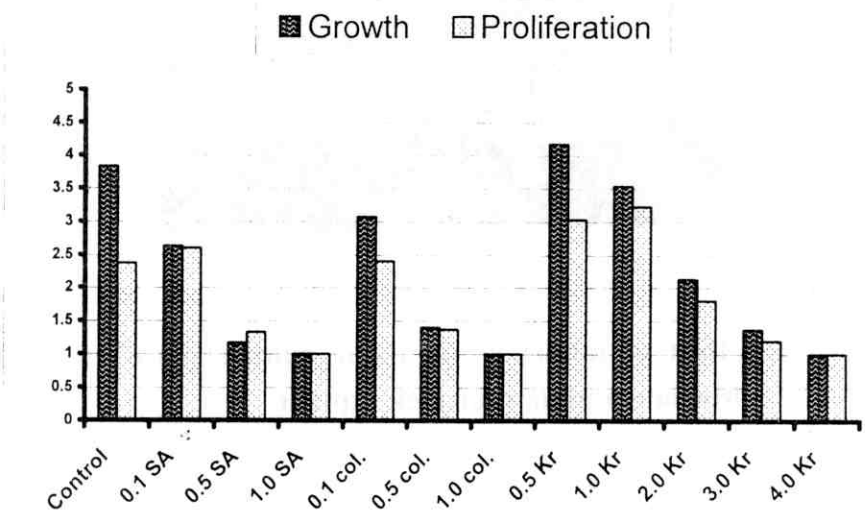


Fig. (3): Effect of different mutagens on growth and proliferation of plum explants.

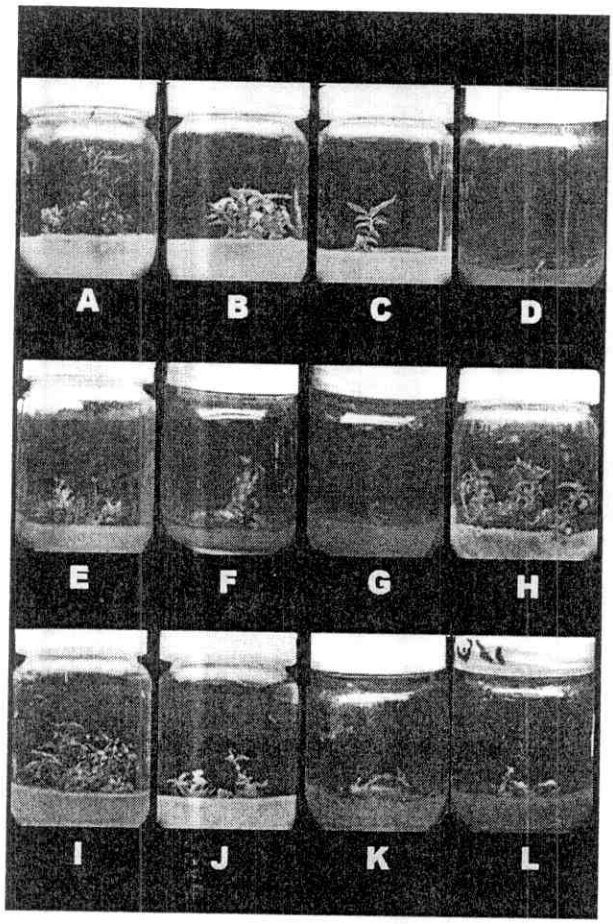


Photo (2): Effect of mutagens on tissue culture parameters of Marianna plum rootstock explant.

A = Control	E = 0.1 g/L Col	H = 0.5 Kr
B = 0.1 g/L SA	F = 0.5 g/L Col	I = 1.0 Kr
C = 0.5 g/L SA	G = 1.0 g/L Col	J = 2.0 Kr
D = 1.0 g/L SA		K = 3.0 Kr
		L = 4.0 Kr

Table (6): Phenotypic variable plantlets of Marianna plum rootstock explants as affected by different mutagens.

Parameters / Treatments		Percentage of variable plantlets
Control		0.0
0.1 SA	g/L	30
0.5 SA		50
1.0 SA		0.0
0.1 Col.	g/L	20
0.5 Col.		20
1.0 Col.		0.0
0.5 Kr		0.0
1.0 Kr		70
2.0 Kr		90
3.0 Kr		30
4.0 Kr		0.0

The above results explain that physical mutagens (irradiation with gamma rays) enhanced the highest percentages of variabilities among treated plantlets specially when 2.0 Kr dose was used.

4. I.2.c. Effect on plantlets organ variabilities:

It is clear from **Table (7)** that colchicine and sodium azide were effective in inducing variabilities in leaf area but colchicine was more effective than sodium azide and gamma irradiation in this respect. However, gamma rays were efficient in inducing phenotypic variabilities in different organs *i.e.*, dwarfed stem, large leaf area, and vertification (hyperhydericity). Beside, increasing irradiation dose up to 2.0 Kr maximized percentage of plantlets with large leaf area and vertified plantlets while the percentage of plantlets with dwarfed stem were increased when 3 Kr dose was used.

Table (7): The effect of different mutagens on plantlets of Marianna plum rootstock explants.

Parameters / Treatment		Dwarfed stem	Large leaf area	Vertification (hyperhydericity)
Control		0.0	0.0	0.0
0.1 SA	g/L	0.0	5.0	0.0
0.5 SA		0.0	6.0	0.0
1.0 SA		0.0	0.0	0.0
0.1 Col.	g/L	0.0	3.0	0.0
0.5 Col.		0.0	4.0	0.0
1.0 Col.		0.0	1.0	0.0
0.5 Kr		0.0	0.0	0.0
1.0 Kr		1.0	3.0	4.0
2.0 Kr		3.0	4.0	8.0
3.0 Kr		5.0	0.0	3.0
4.0 Kr		0.0	0.0	0.0

4. I.2.d. Efficiency of different mutagens:

Table (8) reflect the efficiency of different mutagens either chemical mutagens (sodium azide and colchicine) or physical mutagen (gamma rays) on inducing phenotypic variabilities. It is quite evident that physical irradiation is the best mutagen inducing the highest variabilities followed by sodium azide then colchicine.

Table (8): Efficiency of different mutagens in inducing phenotypic variations in Marianna plum rootstock explant.

Parameters / Treatment	% variable plantlets
Sodium azide	26.67
Colchicine	13.33
Irradiation	30.0

4.I.3. Proliferation:

4.I.3.a. Effect of cytokinin type:

The outlined data in **Table (9)** and **Photo (3)** show a significant increase in proliferation when 2-iP was used instead of either kinetin or BAP. However, both growth and greening criteria were significantly maximized when kinetin was supplemented to the culture medium as compared with either 2-iP or BAP. On the other hand, different cytokinins under study (kinetin, 2-iP and BAP) failed to induce statistical differences when either necrosis or callus production parameter was concerned.

The aforementioned results conclude that 2-iP was superior in enhancing proliferation while kinetin for improving growth and greening parameters. This may be due to that BAP is more efficient as cytokinin at certain concentrations than both kinetin and 2-iP but the concentration used in this experiment was not suitable while the reveres was true in case of 2-iP.

These results confirm the findings of **Abo El-Soaud (1999)** who reported that proliferation of the cultured plum responded well to low concentrations of BA (0.01 to 1.0 mg/L) while decreased gradually by increase BA concentration.

Table (9): Effect of cytokinin type on growth and proliferation of Marianna plum root stock explant.

Parameters / Cyto. type	Necrosis	Callus production	Proliferation	Growth	Greening
Ki	1.50 A	1.73 A	1.90 C	4.0 A	4.20 A
2-iP	1.60 A	1.56 A	4.17 A	3.10 B	3.27 B
BAP	1.77 A	1.83 A	2.67 B	3.27 B	3.37 B

Means of cytokinin type followed by the same letter within each column are not significantly different from each other at 1% level.

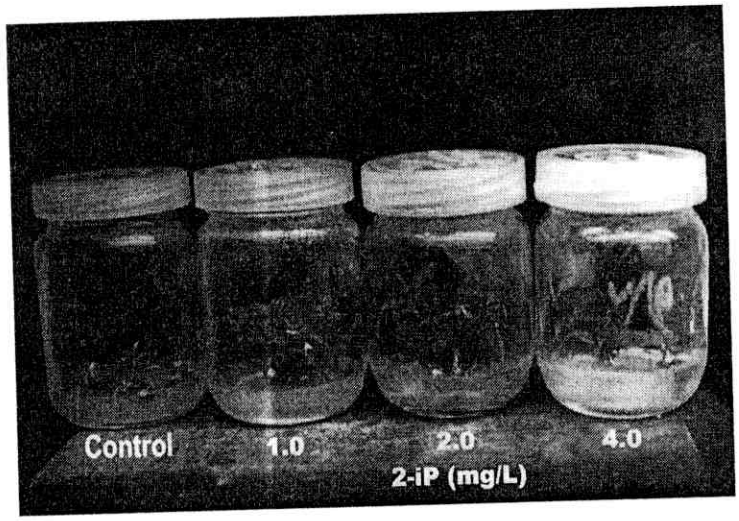


Photo (4): Effect of 2-iP concentration on growth and proliferation of Marianna plum rootstock explants.

4.I.4. Rooting stage:

4.I.4.1. Shoot elongation:

4.I.4.1.a. Effect of medium strength:

The results of **Table (11)** and **Photo (5)** indicate the effect of different medium strengths on necrosis, shoot elongation and rooting. It appears that half strength MS medium resulted in significant increase of both shoot elongation and greening parameters as compared with the other medium strengths. However, continuous diluting of the medium caused a significant reduction in necrosis up to one-fourth and one-eighth medium strengths which showed no statistical differences between both.

In general, half medium strength enhanced shoot elongation and greening parameters. This may be due to the reduction in medium strength which resulted in reducing osmotic potential of the culture

medium and in turn increased absorption rates from the nutrient medium which improved growth. These results are in harmony with the findings of **Gamage *et al.* (2000).** They stated that the best shoot elongation of apple (*Malus domistica* L.) was obtained on half strength Murashige and Skoog.

Table (11): Effect of medium strength on shoot elongation of (Marianna) plum root stock explant.

Parameters / Medium strength	Necrosis	Shoot elongation	Greening
Full	2.67 A	1.33 C	1.87 C
Half-strength	2.03 B	3.43 A	3.27 A
Quarter strength	1.40 C	2.07 B	2.56 B
Eighth strength	1.23 C	1.47 C	1.63 D

Means of medium strength followed by the same letter within each column are not significantly different from each other at 1% level.

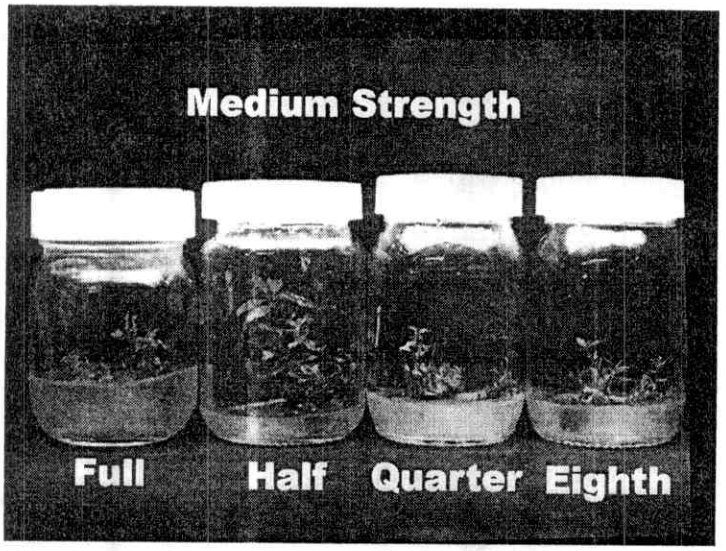


Photo (5): Effect of medium strength on shoot elongation of Marianna plum rootstock.

4.I.3.1.b. Effect of GA_3 concentration:

Table (12) and **Photo (6)** pointed out that increasing GA_3 concentrations encouraged a significant increase of shoot elongation and greening parameters up to 0.3 mg/L which showed the greatest significant increase of shoot elongation and greening. However, necrosis was significantly reduced as low concentrations of GA_3 were used.

The above results disagree with the findings of **Turk *et al.* (1992)**. They reported that addition of 0.1 and 0.5 mg/L GA_3 to the medium have no effect on shoot proliferation or elongation of plum ectotype (*Prunus domestica* L.).

Table (12): Effect of GA_3 concentration on shoot elongation of Marianna plum root stock explant.

Parameters / GA_3 Conc. (mg/L)	Necrosis	Shoot elongation	Greening
Control	1.27 D	1.23 D	1.40 DE
0.1	1.40 D	2.50 B	1.93 C
0.2	2.03 C	2.60 B	2.83 B
0.3	2.67 B	3.73 A	3.73 A
0.4	3.03 A	1.80 C	1.57 D
0.5	3.10 A	1.24 D	1.27 E

Means of GA_3 concentration followed by the same letter within each column are not significantly different from each other at 1% level.

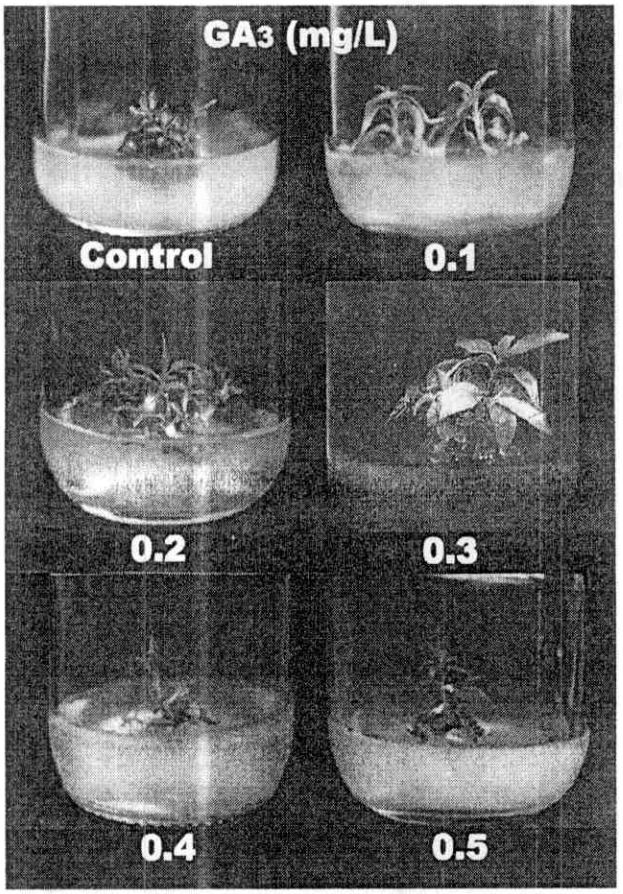


Photo (6): Effect of GA_3 concentrations on shoot elongation of Marianna plum rootstock explants.

4.1.4.2. Root formation:

4.1.4.2.a. Effect of auxin type:

It is clear from **Table (13)** and **Photo (7)** that indole-3-butyric acid (IBA) was significantly surpassed naphthalene acetic acid in increasing necrosis, callus production, greening and rooting parameters. However, the growth parameter was not statistically affected by either IBA or NAA. On the contrary, **Table (14)** and **Photo (8)** reflects that addition of low auxin concentration (1.0 mg/L) to the culture medium enhanced statistical improvement as reduced necrosis and callus production while maximized rooting parameter. Moreover, supplementation of the culture medium with either 1.0 or 2.0 mg/L auxin resulted in a significant increase in both growth and greening parameters in comparison with the other auxin concentrations.

The abovementioned results summarized that addition of low IBA concentration (1.0 mg/L) maximized rooting. These results are in general agreement with the findings of **Marino (1983)** on Japanese plum; **Bassi (1984)** on European plum; and **Bondok *et al.* (1989)** on Marianna 2624. They found that the best rooting occurred when 0.5-0.8 IBA were added to the culture medium.

Table (13): Effect of auxin type on growth and rooting of Marianna plum rootstock explant.

Parameters / Auxin type	Necrosis	Callus	Growth	Greening	Rooting
NAA	2.10 B	1.23 B	3.13 A	1.27 B	1.27 B
IBA	2.73 A	1.80 A	2.40 A	2.10 A	3.20 A

Means of auxin type followed by the same letter within each column are not significantly different from each other at 1% level.

Table (14): Effect of IBA concentration on growth and rooting of Marianna plum rootstock explant.

Parameters / IBA Conc. (mg/L)	Necrosis	Callus	Growth	Greening	Rooting
Control	1.23 D	1.20 C	2.07 B	2.20 B	1.23 D
1.0	1.57 C	1.40 C	2.23 A	2.53 A	3.57 A
2.0	2.20 B	2.50 B	2.30 A	2.63 A	2.20 B
3.0	2.83 A	3.67 A	1.73 C	1.43 C	1.67 C

Means of IBA concentration followed by the same letter within each column are not significantly different from each other at 1% level.

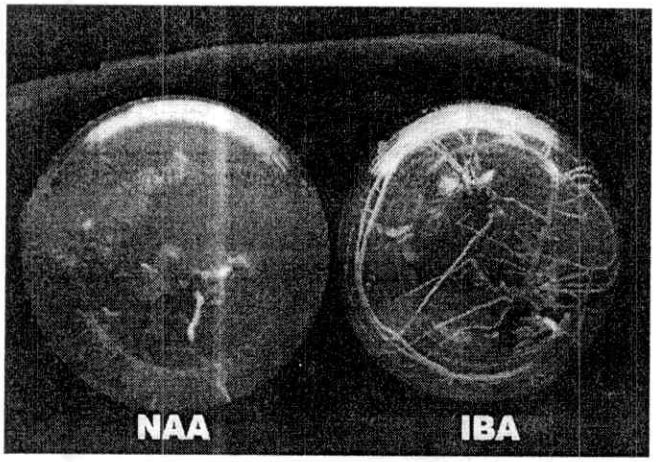


Photo (7): Effect of auxin type on growth and rooting of Marianna plum rootstock explants.

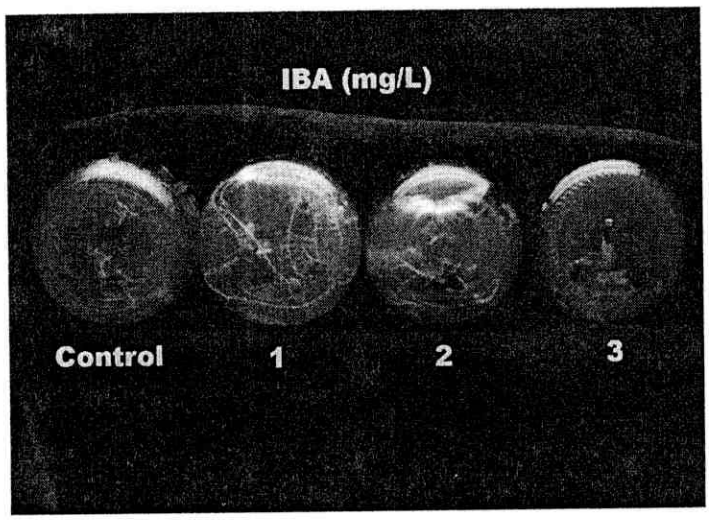


Photo (8): Effect of IBA concentration on growth and rooting of Marianna plum rootstock explants.

4.I.5. Stress tolerance:

4.I.5.1. Drought tolerance:

4.I.5.1.a. Effect of mannitol:

4.I.5.1.a.1. Tissue culture parameters:

The results of **Table (15)**, **Fig. (4)** and **Photo (9)** explain the effect of different mannitol concentrations on the tissue culture parameters. It is noticed that supplementation the culture medium with 20 g/L encouraged significant increase of number of shoots, shoot length, and root length compared with the other concentrations used. The reverse was true when necrosis and callus production were taken in consideration. Meanwhile, growth, number of leaves, and number of roots were statistically higher when either 20 or 40 g/L mannitol was used. However, callus production and shoot thickness were

significantly increased as concentration of mannitol increased up to the highest level used (100 g/L) which encouraged the best results in these mentioned parameters. Beside, harmful effect appeared on the most parameters under study but the plantlets still survived although of high osmotic potentials of the culture medium and in turn a great reduction of free water occurred. This mean that these plantlets showed tolerance to drought stress.

These results are in harmony with the findings of **Fernandez *et al.* (1997).** They found that Gala apple trees grafted on Mark rootstock was tolerant to great drought stress. Reduction in growth and leaf growth, shoot length while leaf emergence and trunk cross were inconsistent were in response to stress. Moreover, **Geeta *et al.* (1995)** declared that growth of Marianna 2624 was increased when two levels of mannitol 275 µM and 550 µM were added to the culture medium as compared with the control.

Table (15) Effect of different mannitol concentrations on the tissue cultural parameters of *in vitro* Marianna plum rootstock plantlets.

Parameters / Mannitol Conc. (g/L)	Necrosis	Callus	Growth	No. of shoots	Shoot length	Shoot thickness	No. of leaves / plant	Root length	No. of roots
20	1.33 E	1.20 D	3.90 A	4.67 A	2.90 A	0.26 D	8.00 A	3.10 A	3.17 A
40	2.57 D	1.80 C	3.60 A	4.07 B	2.60 B	0.27 C	7.10 A	2.73 B	2.83 AB
60	3.27 C	2.57 B	2.60 B	1.90 C	2.17 C	0.30 A	4.33 B	2.43 C	2.50 AB
80	3.83 B	2.77 B	1.33 C	1.47 CD	1.47 D	0.29 B	1.67 C	1.33 D	2.43 B
100	4.57 A	3.17 A	1.07 C	1.00 D	1.27 E	0.31 A	1.00 C	1.17 D	2.16 B

Means of different mannitol concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

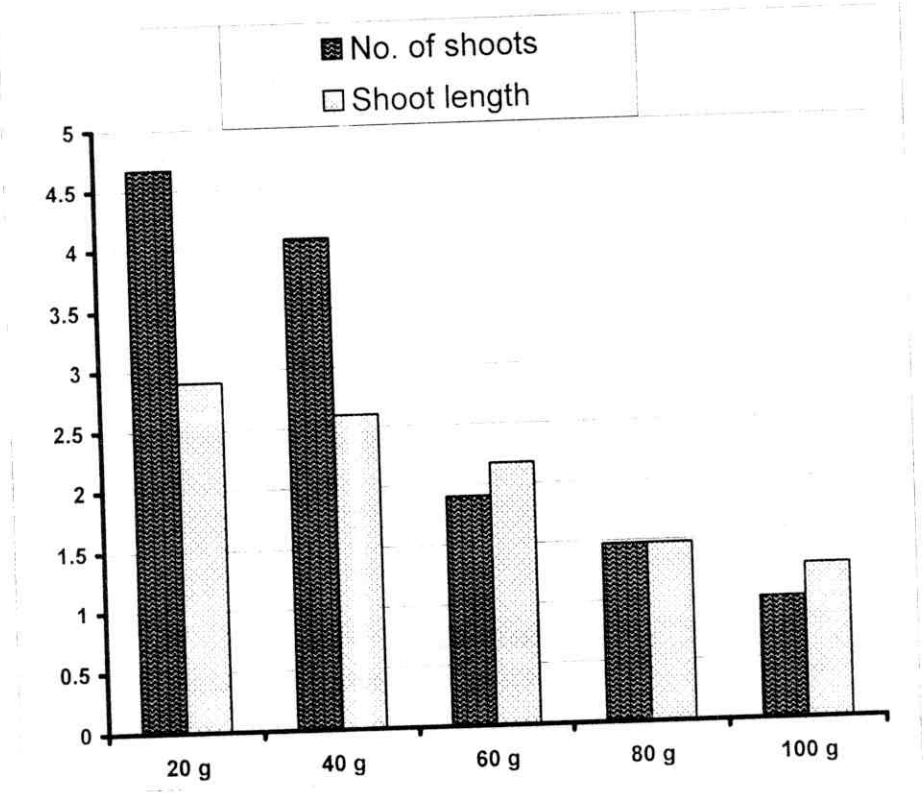


Fig. (4): Effect of different mannitol concentrations on the No. of shoot and shoot length of *in vitro* plum plantlets.

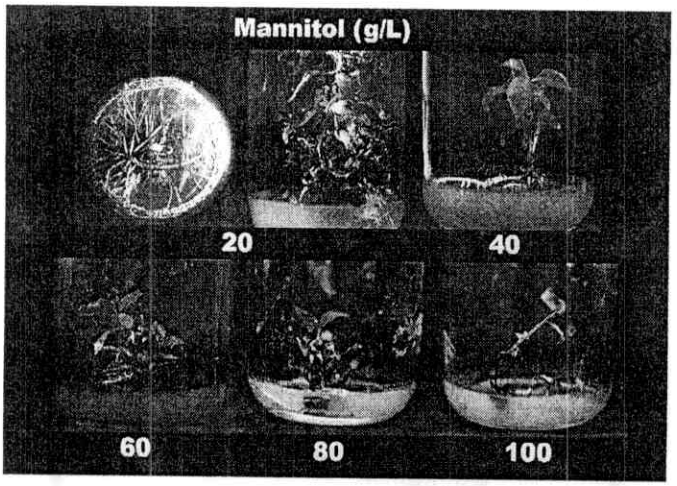


Photo (9): Effect of different mannitol concentration on tissue culture parameters of Marianna plum rootstock explants.

4.I.5.1.a.2. Chemical composition:

Table (16) reflect the effect of different mannitol concentrations on chemical composition. It is obvious that chlorophyll A and non reducing sugars levels were increased significantly as 20 g/L mannitol was added to the culture media. However, supplementation of the culture medium with either 40 or 60 g/L was significantly effective in increasing the accumulation of total sugars or reducing sugars respectively with regard to other mannitol levels. Furthermore, increasing mannitol concentrations enhanced significant increase of chlorophyll-B, total indoles and total phenols up to the highest level at 100 g/L level.

The above results indicate that there was a direct relationship between increase in mannitol concentrations and total indoles and

phenols accumulation in the cultured plantlets. These results explain the mechanism by which the plantlets tolerated higher levels of mannitol in the medium as increasing indoles and phenols encouraged the cultured plantlets to tolerate drought. These results are somewhat in accordance with the findings of **Chandel and Chouyhan (1991).** They detected that proline and ABA contents of the plants grafted on MM111, M9 and M106 rootstocks were significantly higher at 5.0 and 10.0 bar soil moisture tension.

Table (16): Effect of different mannitol concentrations on the chemical composition of *in vitro* Marianna plum rootstock plantlets.

Parameters / Mannitol Conc. (g/L)	Chlorophyll A	Chlorophyll B	Non reducing sugars	Reducing sugars	Total sugars	Total indoles	Total phenols
20	7.72 A	2.58 E	8.36 A	3.01 D	11.37 C	0.74 E	6.57 E
40	7.63 B	2.95 D	7.86 B	4.60 C	12.46 A	0.94 D	7.72 D
60	6.01 C	3.34 C	7.03 C	4.94 A	11.97 B	1.36 C	8.54 C
80	4.32 D	4.03 B	5.76 D	4.73 B	10.49 D	1.63 B	8.94 B
100	1.37 E	4.75 A	5.03 E	4.72 B	9.76 E	1.76 A	9.36 A

Means of different mannitol concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

4.I.5.1.b. Effect of polyethylene glycol (PEG):

4.I.5.1.b.1.Tissue culture parameters:

Table (17), Fig. (5) and **Photo (10)** showed the effect of different PEG concentrations on tissue culture parameters *i.e.,* necrosis, callus production, growth, number of shoots, shoot length, number of leaves/plant, root length, and root number. It is clear that addition of 2 g/L PEG to the culture medium encouraged a significant increase of growth, number of shoots, shoot length, and number of leaves/plant parameters in comparison with the other PEG concentrations. However, the reverse was true when necrosis and callus parameters were considered. Meanwhile, root length was significantly maximized when

either 2 or 4 g/L PEG was added to the culture medium. On the other hand, statistical differences among different PEG concentrations were absent when number of roots was concerned.

The aforementioned results indicate that increasing PEG concentration resulted in harmful effect on most tissue culture parameters. This may be due to the osmotic effect as increasing PEG concentrations increased the osmotic pressure of the culture medium and in turn free water suitable for absorption was decreased which resulted in reducing cell division and elongation. These led to induce a bad effect on most tissue culture parameters of cultured plantlets. These results go in line with the findings of **Imed Dami and Hassison (1997).** They reported that adding 2 g/L polyethylene glycol to the rooting medium encouraged the highest deposition of epicuticular wax compared with the control. However, these results disagree with the findings of **Tewary *et al.* (2000)**. They concluded that supplementation of the medium with 1.0-10.0 g/L PEG was valuable in maximizing sprouting percentage and shoot growth parameters.

Table (17): Effect of different PEG concentrations on the tissue culture parameters of *in vitro* Marianna plum rootstock plantlets.

Parameters / PEG Conc. (g/L)	Necrosis	Callus	Growth	No. of shoots	Shoot length	Shoot thickness	No. of leaves / plant	Root length	No. of roots
2	1.30 E	1.13 D	3.73 A	5.03 A	3.57 A	0.13 C	8.00 A	3.23 A	2.77 A
4	2.27 D	1.73 BC	3.10 B	3.17 B	3.10 B	0.16 BC	6.00 B	3.10 A	2.47 A
6	3.23 C	1.97 B	2.56 C	1.66 C	1.60 C	0.24 AB	3.33 C	2.30 B	2.80 A
8	4.17 B	2.57 A	1.57 D	1.00 D	1.202 D	0.30 A	2.03 D	2.03 B	2.76 A
10	4.93 A	1.47 CD	1.00 E	1.00 D	1.23 D	0.21 ABC	1.00 D	1.47 C	2.33 A

Means of different PEG concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

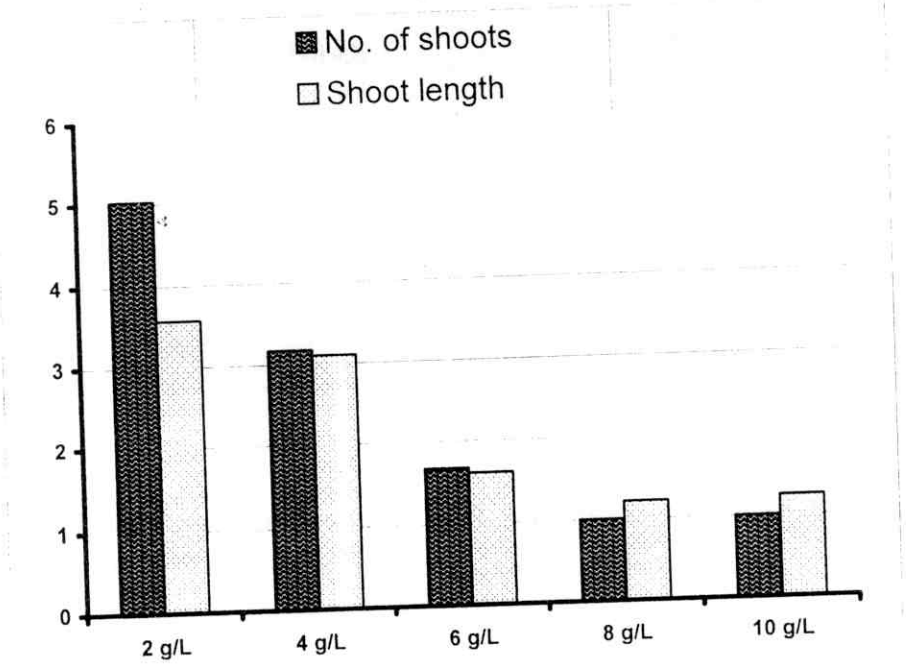


Fig. (5): Effect of different PEG concentrations on No. of shoot and shoot length of *in vitro* plum plantlets.

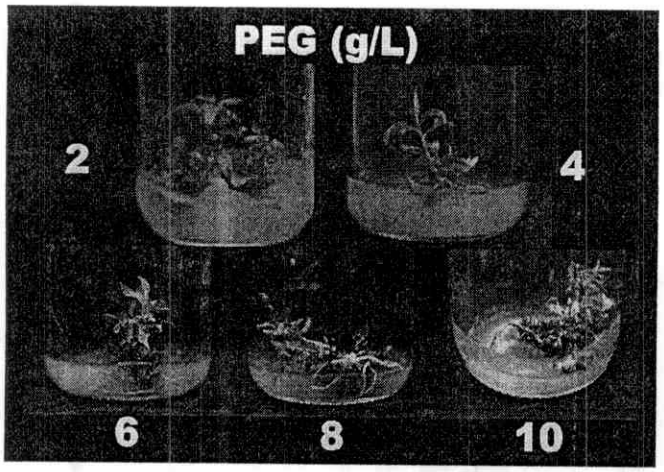


Photo (10): Effect of different PEG concentration on tissue culture parameters of Marianna plum rootstock explants.

4.I.5.1.b.2. Chemical composition:

Data in **Table (18)** reflect the effect of different PEG concentrations on chemical composition *i.e.,* chlorophyll-A & B, non reducing sugars, reducing sugars, total sugars, total indoles and total phenols. It appears that the supplementation of the culture medium with 2.0 g/L PEG enhanced significant increase of chlorophyll-A, non-reducing sugars, and total sugars as compared with the other concentrations. However, increasing PEG-concentration resulted in significant increase in chlorophyll-B, reducing sugars, total indoles and total phenols up to the highest level when 10.0 g/L PEG concentration was used.

Table (18): Effect of different PEG concentrations on the chemical composition of *in vitro* Marianna plum root stock plantlets.

Parameters / PEG Conc. (g/L)	Chlorophyll A	Chlorophyll B	Non reducing sugars	Reducing sugars	Total sugars	Total indoles	Total phenols
2	8.77 A	2.56 E	9.36 A	3.26 E	12.62 A	0.84 E	6.38 D
4	7.02 B	3.76 D	8.57 B	3.66 D	12.24 B	0.94 D	7.04 C
6	6.57 C	3.96 C	6.54 C	4.01 C	10.56 C	1.16 C	7.73 B
8	3.57 D	4.56 B	4.59 D	4.37 B	9.96 D	1.27 B	7.75 B
10	1.56 E	4.76 A	4.32 E	4.47 A	8.79 E	1.33 A	7.93 A

Means of different PEG concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

4.I.5.1.c. Effect of agar:

4.I.5.1.c.1. Tissue culture parameters:

Table (19), Fig. (6) and **Photo (11)** explain the effect of different agar concentrations on tissue culture parameters *i.e.*, necrosis, callus production, growth, number of shoots, shoot length, shoot thickness, number of leaves/plant, root length and number of roots. It is quite evident that addition of agar at the rate of 6 g/L induced significant reduction of necrosis and callus production significantly while significantly maximized number of leaves/plant. Meanwhile, increasing agar concentration in the culture medium from 6 up to 8 g/L encouraged significant increase of growth, number of shoots, shoot length, and root length. On the contrary, most agar concentrations under study increased number of roots. On the other hand, increasing agar concentrations encouraged significant increase of shoot thickness up to the highest level at the highest concentration (14 g/L) in comparison with the other concentrations.

Table (19): Effect of different agar concentrations on the tissue cultural parameters of *in vitro* Marianna plum rootstock plantlets.

Parameters / Agar Conc. (g/L)	Necrosis	Callus	Growth	No. of shoots	Shoot length	Shoot thickness	No. of leaves / plant	Root length	No. of roots
6	1.14 E	1.07 D	3.73 A	5.00 A	3.17 A	0.13 E	10.67 A	3.43 A	2.73 A
8	2.03 D	1.57 BC	3.67 A	4.33 A	3.33 A	0.17 D	7.33 B	3.47 A	3.10 A
10	3.30 C	1.87 AB	2.40 B	2.07 B	2.40 B	0.19 C	3.67 C	1.93 B	3.27 A
12	4.33 B	2.17 A	1.83 C	1.00 C	1.57 C	0.23 B	1.33 D	1.50 BC	2.67 AB
14	4.90 A	1.30 CD	1.03 D	1.00 C	1.13 C	0.24 A	1.04 D	1.30 C	1.83 B

Means of different agar concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

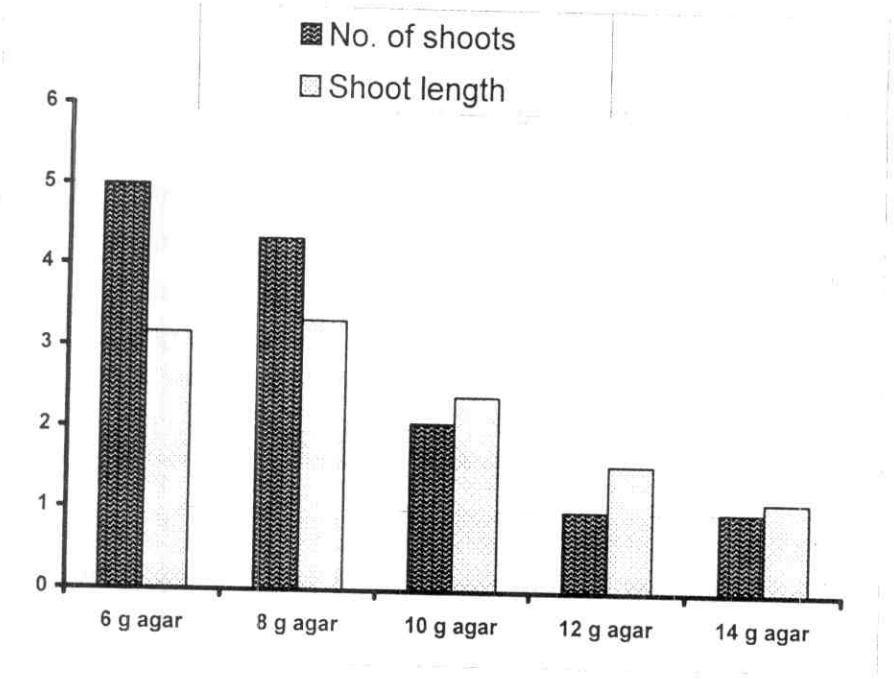


Fig. (6): Effect of different agar concentrations on No. of shoot and shoot length of *in vitro* plum plantlets.

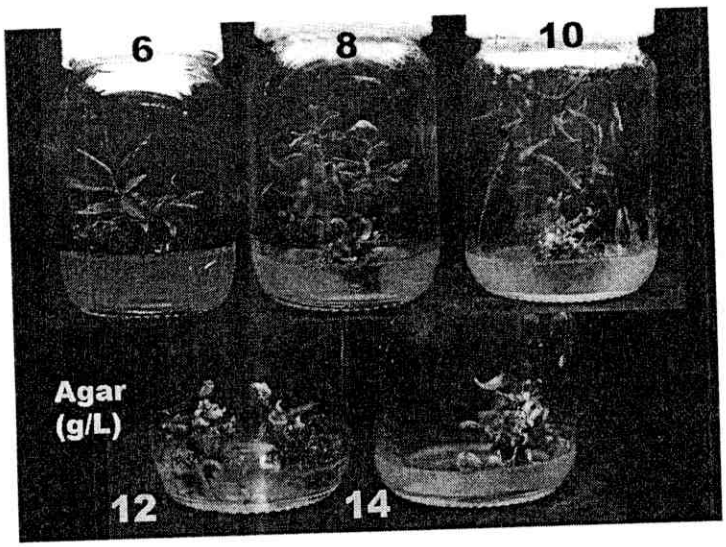


Photo (11): Effect of different agar concentration on tissue culture parameters of Marianna plum rootstock explants.

4.1.5.1.c.2. Chemical composition:

Results in **Table (20)** clarify the effect of different agar concentrations on chemical composition *i.e.*, chlorophyll-A & B, non-reducing sugars, reducing sugars, total sugars, total indoles and total phenols. The table reflects that chlorophyll-A, non reducing sugars and total sugars showed significant increase when 6 g/L agar was added to the culture medium. Meanwhile, addition of either 10 or 12 g/L agar was effective in significant increase of total indoles compared with the other concentrations. However, higher concentrations of agar (14 g/L) succeeded in significant increase of chlorophyll B and total phenols in relation to the other concentrations of agar.

The abovementioned results conclude that increasing of agar as gelling agent in the culture medium up to 8 g/L was suitable to the plantlets either for tissue culture or chemical composition but further increase of agar resulted in harmful effect on either tissue culture or most chemical composition. However, increasing of shoot thickness, chlorophyll B, total indoles, and total phenols may explain the increase of drought tolerance in these plantlets.

These results are in harmony with the findings of **Suksa *et al.* (1997)**. They stated that *in vitro* shoots conservation of *Carica papaya* by osmotic stress via increasing agar concentration up to 20 g/L was effective in suppressing shoot length.

Table (20): Effect of different agar concentrations on the chemical composition of *in vitro* Marianna plum rootstock plantlets.

Parameters / Agar Conc. (g/L)	Chlorophyll A	Chlorophyll B	Non reducing sugars	Reducing sugars	Total sugars	Total indoles	Total phenol
6	8.73 A	2.55 E	7.55 A	3.76 E	11.30 A	0.78 D	6.44 E
8	8.01 B	3.01 D	7.96 B	3.14 D	11.20 B	0.94 C	7.23 D
10	6.37 C	4.02 C	5.74 C	4.36 C	10.10 C	1.25 A	7.58 C
12	4.38 D	4.27 B	4.78 D	4.57 A	9.35 D	1.25 A	7.81 B
14	1.53 E	4.74 A	4.53 E	4.43 B	8.98 E	1.07 B	7.95 A

Means of different agar concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

4.I.4.2. Salinity stress:

4.I.4.2.a. Tissue culture parameters:

The tabulated data of **Table (21)**, **Fig. (7)** and **Photo (12)** show the effect of different concentrations of NaCl, $CaCl_2$ and the combination of NaCl and $CaCl_2$ on tissue culture parameters *i.e.*, necrosis, callus production, growth, number of shoots, shoot length, shoot thickness, number of leaves/plant, root length, and number of roots. It is clear that

different NaCl concentrations had a harmful effect on tissue culture parameters as compared with $CaCl_2$ and the combination of Nacl + $CaCl_2$. Increasing NaCl concentration more than 1000 ppm had a detrimental effect on all parameters under study up to the lethal concentration 2000 ppm which caused almost death of the *in vitro* plantlets. However, the effect of $CaCl_2$ with different levels was less toxic to the plantlets as compared with the similar levels of NaCl. Meanwhile, combination of NaCl + $CaCl_2$ reduced the toxicity of NaCl. Beside, vegetative growth parameters were greatly affected by different salinity agents (NaCl and $CaCl_2$) than rooting parameters.

Table (21): Effect of different salinity concentrations on the tissue cultural parameters of *in vitro* Marianna plum rootstock plantlets.

Parameters / Salinity Conc. (ppm)	Necrosis	Callus	Growth	No. of shoots	Shoot length	Shoot thickness	No. of leaves / plant	Root length	No. of roots
Control	1.10 J	1.00 G	4.57 A	8.24 A	5.34 A	0.23 I	18.43A	3.76 A	3.74 A
500 NaCl	2.10H	1.55 DE	3.54 C	6.08 B	2.23 G	0.26H	9.05 C	2.90CDE	3.65 AB
1000 NaCl	3.51E	1.74BCD	2.17 E	4.04 E	2.06 H	0.27 G	7.00 E	2.54EF	2.03 E
1500 NaCl	4.11C	1.82 BC	1.58 G	2.10G	1.86 I	0.28 F	3.03HI	2.34FG	1.53 F
2000 NaCl	4.93A	1.02 G	1.10 H	1.00 I	1.74 I	0.28 F	1.67 J	1.37 H	1.03 G
500 $CaCl_2$	1.30 I	1.30 F	4.13 B	6.02 B	3.13BC	0.28 F	12.30B	3.51AB	3.73 A
1000 $CaCl_2$	2.63G	1.62 CD	3.63 C	4.63D	3.01CD	0.29 E	8.03 D	3.47AB	3.52ABC
1500 $CaCl_2$	3.57E	1.81 BC	2.53 D	4.13 E	2.94 D	0.31 B	5.50 F	3.23BC	3.40 C
2000 $CaCl_2$	4.10C	1.82 BC	1.90 F	2.70 F	2.75 E	0.32 A	3.70GH	2.10 G	2.60 D
250 NaCl +250 $CaCl_2$	2.03H	1.37 EF	3.59 C	5.18 C	3.23 B	0.26 H	9.27 C	3.49 AB	3.52ABC
500 NaCl +500 $CaCl_2$	3.03 F	1.83 B	2.67 D	3.97 E	2.60EF	0.27 G	6.33 E	3.43 AB	3.48 BC
750 NaCl +750 $CaCl_2$	3.80D	2.23 A	1.70FG	2.03G	2.52 F	0.30 D	4.27 G	2.77 DE	2.67 D
1000 NaCl +1000 $CaCl_2$	4.50B	2.37 A	1.53 G	1.63H	2.50 F	0.31 C	2.33 IJ	3.13BCD	2.57 D

Means of different salinity concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

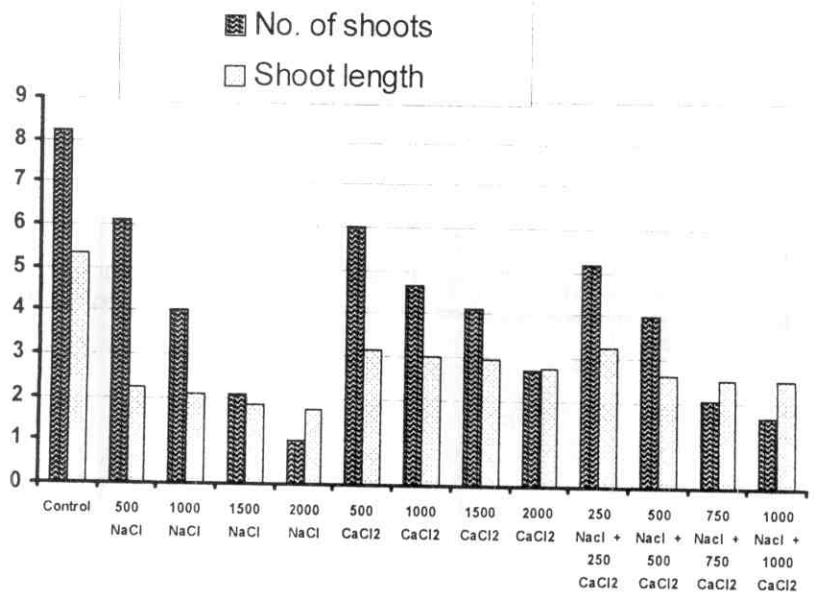


Fig. (7): Effect of different salinity concentrations on No. of shoot and shoot length of *in vitro* plum plantlets.

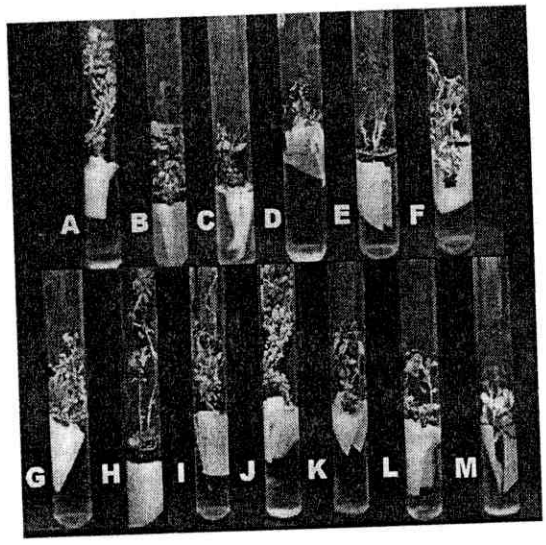


Photo (12): **Effect of salinity on tissue culture parameters of Marianna plum rootstock explants.**

A = Control

B = 500 NaCl	F = 500 $CaCl_2$	J = 250 NaCl + 250 $CaCl_2$
C = 1000 NaCl	G = 1000 $CaCl_2$	K = 500 NaCl + 500 $CaCl_2$
D = 1500 NaCl	H = 1500 $CaCl_2$	L = 750 NaCl + 750 $CaCl_2$
E = 2000 NaCl	I = 2000 $CaCl_2$	M = 1000 NaCl + 1000 $CaCl_2$

4.I.4.2.b. Chemical composition:

Table (22) pointed out that control plants showed superiority in chemical composition than those treated with different concentrations of either NaCl or $CaCl_2$ or both NaCl + CaCl. Meanwhile, adding 500 ppm of $CaCl_2$ to the culture medium reduced the harmful effect of salinity on chlorophyll-A and non-reducing sugars as compared with the other salinity treatments. However, chlorophyll B was significantly maximized in plantlets treated with 2000 ppm NaCl in relation to the other treatments and control. Similarly, the highest significant contents of reducing sugars and total indoles were detected in plantlets treated with combination of 750 NaCl + 750 $CaCl_2$ and 1000 NaCl + 1000 ppm $CaCl_2$ treatments as compared to the other treatments and control. On the other hand, both phenols and proline contents were significantly increased by increasing the concentrations of salinity compared with the lower concentrations.

The above results summarized that salinity is harmful to tissue culture and chemical composition. Also, total indoles, total phenols, and proline had a direct relationship with increasing of salinity. In addition, NaCl is more toxic than $CaCl_2$ and the combination treatments. These results assured the findings of **Shahin (1989)**. He stated that sodium chloride caused the most deleterious effect on vegetative growth followed by mixed salts (NaCl + $CaCl_2$) while $CaCl_2$ was less effective on plum rootstocks. Also, **El-Hammady *et al.* (1993)** on mango; **Bondok *et al.* (1996)** on peach and **El-Hammady *et al.* (1999)** on grape. They found that salinity stress caused an increase in proline contents.

Table (22): Effect of different salinity concentrations on the chemical composition of *in vitro* Marianna plum rootstock plantlets.

Parameters / Salinity Conc. (ppm)	Chlorophyll A	Chlorophyll B	Non reducing sugars	Reducing sugars	Total sugars	Total indoles	Total phenol	Proline
Control	8.83 A	3.20 H	9.73 A	4.27 GH	14.01 A	0.86 H	8.27 G	0.132D
500 NaCl	6.14 C	5.35 EF	7.37 C	5.26 F	12.63 B	1.64 G	10.17 E	0.151CD
1000 NaCl	4.40 F	6.33 C	4.95 E	6.58 E	11.52 D	2.42 D	11.35 C	0.223AB
1500 NaCl	0.006 I	7.13 A	2.36 G	7.56 CD	9.92EFG	2.59 C	12.81 A	0.258A
2000 NaCl	0.001 I	7.23 A	1.60 I	7.75 BC	9.35 H	2.64 BC	12.74	0.263A
500 $CaCl_2$	7.19 B	4.37 G	7.79 B	4.08 H	11.87 C	2.10 F	9.60 F	0.146CD
1000 $CaCl_2$	5.32 D	5.23 F	5.33 D	4.53 G	9.86 FG	2.23 E	10.52 D	0.165BCD
1500 $CaCl_2$	1.53 G	6.23 C	2.52 F	7.34 D	9.86 FG	2.26 E	12.71 A	0.192BC
2000 $CaCl_2$	1.34 H	6.52 B	2.10 H	7.53 CD	9.63 GH	2.73 B	12.74 A	0.210AB
250 NaCl + 250 $CaCl_2$	7.33 B	4.22 G	7.34 C	4.10 H	11.44 D	1.76 G	11.84 B	0.177BCD
500 NaCl + 500 $CaCl_2$	4.73 E	5.51 E	5.24 D	7.52 CD	12.76 B	2.74 B	11.93 B	0.223AB
750 NaCl + 750 $CaCl_2$	0.003 I	6.01 D	2.32 G	7.93 AB	10.22 E	2.93 A	12.70 A	0.267A
1000 NaCl + 1000 $CaCl_2$	0.001 I	6.18CD	2.0 H	8.01 A	10.03EF	2.91 A	12.70 A	0.267A

Means of different salinity concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

4.I.4.2.c. Leaf mineral content:

Table (23) deals with the effect of salinity treatments on leaf macroelement contents. It is clear that the highest N accumulation percentage was significantly obtained with control treatment followed with 500 ppm $CaCl_2$ then combination treatment of (250 NaCl + 250 $CaCl_2$). Similarly, K percentage was significantly maximized with control treatment then followed with 500 ppm NaCl treatment then combination treatment 250 NaCl + 250 $CaCl_2$. However, P was significantly increased when 1500 ppm NaCl was used while Ca was significantly maximized as 2000 ppm $CaCl_2$ was used. Meanwhile, increasing salinity up to 2000 ppm NaCl was significantly effective in raising of Mg percentage as compared with the other treatments. Beside, combination treatment as compared with other treatments. On the other

hand, Na was significantly increased as NaCl concentration increased up to 2000 ppm NaCl in relation to the other salinity treatments.

Furthermore, **Table (24)** clarifies the effect of different salinity treatments on leaf microelement percentage. It showed that increasing concentration of the combination treatment (1000 ppm NaCl + 1000 $CaCl_2$) resulted in significant increase of Fe and Cu percentages as compared with the other treatments. On the contrary, control treatment and using low $CaCl_2$ (500 ppm) were significantly successful in increasing of Zn percentages as compared with the other salinity treatments.

Table (23): Effect of salinity on Macroelements content of Marianna plum rootstock plantlets.

Treatment	Macroelements (%) in dry plantlets						
	N	P	K	Ca	Mg	Na	Cl
Control	7.13 A	0.18 F	21.53A	1.63K	0.58 L	0.86 J	3.05 M
500 NaCl	6.06 G	0.15GH	19.52B	1.84J	0.86 I	1.05 H	4.94 J
1000 NaCl	5.74 J	0.12 IJ	15.14F	1.94 I	1.15 D	1.24 E	6.73 G
1500 NaCl	5.42 K	0.96 A	13.22 I	2.07G	1.26 B	1.86 C	7.33 F
2000 NaCl	5.26 L	0.58 D	9.57 L	2.14F	1.53 A	2.32 A	8.22 E
500 $CaCl_2$	7.06 B	0.16 G	17.34D	2.02H	0.63 K	0.95 I	4.03 L
1000 $CaCl_2$	6.61 D	0.14 H	13.33H	2.26E	0.93 H	1.12 G	4.92 K
1500 $CaCl_2$	5.94 I	0.11 J	10.97J	2.35D	1.03 G	1.13 G	5.93 I
2000 $CaCl_2$	5.73 J	0.93 B	9.04M	2.56A	1.12 E	1.70 F	6.55 H
250 + 250	6.93 C	0.14 HI	19.25C	2.02H	0.76 J	1.26 E	8.83 D
500 + 500	6.36 E	0.11 J	16.71E	2.36D	1.07 F	1.53 D	10.14 C
750 + 750	6.16 F	0.83 C	14.14G	2.42C	1.24 C	1.86 C	13.32 B
1000 + 1000	5.96H	0.45 E	10.25K	2.47B	1.53 A	2.05 B	16.52 A

Means of different salinity concentrations on macroelement content followed by the same letter within each column are not significantly different from each other at 1% level.

Generally, the results reflected the deleterious effect of salinity on leaf minerals levels as a result of increasing of medium pH and in turn reduce their availability and absorption. Also, may be due to the antagonism between different elements due to the increase of Na and Cl

levels in the leaves. These results are in agreement with the findings of **Zekri (1987)** who found that leaf Na^+ and Cl^- contents of citrus rootstocks increased with increasing NaCl in the nutrient solution.

Table (24): Effect of salinity on Microelement content of Marianna plum rootstock plantlets.

Salinity conc. (ppm)	Microelement (ppm) in dry plantlets			
	Fe	Mn	Zn	Cu
Control	571.67 L	705.00A	143.33A	242.00L
500 NaCl	734.33 I	622.00C	122.67C	320.00H
1000 NaCl	951.00 F	592.67E	112.33D	371.67F
1500 NaCl	1102.67D	470.33I	102.67E	426.00D
2000 NaCl	1175.67C	402.33K	93.33F	441.67B
500 $CaCl_2$	622.33K	651.67B	134.00B	265.00K
1000 $CaCl_2$	702.67 J	604.00D	122.67C	272.00J
1500 $CaCl_2$	855.67G	551.67F	105.00E	293.33 I
2000 $CaCl_2$	953.33F	531.00G	102.00E	321.00H
250 + 250	848.63H	591.33E	109.00D	366.00G
500 + 500	967.00E	519.00H	92.67F	414.33H
750 + 750	1259.67B	467.00I	82.67G	432.67C
1000 + 1000	1373.67A	450.00J	72.00H	447.00A

Means of different salinity concentrations on Microelement content followed by the same letter within each column are not significantly different from each other at 1% level.

4.1.5. DNA finger printing using RAPD-PCR technique:

RAPD assay, for randomly amplified polymorphic DNA, was developed independently by two different laboratories **(Welsh and McClelland, 1990; Williams *et al.*, 1990).** It detects nucleotide sequence polymorphism in a DNA amplification assay using only a single primer of arbitrary nucleotide sequence, RAPD assay was used as a tool to identify molecular markers linked to a trait of interest.

For primer; OP-A-16 (**Photo, 13**), the band with molecular weight 1 K bp appeared in both two irradiated treatments (1 & 2 K-

rad) (lane 3 & 4) while this band disappeared from the control sample (lane 2), while in primer OP-B6; the band with molecular weight 500 bp which found in both control (non-irradiated treatment) and 1 K-rad irradiation treatment (lane 2 & 3) disappeared from the 2K-rad irradiation treatment (lane 4) **(Photo 14).**

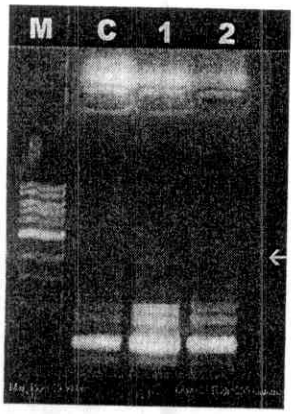
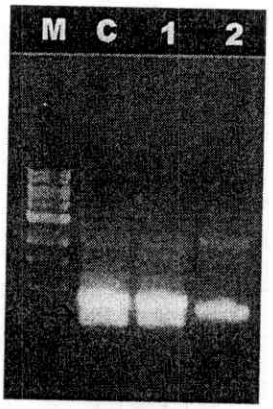


Photo (13): Primer **A-16**



M = Marker
C = Control
(non-irradiated)
1 = 1 Krad
2 = 2 Krad

Photo (14): Primer **B6**

The same results were obtained with primer OP-B7 **(Photo 15).** In this figure we found that the band with molecular size 600 bp found in both control and 1K-rad irradiation treatment (lanes 2 & 3) while this band was not found in the other treatment; 2 K-rad (lane 4).

Also, with primer OP-E7; the 2-K-rad treatment led to avoidance of band with molecular weight 500 bp (**Photo 16**, lane 4) while this band appeared in both control and 1K-rad treatment (**Photo 17**, lane 2 & 3).

For primer OP-E6 **(Photo 17),** no differences found among the three samples; control (not irradiated) and two treatments (1 and 2 K-rad).

From all above results, RAPD analysis can detect differences which occurred from irradiation treatments with different doses of gamma rays.

The detection of RAPD markers on the genomic map of different plants is beneficiate to improve breeding programs for these

plants. It offers the simplest and fastest methods for detecting a great number of genomic markers in the shortest period of time **(Welsh and McClelland, 1990; Williama *et al.*, 1990; Novy and Vorsa, 1996; Autio *et al.*, 1998 and Al-Tiraif *et al.*, 1998).**

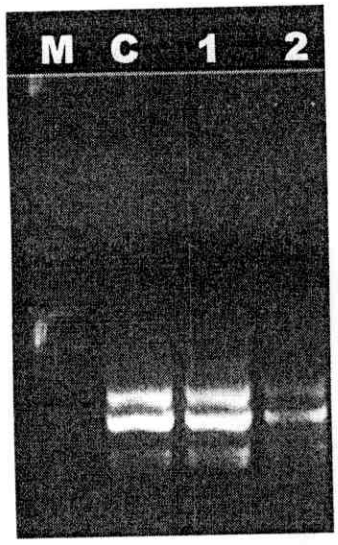


Photo (15): Primer B7

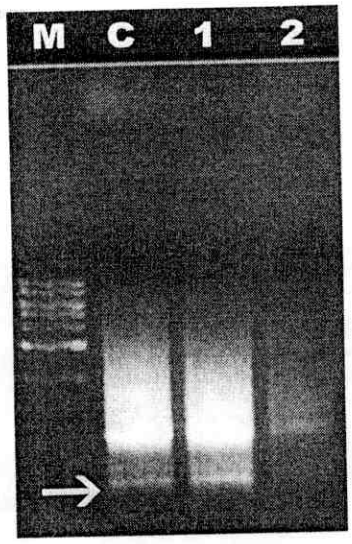


Photo (16): Primer E7

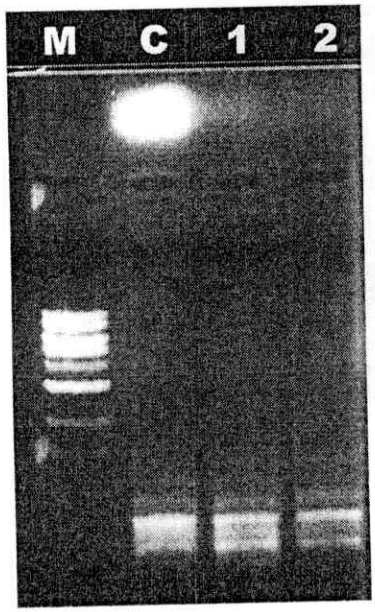


Photo (17): Primer E6

M = Marker
C = Control
(non-irradiated)
1 = 1 Krad
2 = 2 Krad

4.1.6. Acclimatization:

It is appear from **(Photo 18)** that Marianna 2624 plum rootstock) was successfully acclimatized by using a combination of peatmoss and sand at rate 3:1 (v/v), respectively.

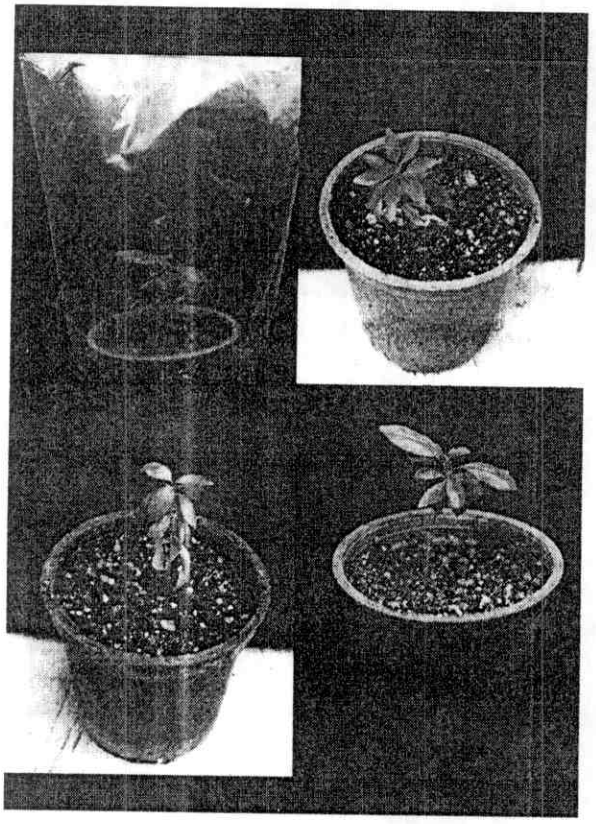


Photo (18): The acclimatization of Marianna rootstock plantlets.

4.II. *Pyrus betulaefolia*:

4.II.1. Induction of mutations and variations:

4.II.1.a. Effect on tissue culture parameters:

Table (25), **Fig. (8)** and **Photo (19)** deals with the effect of different mutagens either chemical (colchicine or sodium azide) or physical (gamma rays) on tissue culture parameters *i.e.*, necrosis, callus production, proliferation, growth and greening. It is clear that increasing concentrations of mutagens induced harmful effect on most parameters under study. Control explants surpassed those treated with mutagens in most parameters under study. The lowermost levels of necrosis occurred in the control and mutagens level *i.e.*, 0.1g/L colchicine, sodium azide as well as 0.5 Kr gamma rays. Also, proliferation was significantly increased when 0.1g/L sodium azide, 0.5 Kr gamma rays treatments and control were concerned as compared with the other treatments. Moreover, control and 1.0 Kr gamma rays showed significant improvement in greening parameter in relation to the other treatments. Meanwhile, 0.5 Kr gamma rays resulted in significant increase of growth compared with the others. However, callus production was statistically maximized when 0.5g/L colchicine was added to the culture medium.

Generally, the beforementioned results show that the lowest concentrations were preferred in reducing the bad effect of mutagens on tissue culture parameters. These results are in agreement with the findings of **Youssef *et al.* (1998)**. They pointed out that low concentration of colchicine gave the greatest shoots fresh weight while the higher concentration increased leaf area and leaf length of *in vitro Melaleuca armillaris* shoots.

Table (25): Effect of different mutagens on tissue culture parameters of betulaefolia pear rootstock explants.

Parameters / Treatment		Necrosis	Callus production	Growth	Proliferation	Greening
Control		1.13 G	1.00 F	4.03 A	2.67 C	3.57 A
0.1 SA	g/L	2.40 D	1.23 DE	3.97 A	2.81 C	2.67 D
0.5 SA	g/L	3.70 B	1.37 D	1.73 E	2.23 D	1.97 E
1.0 SA	g/L	5.00 A	1.00 F	1.00 F	1.00 E	1.00 F
0.1 Col.	g/L	1.27 FG	1.57 C	3.33 B	3.23 B	3.20 BC
0.5 Col.	g/L	2.83 C	2.10 A	2.30 D	2.23 D	2.10 E
1.0 Col.	g/L	5.00 A	1.00 F	1.00 F	1.00 E	1.00 F
0.5 Kr		1.43 F	1.10 EF	4.20 A	3.73 A	3.43 AB
1.0 Kr		2.10 E	1.17 E	3.50 B	3.13 B	3.47 A
2.0 Kr		2.43 D	1.73 B	2.67 C	3.10 B	3.03 C
4.0 Kr		3.87 B	1.80 B	1.97 E	2.43 D	1.87 E
8.0 Kr		5.00 A	1.00 F	1.00 F	1.00 E	1.00 F

Means of different mutagens followed by the same letter within each column are not significantly different from each other at 1% level.

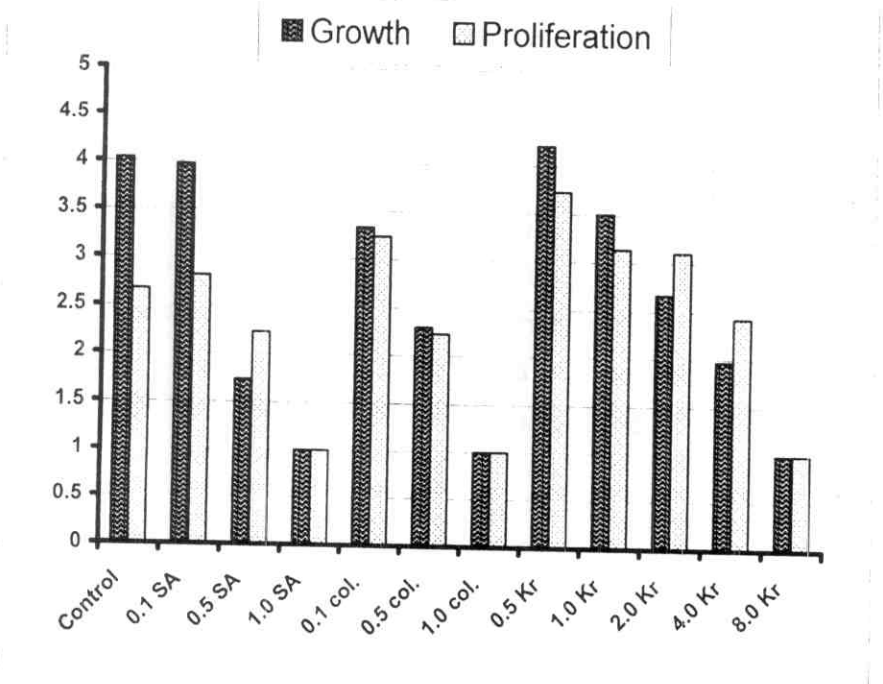


Fig. (8): Effect of different mutagens on growth and proliferation of betulaefolia pear rootstock explants.

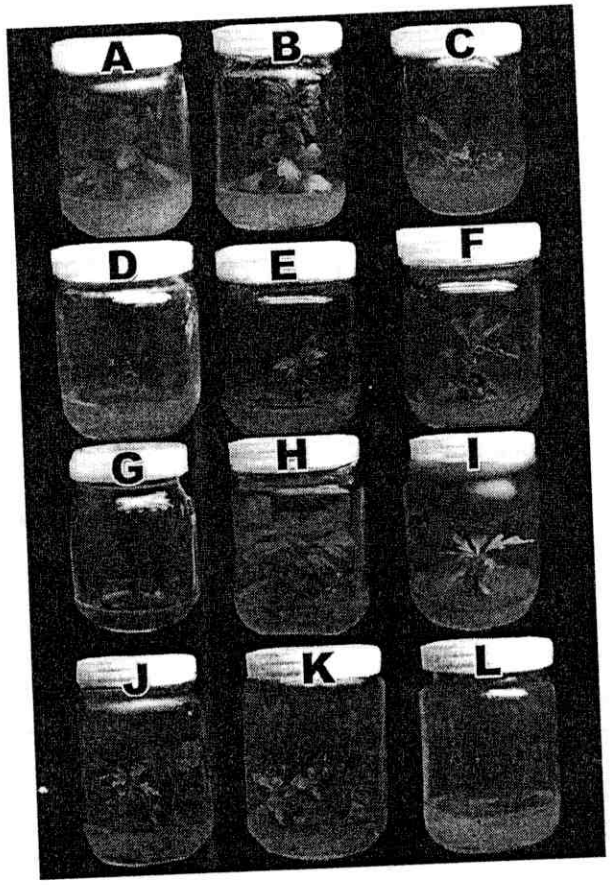


Photo (19): Effect of different mutagens on tissue culture parameters of betuleafolia pear rootstock explants.

A = Control
B = 0.1 g/L SA
C = 0.5 g/L SA
D = 1.0 g/L SA
E = 0.1 g/L Col.
E = 0.5 g/L Col.
E = 1.0 g/L Col.
H = 0.5 kr
I = 0.5 kr
J = 0.5 kr
K = 0.5 kr
L = 0.5 kr

4.II.1.b. Effect on phenotypic variabilities:

Data in **Table (26)** and **Photo (20)** reflect the effect of different mutagens on percentage of variant plantlets. It showed that gamma rays were superior in inducing high percentage of variable plantlets in relation to either colchicine or sodium azide treatments. Meanwhile, the highest percentage of variable plantlets appeared with those treated with 4.0 Kr ,followed by 2.0 Kr of gamma rays. However, sodium azide treatments were superior than colchicine treatments in inducing variabilities as adding 0.5g/L of sodium azide to the culture medium encouraged 65 variable plantlets while the same concentration of colchicine induced 25 variable plantlets.

Table (26): Phenotypic variable plantlets as induced by different mutagens in betulaefolia pear rootstock explants.

Parameters / Treatment		Percentage of variable plantlets
Control		0.0
0.1 SA	g/L	40
0.5 SA		65
1.0 SA		0.0
0.1 Col.	g/L	20
0.5 Col.		25
1.0 Col.		0.0
0.5 Kr		0.0
1.0 Kr		30
2.0 Kr		70
4.0 Kr		95
8.0 Kr		10

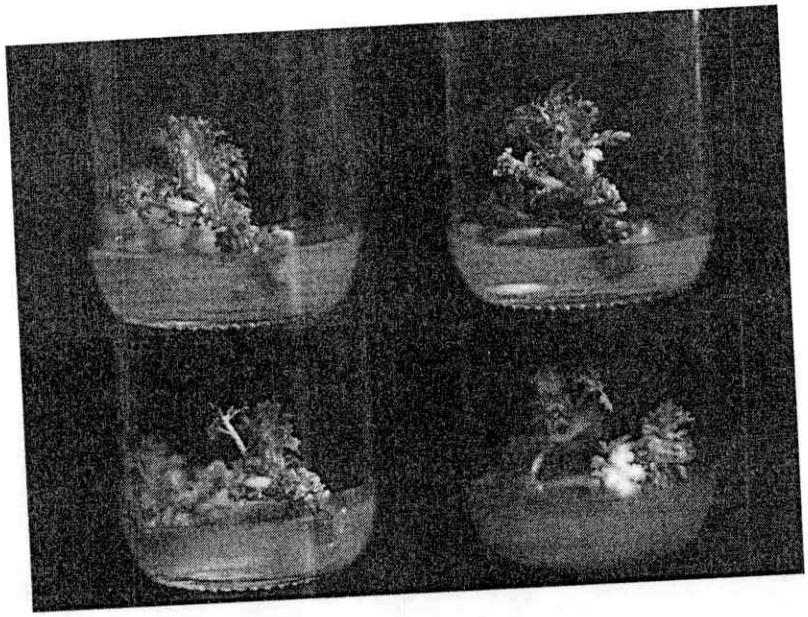


Photo (20a): Effect of different mutagens on vitrification (hyperhydericity) of betuleafolia pear rootstock explants.

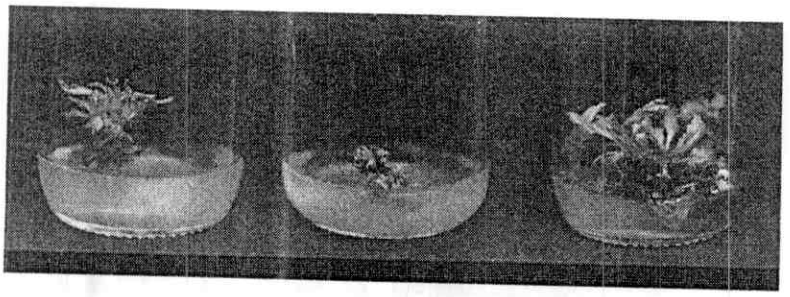


Photo (20b): Effect of different mutagens on dwarfism of betuleafolia pear rootstock explants.

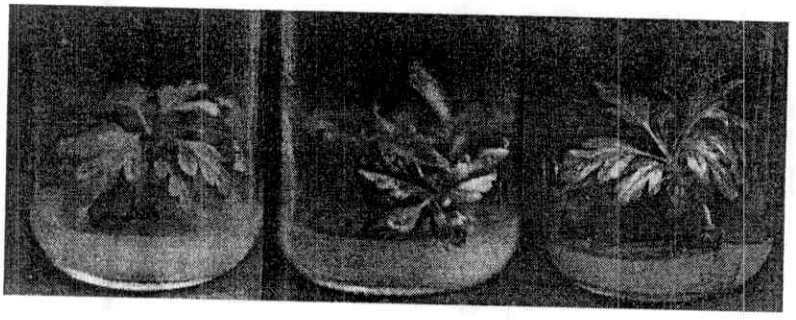


Photo (20c): Effect of different mutagens on leaves of betuleafolia pear rootstock explants.

4.II.1.c. Effect on plantlets organ variabilities:

Table (27) clarifies that leaf area was the most organ greatly affected by mutagens in response to sodium azide, colchicine and irradiation treatments. However, plantlets with dwarf stem and vertification (hyperhydericity) appeared only with irradiation

treatments. Beside the highest percentage of plantlets with dwarf stem occurred with higher dose of gamma rays (8.0 Kr), followed by 4.0 Kr, and 3.0 Kr. On the other hand, 0.5 Kr dose of gamma rays induced the highest percentage of large leaf area followed by 0.1 g/L sodium azide and 2.0 Kr dose of gamma rays. Furthermore, vertified plantlets percentage were increased when subjected to 4.0 Kr and 2.0 Kr of gamma rays.

The above results conclude that gamma rays are effective in inducing variabilities (hyperhydericity) in different organs [leaf area, dwarf stem and vertification (hyperhydericity)]. These results somewhat agreed with the findings of **Masuda and Yoshioka (1997)** They found that the highest mutations in apple shoots were obtained when the shoots were irradiated with 5.0 Gy/h dose.

Table (27): The effect of different mutagens on plantlets of betuleafolia (Pear rootstock) explants.

Parameters / Treatment		Dwarfed stem	Large leaf area	Vertification (hyperhydericity)
Control		0.0	0.0	0.0
0.1 SA	g/L	0.0	6.0	0.0
0.5 SA		0.0	7.0	0.0
1.0 SA		0.0	1.0	0.0
0.1 Col.	g/L	0.0	4.0	0.0
0.5 Col.		0.0	5.0	0.0
1.0 Col.		0.0	0.0	0.0
0.5 Kr		0.0	0.0	0.0
1.0 Kr		0.0	4.0	5.0
2.0 Kr		2.0	6.0	7.0
4.0 Kr		3.0	2.0	9.0
8.0 Kr		5.0	0.0	0.0

4.II.1.a.4. Efficiency of different mutagens:

Table (28) showed a comparison between efficiency of sodium azide, colchicine, and irradiation with gamma rays in inducing phenotypic variabilities. It is clear that physical mutagen (irradiation with gamma rays) is greatly effective in maximizing percentage of variabilities as compared with either sodium azide or colchicine. Also, sodium azide as chemical mutagen surpassed colchicine in inducing variabilities.

Table (28): Efficiency of the different mutagens in inducing phenotypic variations in betulaefolia pear rootstock explants.

Parameters / Treatment	% variable plantlets
Sodium azide	35.00
Colchicine	15.00
Irradiation	41.00

4.II.2. Proliferation stage:

4.II.2.a. Effect of cytokinin types:

The outlined data in **Table (29)** and **Photo (21)** describe the effect of different cytokinin types (kinetin, 2-iP (2-isopentenyl-adenine), and BAP (6-benzylamino purine) on necrosis, callus production, growth, proliferation, and greening parameters. It is obvious that both kinetin and 2-iP were significantly effective in reducing necrosis and callus production as compared with BAP. However, the reverse was true when greening parameter was considered. Similarly, growth was significantly increased when kinetin was added to the culture medium in relation to 2-iP and BAP. On the other hand, proliferation was significantly maximized as BAP was added to the culture medium as compared with the others.

The aforementioned results showed that BAP could be recommended for maximizing proliferation while kinetin is more preferable for improving growth and greening parameters. These

results are in agreement with the findings of **Sriskandarajah *et al.* (1990)**. They indicated that shoot proliferation was better with BA while kinetin was superior than 2-iP in improving growth.

Table (29): Effect of cytokinin type on growth and proliferation of betulaefolia pear rootstock explants.

Parameters / Cytokinin type	Necrosis	Callus production	Growth	Proliferation	Greening
Ki	1.32 B	1.00 B	4.17 A	1.30 C	4.17 A
2-iP	1.33 B	1.00 B	3.63 B	2.80 B	3.80 A
BAP	2.57 A	1.27 A	2.40 C	4.07 A	2.73 B

Means of cytokinin type followed by the same letter within each column are not significantly different from each other at 1% level.

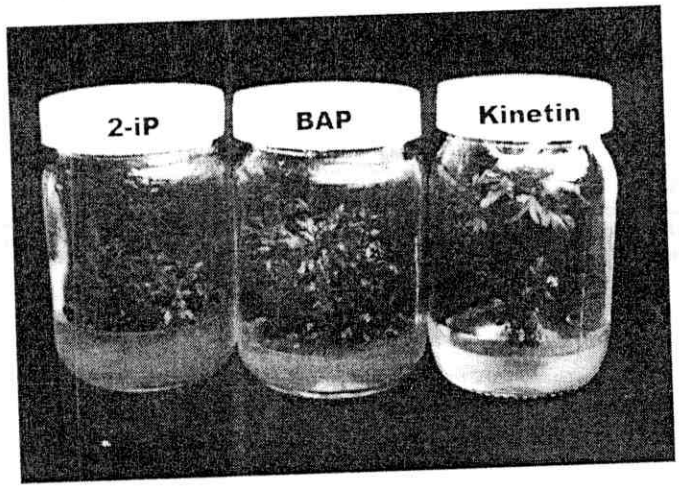


Photo (21): Effect of cytokinin type on growth and proliferation of betuleafolia pear rootstock explants.

4.II.2.b. Effect of BAP concentration:

Table (30) and **Photo (22)** verifies that growth and greening parameters were significantly improved when lower concentrations of BAP (0.0 and 1.0 mg/L) were used compared with higher concentrations. However, the reverse was true when necrosis and callus production parameters were involved. On the other hand, supplementation of the culture medium with 2.0 mg/L BAP enhanced significant increase of proliferation in comparison with the other concentrations.

The results conclude that addition of 2.0 mg/L BAP to the medium maximized proliferation. These results go in line with the findings of **Farid (1997)** on pecan and pistachio and **Zaied (1997)** on stone fruits. They stated that addition of 2.0 mg/L BAP to the culture medium enhanced the highest proliferation.

Table (30): Effect of BAP concentrations on growth and proliferation of betulaefolia pear rootstock explants.

Parameters / BAP conc. (mg/L)	Necrosis	Callus production	Growth	Proliferation	Greening
Control	1.67 D	1.00 D	3.33 A	1.36 D	3.90 A
1.0	1.70 C	1.43 C	3.63 A	2.57 B	4.07 A
2.0	2.50 B	1.73 B	2.07 B	4.10 A	2.83 B
4.0	3.00 A	2.77 A	1.47 C	2.10 C	1.76 C

Means of BAP concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

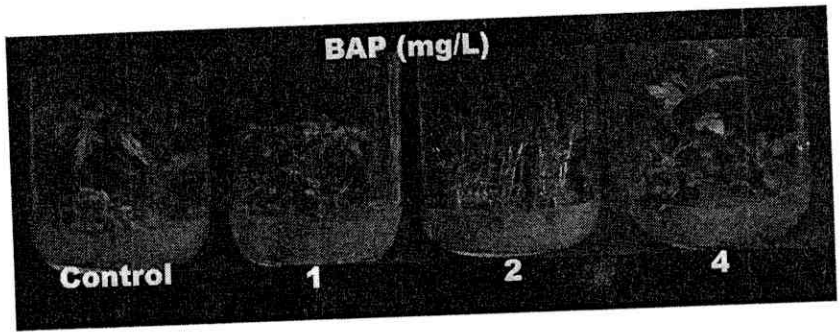


Photo (22): Effect of BAP concentration on growth and proliferation of betulaefolia pear rootstock explants.

4.II.3. Rooting stage:

4.II.3.1. Shoot elongation:

4.II.3.1.a. Effect of medium strength:

Data in **Table (31)**, **Fig. (9)** and **Photo (23)** indicated that shoot elongation and greening parameters were statistically increased when half Murashige and Skoog medium strength was used as compared with the other medium strengths. However, continuous dilution of medium strength resulted in a significant reduction in all parameters under study *i.e.*, necrosis, shoot elongation, and greening as compared with full medium strength.

The above results showed that half medium strength is superior for increasing shoot elongation and greening. These results confirm the findings of **Gamage *et al.* (2000)**. They reported that the best shoot elongation of apple (*Malus domastica* L.) was obtained on half-strength MS medium.

Table (31): Effect of medium strength on shoot elongation of betulaefolia pear rootstock explants.

Parameters / Medium strength	Necrosis	Shoot elongation	Greening
Full	1.87 A	2.83 B	2.43 B
Half-strength	1.33 B	3.83 A	3.83 A
Quarter strength	1.13 B	1.90 C	2.20 C
Eighth strength	1.10 B	1.23 D	1.50 D

Means of medium strength followed by the same letter within each column are not significantly different from each other at 1% level.

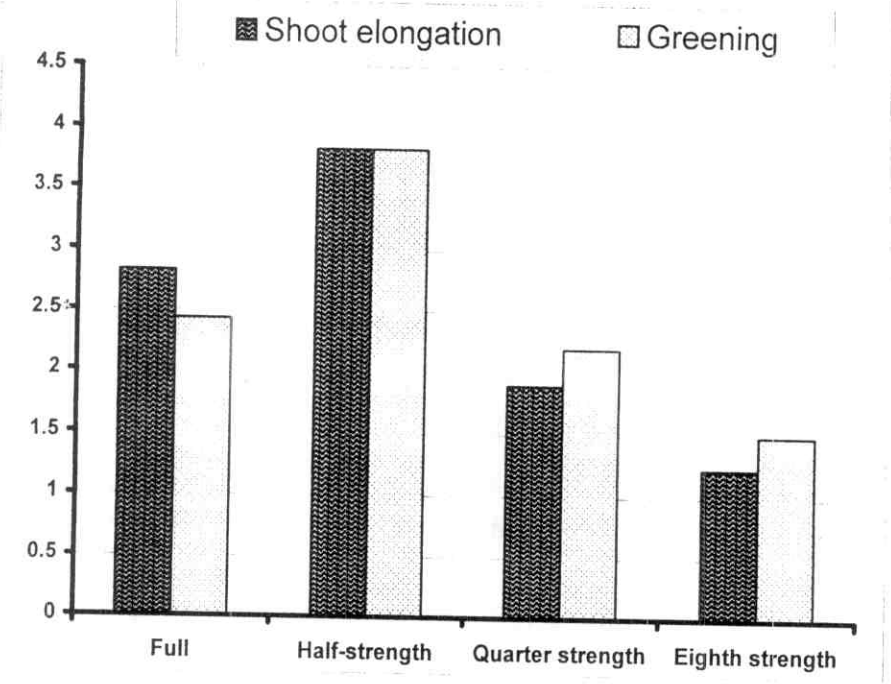


Fig. (9): Effect of medium strength on shoot elongation and greening of betulaefolia pear rootstock explants.

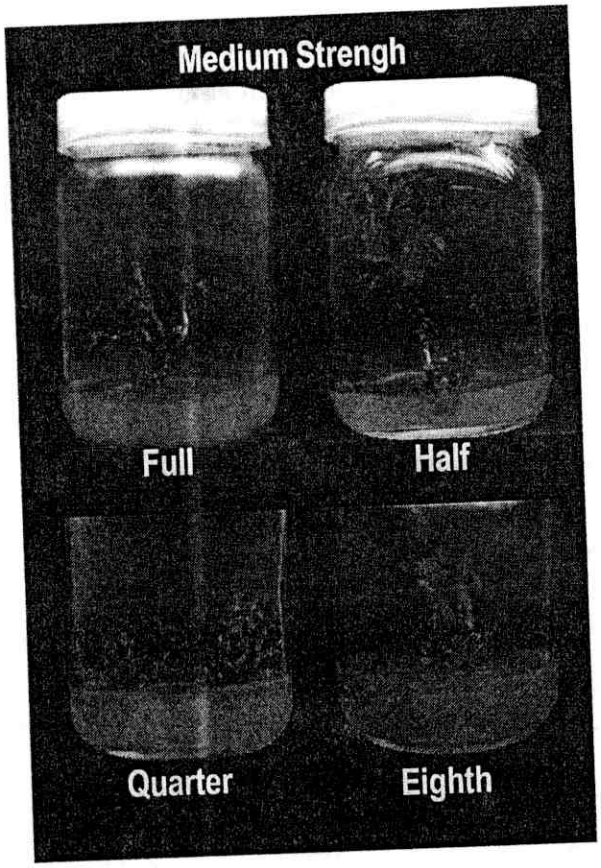


Photo (23): **Effect of medium strength shoot elongation of betulaefolia pear rootstock explants.**

4.II.3.1.b. Effect of GA_3 concentration:

Table (32) and **Photo (24)** reflects the effect of different GA_3 concentrations on necrosis, shoot elongation, and greening parameters. It is clear that supplementation of the culture medium with 2.0 mg/L GA_3 succeeded in inducing the most significant increase of shoot elongation and greening parameters in comparison with the other GA_3 concentrations. However, the lower most significant necrosis, shoot elongation and greening were occurred as control and 1.0 mg/L GA_3 were considered.

The mentioned results assured the importance of 2.0 mg/L GA_3 in maximizing shoot elongation and greening. These results confirm the findings of **Ali (2000)**. She declared that supplementing of the culture medium with 1.0 or 2.0 mg/L GA_3 induced the best shoot elongation of citrus rootstocks.

Table (32): Effect of GA_3 concentration on shoot elongation of betulaefolia pear rootstock explants.

Parameters / GA_3 Conc. (mg/L)	Necrosis	Shoot elongation	Greening
Control	1.20 D	1.43 C	1.67 C
1.0	1.93 C	2.26 B	2.43 B
2.0	2.40 B	3.70 A	3.73 A
3.0	2.83 A	2.17 B	2.43 B

Means of GA_3 concentration followed by the same letter within each column are not significantly different from each other at 1% level.

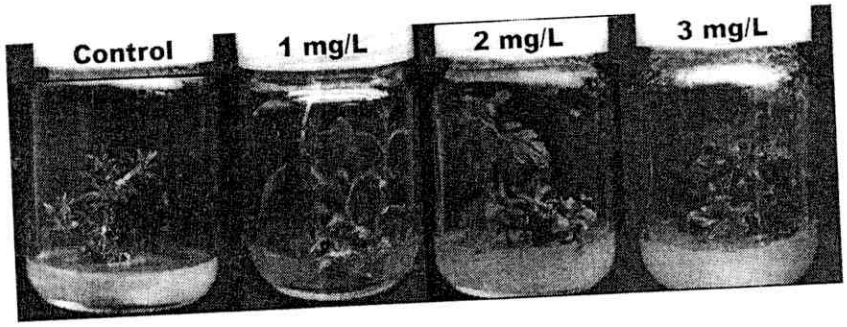


Photo (24): Effect of GA_3 concentration on shoot elongation of betulaefolia pear rootstock explants.

4.II.3.2. Root formation:

4.II.3.2.a. Effect of auxin type:

Table (33) and **Photo (25)** clarifies that NAA significantly surpassed IBA in reducing necrosis while significantly increased growth and greening parameters. However, adding IBA to the culture medium encouraged the best root formation in relation to NAA.

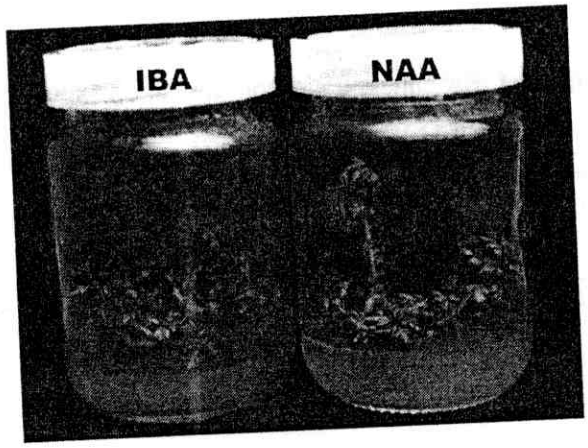


Photo (25): Effect of auxin type on growth and rooting of betulaefolia pear rootstock explants.

4.II.3.2.b. Effect of IBA concentration:

It is clear from **Table (34)** and **Photo (26)** that either the control or addition of 1.0 mg/L IBA to the culture medium resulted in a significant increase of growth and greening parameters and reduced necrosis in comparison with the other IBA concentrations. However, supplementation of the culture medium with 2.0 mg/L encouraged significant increase of rooting. On the other hand, increasing the concentration of IBA up to 3.0 mg/L enhanced the highest significant increase of callus production.

The aforementioned results explain that addition of 2.0 mg/L IBA induced the highest rooting. These results assured the findings of **Welander (1991)** who reported that using of 2.0 mg/L IBA enhanced root formation.

Table (33): Effect of auxin type on growth and rooting of betulaefolia pear rootstock explants.

Parameters / Auxin type	Necrosis	Callus	Growth	Greening	Rooting
NAA	2.03 B	1.43 B	3.13 A	3.10 A	1.43 B
IBA	2.73 A	1.60 A	2.50 B	2.33 B	3.27 A

Means of auxin type followed by the same letter within each column are not significantly different from each other at 1% level.

Table (34): Effect of IBA concentration on growth and rooting of betulaefolia pear rootstock explants.

Parameters / IBA Conc. (mg/L)	Necrosis	Callus	Growth	Greening	Rooting
Control	1.20 C	1.00 C	3.30 A	3.10 A	1.23 C
1.0	1.37 C	1.30 B	2.43 B	3.10 A	2.20 B
2.0	2.17 B	1.40 B	2.03 C	2.57 B	3.67 A
3.0	2.63 A	2.83 A	2.07 C	2.03 C	1.20 C

Means of IBA concentration followed by the same letter within each column are not significantly different from each other at 1% level.

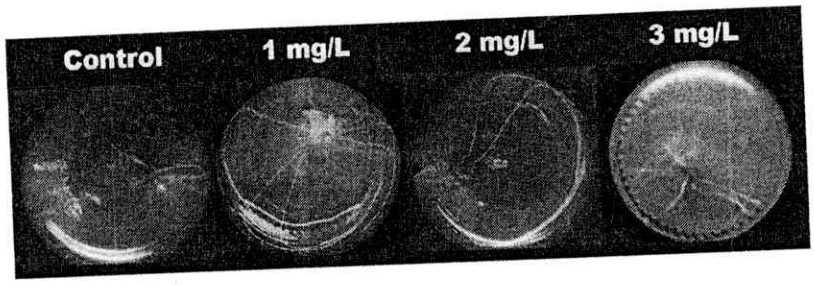


Photo (26): Effect of IBA concentrations on growth and rooting of betulaefolia pear rootstock explants.

4.II.4. Stress tolerance:

4.II.4. Drought stress:

4.II.4.1.a. Effect of mannitol:

4.II.4.1.a.1. Tissue culture parameters:

Table (35), **Fig. (10)** and **Photo (27)** deals with the effect of different mannitol concentrations on necrosis, callus production, growth, number of shoots, shoot length, shoot thickness, number of leaves/plant, root length, and root number. It appears that addition of 20 g/L mannitol to the culture medium resulted in significant reduction in necrosis and callus production while significantly increased growth, number of shoots, shoot length, number of leaves/plant and number of roots as compared to the other mannitol concentrations. However, shoot thickness was significantly maximized by increasing mannitol concentrations up to 80 g/L in relation to the others. Moreover, using of either 60 or 80 g/L mannitol in the culture medium enhanced a significant increase of root length in comparison with the other concentrations. Besides, increasing mannitol concentrations induced adverse effect on most parameters under study except callus production and necrosis where they increased significantly.

Table (35): Effect of different mannitol concentrations on the tissue cultural parameters of *in vitro* betulaefolia pear rootstock plantlets.

Parameters / Mannitol Conc. (g/L)	Necrosis	Callus	Growth	No. of shoots	Shoot length	Shoot thickness	No. of leaves / plant	Root length	No. of roots
20	1.10 E	1.03 E	4.20 A	4.50 A	5.20 A	0.22 E	18.83 A	3.30 B	3.70 A
40	2.07 D	1.67 D	3.77 B	2.27 B	4.03 B	0.36 C	14.33 B	3.24 B	3.33 B
60	3.03 C	2.03 C	3.07 C	3.60 C	3.83 C	0.37 B	9.00 C	3.54 A	2.56 C
80	3.63 B	2.37 B	3.03 D	2.10 D	3.57 D	0.39 A	4.00 D	3.51 A	2.30 D
100	4.50 A	3.70 A	1.77 D	1.43 E	3.03 E	0.31 D	1.43 E	2.48 C	2.17 D

Means of different mannitol concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

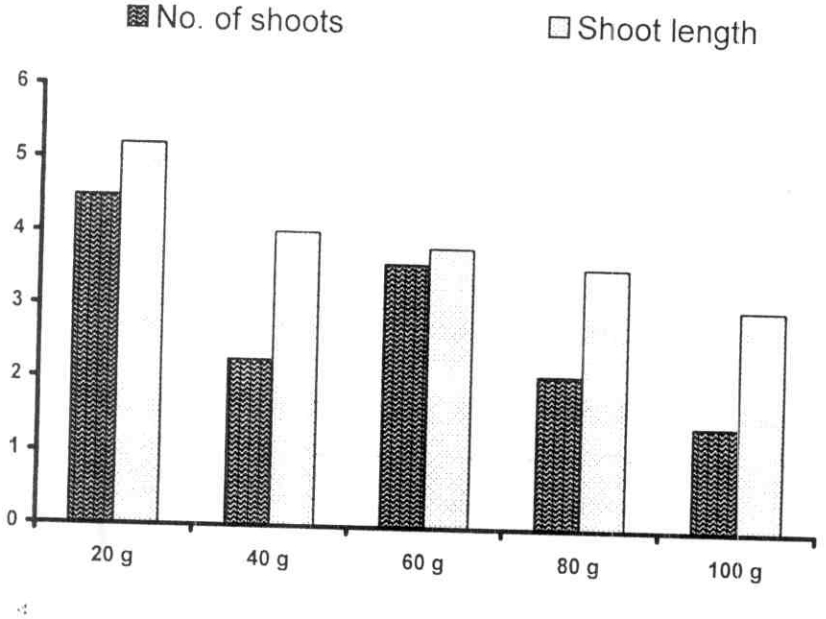


Fig. (10): Effect of different mannitol concentrations on No. of shoot and shoot length of *in vitro* betulaefolia pear rootstock plantlets.

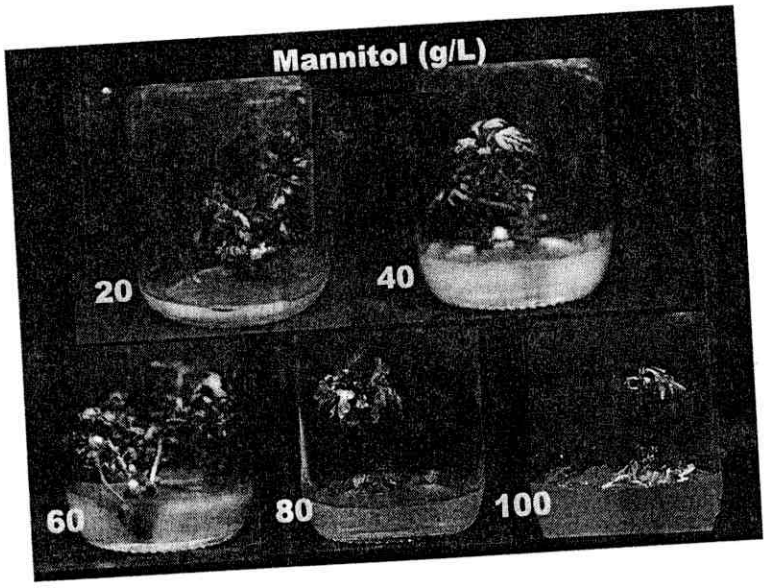


Photo (27): Effect of mannitol concentration on tissue culture parameters of betulaefolia pear rootstock explants.

4.II.4.1.a.2. Chemical composition:

Table (36) refers to the effect of different concentrations of mannitol on chemical composition *i.e.*, chlorophyll-A & B, non reducing sugars, reducing sugars and total sugars, total indoles, and total phenols. It appear that addition of low concentrations (20 or 40 g/L) of mannitol to the culture medium encouraged a significant improvement of chlorophyll-A compared with the higher concentrations (80 and 100 g/L) while the reverse was true with chlorophyll- B. Meanwhile, non-reducing sugars and total sugars were significantly increased when low mannitol concentration (20 g/L) was added to the culture medium while increasing concentration had significantly reduced those parameters to the lowest level. On the other hand, increasing mannitol concentrations in the culture medium resulted in significant increase of reducing sugars, total indoles and total phenols accumulation.

Table (36): Effect of different mannitol concentrations on the chemical composition of *in vitro* betulaefolia pear rootstock plantlets.

Parameters / Mannitol Conc. (g/L)	Chlorophyll A	Chlorophyll B	Non reducing sugars	Reducing sugars	Total sugars	Total indoles	Total phenols
20	9.65 A	3.73 D	9.23 A	3.73 E	13.06 A	0.96 D	9.36 E
40	9.42 A	4.01 C	8.72 B	3.86 D	12.57 B	0.99 D	10.76 D
60	8.07 B	4.20 B	7.54 C	4.32 C	11.00 C	1.38 C	11.27 C
80	5.50 C	4.35 A	7.03 D	4.56 B	11.59 E	1.46 B	11.76 B
100	2.11 D	4.27AB	6.84 E	4.84 A	11.68 D	1.67 A	11.86 A

Means of different mannitol concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

Generally, the results of tissue culture and chemical parameters reflect that increasing mannitol concentrations had harmful effect on most tissue culture and chemical parameters. However, reducing sugars, total indoles and phenols were increased. These results explain that these plantlets have gained an increase drought tolerance ability.

4.II.4.1.b. Effect of polyethylene glycol:

4.II.4.1.b.1. Tissue culture parameters:

Effect of different polyethylene glycol (PEG) concentration on tissue culture parameters were tabulated in **Table (37)**, **Fig.(11)** and **Photo (28).** The data show that increasing PEG percentages from 2 up to 10 g/L resulted in significant decrease of most parameters specially growth, number of shoots, and shoot length which were significantly at the highest level at 2 g/L PEG in the culture medium compared with the other higher concentrations. However, necrosis shoot thickness and callus production had the reverse behaviour. Meanwhile, number of leaves/plant were significantly increased with the addition of either 2.0 or 4.0 g/L PEG in the culture medium in relation to the other PEG percentages. Moreover, root length was positively responded with significant level to increased PEG percentages up to 8.0 g/L compared with 10.0 g/L. On the other hand,

supplementation of the culture medium with either 4.0, 6.0 or 8.0 g/L of PEG enhanced significant increase of root length as compared with 10.0 g/L level.

Table (37): Effect of different PEG concentrations on the tissue cultural parameters of *in vitro* betulaefolia pear rootstock plantlets.

Parameters / PEG Conc. (g/L)	Necrosis	Callus	Growth	No. of shoots	Shoot length	Shoot thickness	No. of leaves / plant	Root length	No. of roots
2	1.33 E	1.03 E	4.17 A	5.03 A	3.87 A	0.17 E	10.33 A	3.34 A	2.57 B
4	2.10 D	1.37 D	3.66 B	4.10 B	3.57 B	0.22 D	9.00 A	3.18 A	3.03 A
6	2.40 C	1.73 C	3.60 B	3.10 C	3.13 C	0.24 C	7.00 B	3.22 A	2.93 A
8	3.10 B	2.13 B	2.10 C	2.33 CD	2.57 D	0.24 B	3.67 C	3.13 A	2.80 AB
10	4.43 A	2.80 A	1.57 D	1.80 D	1.53 E	0.28 A	1.33 D	2.40 B	1.88 C

Means of different PEG concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

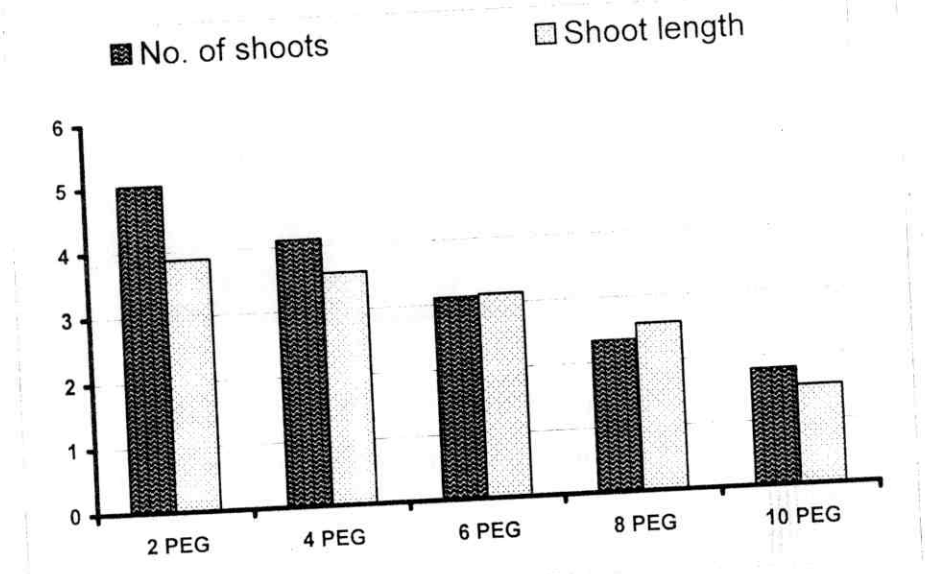


Fig. (11): Effect of different PEG concentrations on No. of shoot and shoot length of *in vitro* betulaefolia pear rootstock plantlets.

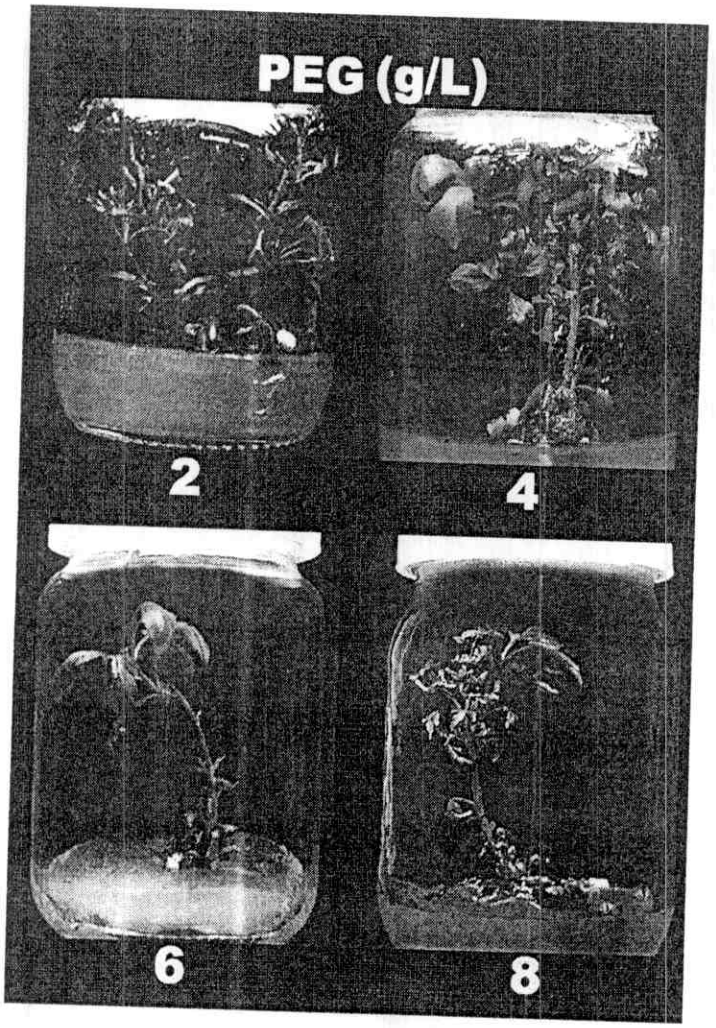


Photo (28): Effect of different PEG concentrations on tissue culture parameters of betulaefolia pear rootstock explants.

4.II.4.1.b.2. Chemical composition:

Table (38) explain the effect of PEG at different percentages in the culture medium on chemical composition. It is clear that chlorophyll-A and non-reducing sugars were significantly maximized as 2.0 g/L PEG was added to the medium involved in comparison with the other percentages of PEG. However, the reverse was true when chlorophyll-B and reducing sugars were concerned. On the contrary, total sugars accumulation was significantly increased as 6.0 g/L of PEG was added to the culture medium in relation to the others. Similarly, total indoles and phenols were significantly higher when 8.0 g/L of PEG was added to the medium.

Table (38): Effect of different PEG concentrations on the chemical composition of *in vitro* betulaefolia pear rootstock plantlets.

Parameters / PEG Conc. (g/L)	Chlorophyll A	Chlorophyll B	Non reducing sugars	Reducing sugars	Total sugars	Total indoles	Total phenol
2	9.23 A	2.757 E	8.963 A	3.250 E	12.213C	0.983 D	9.573 E
4	8.93 B	3.850 D	8.563 B	3.557 D	12.120D	1.467CD	10.627 D
6	8.52 C	4.350C	8.013 C	4.753 C	12.767A	1.770C	11.347C
8	7.54 D	4.550 B	6.557 D	5.863 B	12.420B	3.020 A	12.547 A
10	2.12 E	4.263 A	5.743 E	6.020 A	11.763E	2.350 B	12.503 B

Means of different PEG concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

4.II.4.1.c. Effect of agar:

4.II.4.1.c.1. Tissue culture parameters:

The results of **Table (39)**, **Fig. (12)** and **Photo (29)** indicate the effect of different agar concentrations on tissue culture parameters. It is quite evident that supplementation of the culture medium with 6.0 g/L agar significantly improved most tissue culture parameters *i.e.*, necrosis, growth, number of shoots, shoot length, number of leaves/plant, root length and number of roots in comparison with the other agar

concentrations. Increasing agar concentration in the medium induced significant reduction for these parameters. However, callus production, necrosis, and shoot thickness were significantly increased by increasing agar concentrations up to the highest levels (14.0 g/L) compared with the other concentrations.

Table (39): Effect of different agar concentrations on the tissue cultural parameters of *in vitro* betulaefolia pear rootstock plantlets.

Parameters / Agar Conc. (g/L)	Necrosis	Callus	Growth	No. of shoots	Shoot length	Shoot thickness	No. of leaves / plant	Root length	No. of roots
6	1.07 E	1.03 E	4.27 A	6.00 A	3.67 A	0.19 E	12.67 A	3.03 A	4.03 A
8	2.10 D	1.33 D	3.93 B	4.03 B	3.07 B	0.21 D	10.67 A	2.67 B	3.63 B
10	2.67 C	1.80 C	3.07 C	3.07 C	2.53 C	0.24 C	5.33 B	2.57 B	2.80 C
12	3.90 B	2.50 B	1.63 D	1.57 D	2.03 D	0.27 B	2.33 C	2.17 C	2.67 C
14	4.53 A	4.13 A	1.23 E	1.20 D	1.40 E	0.31 A	1.00 C	2.13 C	2.27 D

Means of different agar concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

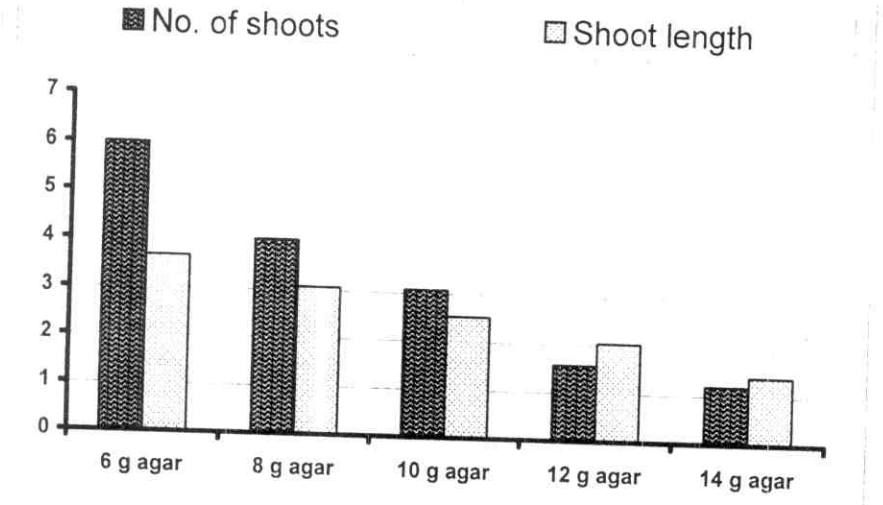


Fig. (12): Effect of different agar concentrations on No. of shoots and shoot length of *in vitro* betulaefolia pear rootstock plantlets.

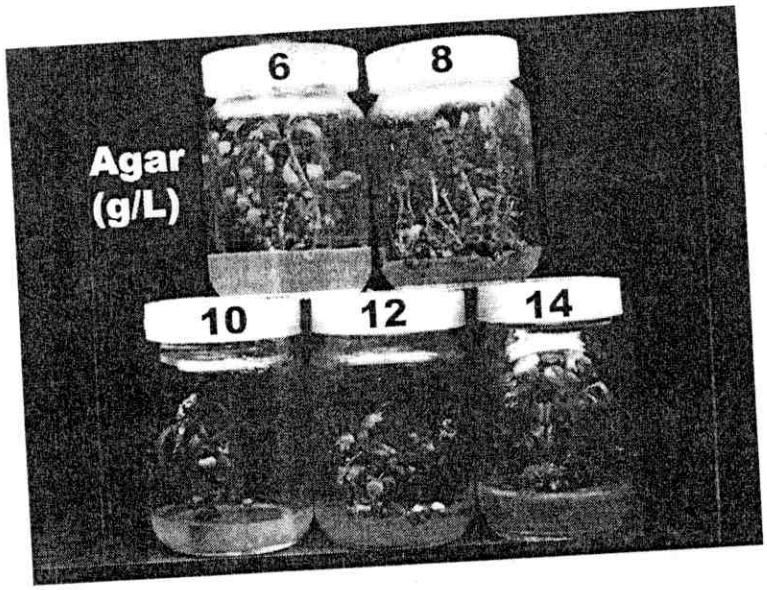


Photo (29): Effect of different agar concentrations on tissue culture parameters of betulaefolia pear rootstock explants.

4.II.4.1.c.2. Chemical composition:

Table (40) reflects the effect of different agar concentrations in the medium under study on chemical composition. The data show that chlorophyll-A and non reducing sugars were statistically increased when lower levels of agar (6.0 g/L) was used in the medium. However, supplementation of the culture medium with higher concentrations up to 14.0 g/L enhanced significant increase in chlorophyll-B, total indoles, and total phenols in relation to the other agar concentrations. Similar results were obtained with reducing sugars when agar concentration was

increased up to 12 g/L in the medium. Meanwhile, addition of 8.0 g/L agar significantly improved total sugar levels.

Table (40): Effect of different agar concentrations on the chemical analysis of *in vitro* betulaefolia pear rootstock plantlets.

Parameters / Agar Conc. (g/L)	Chlorophyll A	Chlorophyll B	Non-reducing sugars	Reducing sugars	Total sugars	Total indoles	Total phenol
6	9.75 A	3.54 D	10.23 A	3.79 E	14.03 B	1.13 E	9.04 E
8	8.78 B	4.14 C	9.72 B	4.37 D	14.10 A	1.25 D	9.77 D
10	8.54 C	4.23 C	8.55 C	4.78 C	13.33 C	1.46 C	10.56 B
12	6.50 D	4.73 B	7.50 D	5.13 A	12.63 D	1.71 B	10.93 B
14	3.19 E	4.91 A	6.01 E	5.09 B	11.10 E	1.93 A	11.34 A

Means of different agar concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

4.II.4.2. Salinity stress:

4.II.4.2.a. Tissue culture parameters:

Referring to the effect of different salinity causal agents (NaCl, $CaCl_2$ and their combination) at various levels 500, 1000, 1500, 2000 ppm on necrosis, callus production, growth, number of shoots, shoot length, shoot thickness, number of leaves/plant, root length, and number of roots parameters were tabulated in **Table (41)**, **Fig. (13)** and **Photo (30)**. The results reflect that increasing levels of salinity induced a harmful effect on most tissue culture parameters. Also, $CaCl_2$ and the combination treatment (NaCl + $CaCl_2$) were less harmful than NaCl treatments at the same salinity levels. However, number of roots was not significantly reduced by increasing salinity in the culture medium as compared with the other parameters studied except callus production which was significantly increased when 1500 ppm NaCl was added to the medium.

Table (41): Effect of different salinity concentrations on the tissue cultural parameters of *in vitro* betulaefolia pear rootstock plantlets.

Parameters / Salinity Conc. (ppm)	Necrosis	Callus	Growth	No. of shoots	Shoot length	Shoot thickness	No. of leaves / plant	Root length	No. of roots
Control	1.07 H	1.00 H	4.78 A	7.04 A	4.58 AB	0.20 I	21.00 A	4.24 A	4.52 A
500 NaCl	2.00 F	1.33 F	4.23 C	6.02 C	4.51ABC	0.24 G	18.00 C	4.03BC	4.32 AB
1000 NaCl	2.73 E	2.10 C	3.63 E	4.23 E	3.44 H	0.26 E	10.00 F	3.90 CD	4.07 BCD
1500 NaCl	3.60 C	2.73 A	2.57 H	2.17 G	3.10 I	0.29 C	5.33 H	3.57 E	4.00BCDE
2000 NaCl	4.90 A	1.13GH	1.07 K	1.00 H	4.23DEF	0.31 B	1.67 J	3.40 F	3.60 EF
500 $CaCl_2$	1.27 H	1.23FG	4.47 B	6.90AB	4.72 A	0.25 F	19.33 B	4.06 B	4.27 ABC
1000 $CaCl_2$	2.07 F	1.80 D	3.95 D	6.30BC	4.43BCD	0.28 D	16.33 D	4.07 B	4.03BCDE
1500 $CaCl_2$	2.77 E	1.93CD	2.87 G	5.33 D	4.03 FG	0.31 B	8.33 G	3.46 EF	3.40 FG
2000 $CaCl_2$	4.17 B	1.90 D	1.37 J	2.50 G	3.83 G	0.33 A	3.37 I	3.46 EF	3.07G
250 NaCl+250 $CaCl_2$	1.58 G	1.27FG	3.73DE	6.47ABC	4.47BCD	0.23 H	19.10BC	4.10AB	4.31AB
500 NaCl+500 $CaCl_2$	1.97 F	1.37 F	3.17 F	5.33 D	4.17 EF	0.28 D	15.03 E	3.88 CD	3.43 FG
750 NaCl+750 $CaCl_2$	3.13 D	2.53 B	2.10 I	3.33 F	4.30CDE	0.29 C	8.37 G	3.77 D	3.82CDEF
1000 NaCl+1000 $CaCl_2$	4.33 B	1.60 E	1.90 I	2.33 G	4.10 EF	0.28 D	2.33 IH	3.78 D	3.79 DEF

Means of different salinity concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

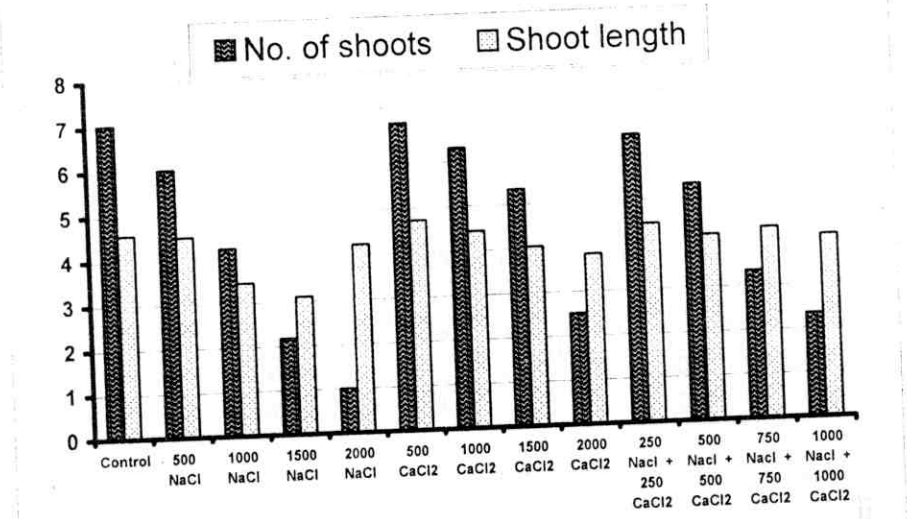


Fig. (13): Effect of different salinity concentrations on No. of shoots and shoot length of *in vitro* betulaefolia pear rootstock plantlets.

4.II.4.2.b. Chemical composition:

Table (42) verifies that increasing the concentration of salinity (NaCl or $CaCl_2$) up to 2000 ppm led to a significant increase in chlorophyll-B, total indoles, total phenols and proline contents, as compared with lower concentrations and control. However, the reverse was noticed when chlorophyll-A and non reducing sugars were considered. However, reducing sugars parameter were significantly increased by increasing NaCl concentration in the culture medium up to 1500 or 2000 ppm. Furthermore, addition of 500 ppm of NaCl to the medium significantly enhanced high accumulation of total sugars in relation to the other salinity causal agents with different concentrations.

Table (42): Effect of different salinity concentrations on the chemical composition of *in vitro* betulaefolia pear rootstock plantlets.

Parameters / Salinity Conc. (ppm)	Chlorophyll A	Chlorophyll B	Non reducing sugars	Reducing sugars	Total sugars	Total indoles	Total phenol	Proline
Control	9.93 A	4.01 D	9.26 A	3.24GH	12.51 B	1.07 E	9.76 G	0.173 D
500 NaCl	8.75 B	4.08 D	8.55 B	4.58 F	13.14 A	1.60 C	11.17 E	0.186 D
1000 NaCl	6.09 C	5.66 C	5.81 C	5.62 E	11.44CD	1.88 B	12.20 C	0.252 BC
1500 NaCl	2.95 D	6.18 B	2.94 F	7.32 B	10.26EF	2.64 A	12.55AB	0.277ABC
2000 NaCl	0.006 F	6.66 A	2.41 G	7.27 B	9.68 GH	2.58 A	12.52ABC	0.282ABC
500 $CaCl_2$	9.10 B	4.27 D	8.83 B	3.40 G	12.20 B	1.38 D	10.61 F	0.177D
1000 $CaCl_2$	6.28 C	5.43 C	6.07 C	4.49 F	10.56 E	1.72 C	11.77 D	0.191D
1500 $CaCl_2$	3.21 D	5.48 C	3.62 E	7.62 A	11.24 D	2.58 A	12.35 BC	0.226CD
2000 $CaCl_2$	1.30 E	6.61AB	2.86 F	7.53 A	10.40EF	2.70 A	12.68 AB	0.233CD
250 NaCl+250 $CaCl_2$	8.67 B	3.32 E	8.67 B	3.13 H	11.80 C	1.31 D	11.17 E	0.192D
500 NaCl+500 $CaCl_2$	5.78 C	4.07 D	4.43 D	4.43 F	8.87 I	1.64 C	11.81 D	0.263 BC
750 NaCl+750 $CaCl_2$	3.52 D	5.33 C	2.98 F	7.07 C	10.05FG	2.76 A	12.73 A	0.296AB
1000 NaCl+1000 $CaCl_2$	1.43 E	5.27 C	2.88 F	6.73 D	9.58 H	2.65 A	12.60 AB	0.323A

Means of different salinity concentrations followed by the same letter within each column are not significantly different from each other at 1% level.

The aforementioned results summarized that increasing salinity concentrations led to reduction in most tissue culture and chemical composition parameters except total phenols, total indoles, reducing sugars, and proline content. This explain the increasing of salt tolerance of betulaefolia pear rootstock. These results go in line with the findings of **El-Hawary (1987)** on grapevine cvs; **Gaser (1992)** on grapevine cvs; **El-Said *et al.* (1995)** on olive and **Hassan (2001)** on banana. They reported that the increase of proline content with increasing salinity levels. Also, **Ahmed (2000)** stated that treating of three banana cvs with NaCl increased total soluble sugars content. In addition, **Sweidan *et al.* (1992)** indicated that total phenols were gradually increased by increasing salt concentrations in apricot seedlings.

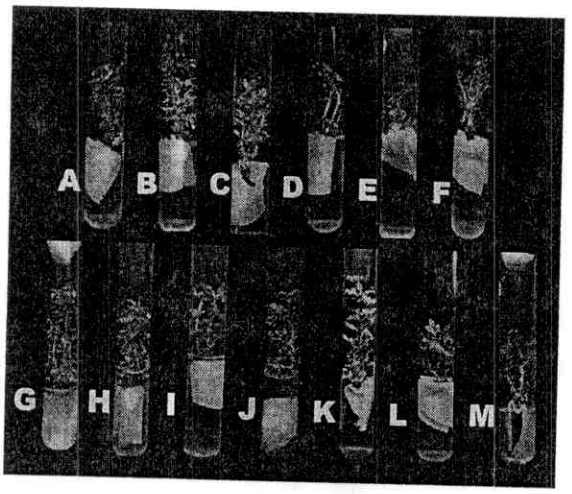


Photo (30): Effect of different salinity concentrations on tissue culture parameters of betulaefolia pear rootstock explants.

A = Control

B = 500 NaCl	F = 500 $CaCl_2$	J = 250 NaCl + 250 $CaCl_2$
C = 1000 NaCl	G = 1000 $CaCl_2$	K = 500 NaCl + 500 $CaCl_2$
D = 1500 NaCl	H = 1500 $CaCl_2$	L = 750 NaCl + 750 $CaCl_2$
E = 2000 NaCl	I = 2000 $CaCl_2$	M = 1000 NaCl + 1000 $CaCl_2$

4.II.4.c. Leaf mineral content:

The data in **Table (43)** explain the effect of salinity on leaf macroelement content of *P. betulaefolia*. It appears that N and K percentages were significantly affected by salinity as control plantlets showed the highest levels. However, P, Mg and Cl percentage were significantly at the highest level when combination treatment (1000 + 1000 of NaCl and $CaCl_2$ respectively) was used. On the other hand, adding of 2000 ppm $CaCl_2$ enhanced significant increase of Ca in the leaves. Furthermore, Na percentage was significantly increased when 2000 ppm NaCl was added to the culture medium.

Regarding the effect of salinity on leaf microelement, it is clear from **Table (44)** that Fe percentage was maximized as combination treatment (1000 + 1000) was used. On the other hand, the reverse was true with Mn and Zn percentages as control contains the highest level.

Table (43): Effect of salinity on Macroelements content of betulaefolia Pear rootstocks plantlets.

Salinity conc. (ppm)	Macroelements (%) in dry plantlets						
	N	P	K	Ca	Mg	Na	Cl
Control	7.65A	0.24B	25.83A	1.96K	0.96 J	0.95L	4.52M
500 NaCl	7.31B	0.19DE	20.32E	2.05 J	1.16 G	1.13J	6.54H
1000 NaCl	6.36C	0.14F	16.83H	2.14 I	1.25 F	1.34F	8.73F
1500 NaCl	5.64G	0.13F	13.30K	2.24H	1.52 D	2.55D	2.55B
2000 NaCl	5.42 I	0.08H	11.52M	2.33G	1.77 B	4.67A	11.52B
500 $CaCl_2$	6.14 D	0.21C	22.33C	2.24H	0.79 K	1.04 K	5.13 L
1000 $CaCl_2$	5.86 E	0.20CD	19.20F	2.55D	1.04 I	1.12 J	5.73 K
1500 $CaCl_2$	5.24 J	0.18DE	15.31 I	2.65C	1.24 F	1.16 I	5.87 J
2000 $CaCl_2$	5.15 K	0.14F	12.53L	3.03A	1.32 E	1.22 H	6.22 I
250 + 250	5.75 F	0.17E	23.32B	2.23H	1.07 H	1.27G	6.56G
500 + 500	5.48 H	0.14F	20.52D	2.43F	1.26 F	1.64E	9.58E
750 + 750	4.67 L	0.09G	17.52G	2.51E	1.63 C	1.90 D	11.14C
1000 + 1000	4.23M	0.56A	13.48 J	2.84B	1.82 A	2.40C	13.12A

Means of different salinity concentrations Macroelement content followed by the same letter within each column are not significantly different from each other at 1% level.

Table (44): Effect of salinity on Microelements content of betulaefolia Pear rootstocks plantlets.

Salinity conc. (ppm)	Microelemets (ppm) in dry plantlets			
	Fe	Mn	Zn	Cu
Control	721.67M	891.67A	170.67A	311.00L
500 NaCl	882.67K	711.67C	154.33C	401.33H
1000 NaCl	1162.67F	681.67E	121.67G	451.00F
1500 NaCl	1231.00E	602.67H	101.33H	521.67D
2000 NaCl	1472.33B	581.00J	84.33I	632.33A
500 $CaCl_2$	832.67L	751.67B	161.67B	351.00K
1000 $CaCl_2$	951.33 J	702.67 D	145.00 D	372.67 J
1500 $CaCl_2$	1002.69H	651.63F	136.67E	394.33 I
2000 $CaCl_2$	1132.63G	603.33 G	132.67 F	411.67G
250 + 250	972.67 I	702.69 D	132.67F	451.69F
500 + 500	1251.00D	591.63I	100.67H	483.67E
750 + 750	1392.33C	422.67K	85.67 I	531.67C
1000 + 1000	2162.67A	361.67L	75.33 J	628.00B

Means of different salinity concentrations Microelement content followed by the same letter within each column are not significantly different from each other at 1% level.

4.11.5. Acclimatization:

It appears from **(Photo 31)** that betulaefolia pear rootstock) was successfully acclimatized by using combination of peatmoss and sand at rate 3:1 (v/v) respectively.

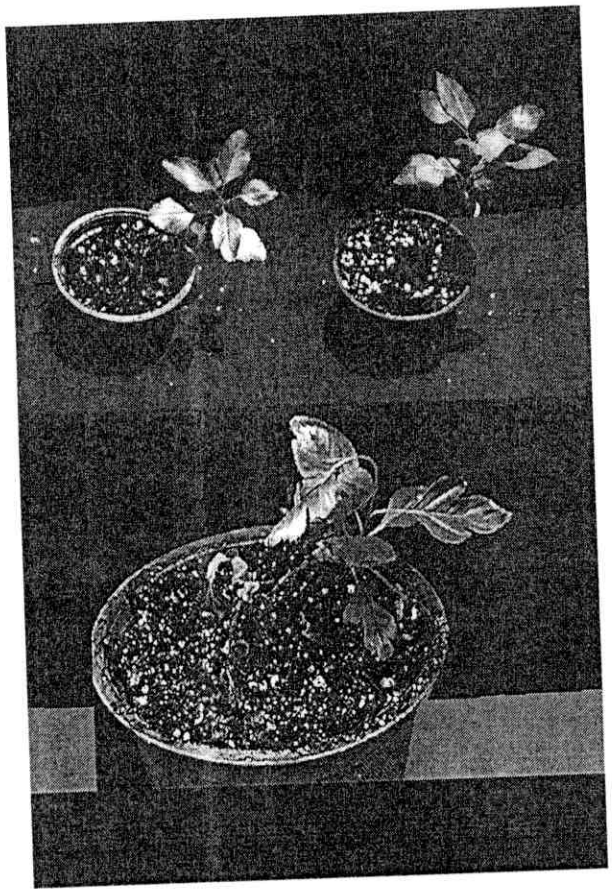


Photo (31): The acclimatization of betulaefolia pear rootstock plantlets.