4- RESULTS

4-1. Micropropagation of apricot plants:

4-1-1. Establishment stage :

4-1-1-1. Effect of nutrient medium:

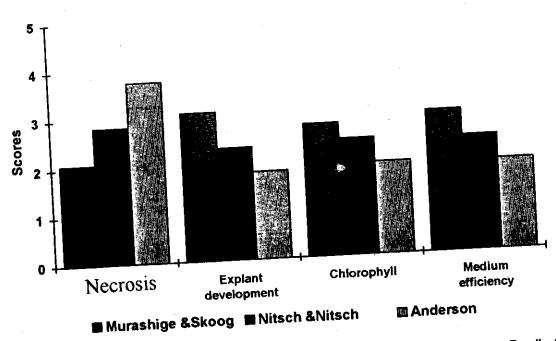
Table (1-A) and Fig. (1) shows the effect of different nutrient media on explant development of apricot explants. It is clear that, necrosis was developed at highest level when Anderson medium was used followed by Nitsch and Nitsch and Murashige and Skoog medium in a descending order. However, Murashige and Skoog medium induced highly significant increase in explant development, chlorophyll and medium efficiency as compared with other used media under study. In contrast, Anderson medium had the poorest effect on all growth criteria of apricot explant.

In addition, it is quite evident from **Table (1-B)** that explant development of Hamawy apricot significantly surpassed Balady explant. On the contrary, necrosis showed highly significant increase in the Balady explant than Hamawy. On the other hand, chlorophyll was not affected from the statistical point of view. Dealing with the effect of the explant, it is clear from **Table (1-C)** and **Fig. (2)** that, shoot-tips had significantly better development in comparison with one-node cuttings explants, however, the interaction between the medium and plant type as shown in **Table (1-D)** and **Fig. (3)** indicate that, Murashige and Skoog medium showed highly significant increase in explant development over other media for both Hamawy and Balady apricot.

Table (1): Effect of different nutrient media and explants on explant development parameters of apricot plants.

1-A: Effect of nutrient medium.

Necrosis explant development		Chiorophyli	Medium efficiency	
2.08	3.08	2.75	2.92	
	2.33	2.42	2.38	
	1.83	1.92	1.88	
3.75		 	0.07	
0.42	0.42	0.53	0.27	
0.57	0.57	0.72	0.36	
	2.08 2.83 3.75	development 2.08	development 2.08 3.08 2.75 2.83 2.33 2.42 3.75 1.83 1.92 0.42 0.42 0.53	



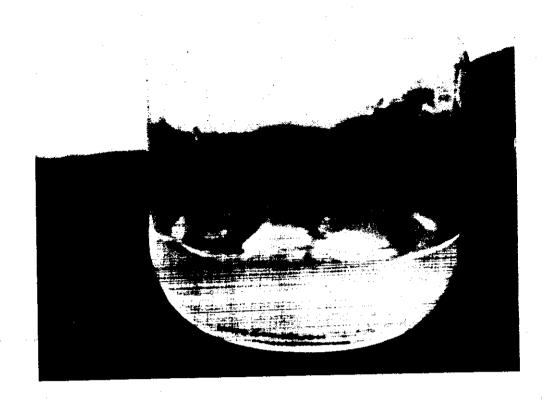
(1): Effect of different nutrient media and explant on explant development parameters of apricot plants Fig.

1-B: Effect of plant type:

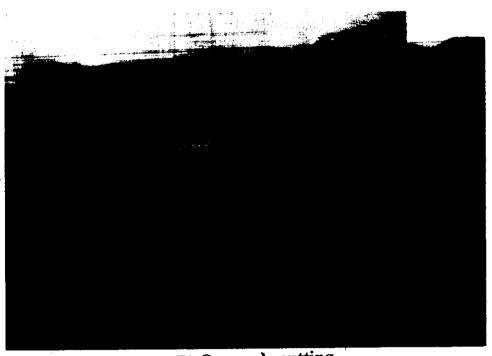
Necrosis	Explant development	Chlorophyll
		2.50
2.56	2.61	2.50
2 22	2.22	2.22
3.22		ļ
0.34	0.34	N.S
0.51	0.51	N.S
	2.56 3.22 0.34	Necrosis development 2.56 2.61 3.22 2.22 0.34 0.34

1-C: Effect of explant.

Explant	Necrosis	Explant development	Chlorophyll
Shoot-tip	2.72	2.67	2.44
One-node cutting	3.06	2.17	2.28
L.S.D. at 0.05	N.S	0.34	N.S
L.S.D. at 0.01	N.S	0.51	N.S



(A) Shoot-tip



(B) One-node cutting

Fig. (2): Effect of different explants [shoot-tip (A) and one-node cutting (B)] on explant development of Hamawy plants.

1-D: Effect of interaction between medium and plant type.

Variety	Necrosis			Explant development		phyli
Medium	Hamawy	Balady	Hamawy	Balady	Hamawy	Balady ————
Murashige and Skoog	1.83	2.33	3.67	2.50	2.83	2.67
Nitsch and Nitsch	2.67	3.00	2.33	2.33	2.67	2.17
Anderson	3.67	2.00	2.00	1.67	1.83	2.00
L.S.D. at 0.05	N.S		0.59		N.S	
L.S.D. at 0.01		ı.s 	0	.80	N.S	

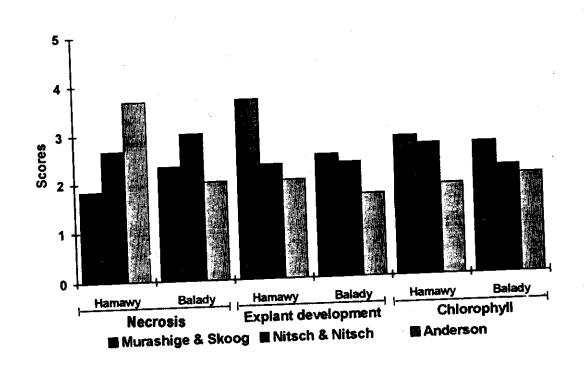


Fig. (3): Effect of interaction between nutrient media and plant type on nicroses, explant development and chlorophyll parameters of apricot plants.

4-1-1-2. Effect of Antioxidant treatments:

Table (2-A) shows the effect of antioxidant treatments on explant development of apricot plants. It is found that, necrosis was reduced with high significancy when the explants were pretreated with antioxidant solution as compared with other Meanwhile, combination of antioxidants solution treatments. pretreament and addition of active charcoal to the medium took the in this respect. Moreover, pretreatment with second rank antioxidant solution enhanced both explant development and chlorophyll with high significancy in relation to other treatments. Considering the effect of variety, it is obvious from Table (2-B) that, Hamawy explants showed slight differences than Balady in both necrosis and chlorophyll, while Hamawy explants gave highly significant increase in explant development in comparison with Dealing with the interaction between antioxidant treatments and plant variety, it is clear from Table (2-C) and Fig. (4) that, interactions between different antioxidant treatments and either Hamawy or Balady plants did not show any significant differences among them. However, interaction between antioxidant solution and Hamawy plants gave the highest significant increase in explant development. However, antioxidant solution treatment for either Hamawy or Balady encouraged the highest significant increase in chlorophyll as compared with the other treatments.

Table (2): Effect of different antioxidant treatments on explant development parameters of apricot plants.

2-A: Effect of antioxidant treatments.

Antioxidant	Necrosis	Explant development	Chlorophyll
	4.92	1.00	1.00
Control Antioxidant solution	3.00	1.83	2.67
Active charcoal	3.92	1.17	1.08
Ant. sol. + Act. Char.	3.66	1.25	1.08
S.D. at 0.05	0.36	0.35	0.31
S.D. at 0.01	0.48	0.47	0.42

2-B: Effect of plant type.

3.80	development 1.50	1.59
3.92	1.12	1.38
N.S	0.25	N.S
	0.33	N.S
	3.92 N.S N.S	N.S 0.25

2-C: Effect of interaction between antioxidant and plant.

Plant	Necrosis		Explant development		Chlorophyll		
	Hamawy	Balady	Hamawy	Balady	Hamawy	Balady	
Antioxidant	4.83	5.00	1.00	1.00	1.00	1.00	
Control	3.00	3.00	2.34	1.33	3.00	2.33	
Antioxidant solution	3,83	4.00	1.33	1.00	1.00	1.17	
Active charcoal Ant. sol. + Act. Char.	3.55	3.83	1.33	1.17	1.33	1.00	
	N.S		0.49		0.44		
L.S.D. at 0.05 L.S.D. at 0.01		N.S N.S		0.66		0.59	

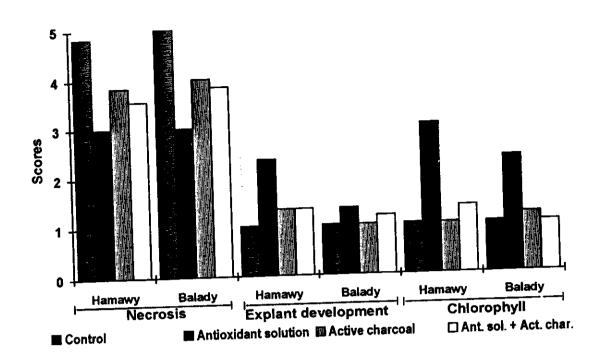


Fig. (4): Effect of interaction between antioxidant and variety on explant development parameters of apricot plants.

4-1-1-3. Effect of medium strength:

It is found from **Table (3-A)** and **Fig. (5)** that, reducing MS medium strength improved survival and growth parameters. In this sphere, one-fourth MS strength induced highly significant less necrosis and highest increase in explant development as well as medium efficiency followed by one-half and one-eighth MS strengths as compared with full MS strength.

Regarding the effect of plants as shown in **Table** (3-B), it is clear that Hamawy apricot had significantly higher necrosis, explant development, chlorophyll and medium efficiency in comparison with Balady apricot.

Table (3): Effect of different Murashige and Skoog medium strengths and explant type on growth of cultured explant and development of apricot plants.

3-A: Effect of medium strength.

Medium strength	Necrosis	explant development	Chlorophyll	Medium efficiency
Full	3.50	1.42	2.08	1.75
One-half	2.17	2.50	1.92	2.21
One-Fourth	1.25	3.50	2.08	2.79
One-eighth	1.50	2.08	1.92	2.00
L.S.D. at 0.05	0.44	0.49	N.S	0.34
L.S.D. at 0.01	0.66	0.66	N.S	0.46

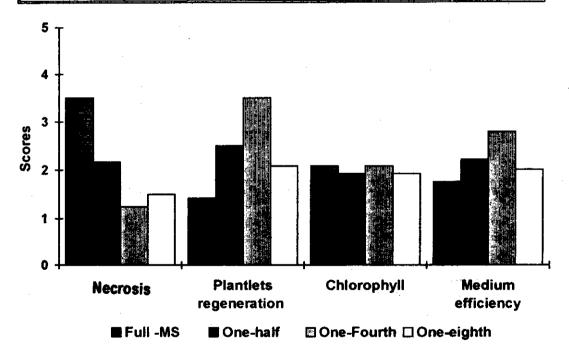


Fig. (5): Effect of different Murashige and Skoog medium strengths and explant type on growth and development of cultured apricot explants.

3-B: Effect of the variety.

Variety	Necrosis	explant development	Chiorophyil	Medium efficiency
Hamawy	3.25	2.96	3.25	3.11
Balady	2.92	2.38	2.92	2.65
L.S.D. at 0.05	0.26	0.30	0.26	0.32
L.S.D. at 0.01	0.66	0.41	0.60	0.44

4-1-1-4. Effect of additives :

Table (4-A) and Fig. (6) involved the effect of additive treatments on explant development of apricot plants. It is obvious that, adenine sulphate induced significantly less necrosis and highest significant increase in explant development and chlorophyll followed by coconut milk and MES hydrolysate in a descending order. However, sphadex treatment was inferior in this respect.

Besides, **Table (4-B)** include the response of apricot plants to additive treatments. It is clear that, Hamawy explants had significantly better growth and proliferation than Balady apricot.

Regarding the effect of the interaction between additives and apricot plants it is clear from **Table (4-C)** and **Fig. (7)** that explant development and chlorophyll increased significantly when adenine sulphate was used with Hamawy explants. Generally, the application of adenine sulphate to Hamawy explants was more promising in increasing survival, growth, proliferation and chlorophyll.

Table (4): Effect of different additives on growth and proliferation of cultured apricot explants.

4-A: Effect of additives.

Necrosis	Growth	Proliferation	Chiorophyll
 	3.50	2.08	3.92
1	2.50	1.92	3.33
1.25	2.00	1.92	3.25
3.50	1.42	2.08	1.67
0.44	0.49	N.S	0.40
0.60	0.66	N.S	0.54
	3.50 0.44	1.50 3.50 2.17 2.50 1.25 2.00 3.50 1.42 0.44 0.49	1.50 3.50 2.08 2.17 2.50 1.92 1.25 2.00 1.92 3.50 1.42 2.08 0.44 0.49 N.S

4-B: Effect of plant variety.

Variety	Necrosis	Necrosis Growth		Chlorophyl	
Hamawy	2.04	2.83	2.50	3.00	
Balady	2.17	1.87	1.50	3.08	
L.S.D. at 0.05	N.S	0.35	0.30	N.S	
L.S.D. at 0.01	N.S	0.47	0.40	N.S	

4-C: Effect of interaction between additive and plant type.

Variety	Necrosis		Gro	wth	Proliferation		Chlorophyli	
Additive	Hamawy	Balady	Hamawy	Balady	Hamawy	Balady	Hamawy	Balady
Adenine sulphate	1.67	1.33	4.17	2.83	2.67	2.00	4.17	3.67
Coconut milk	2.00	2.33	3.33	1.67	2.17	3.67	3.33	3.33
MES hydrolysate	1.17	1.33	2.17	1.78	2.50	1.33	3.17	3.33
Sphadex	3.33	3.67	1.67	1.17	2.67	1.50	1.33	2.00
L.S.D. at 0.05	N	.s	0.	70	N	.S	0.	57
L.S.D. at 0.01	N	.s	0.	94	N	.s	0.	71

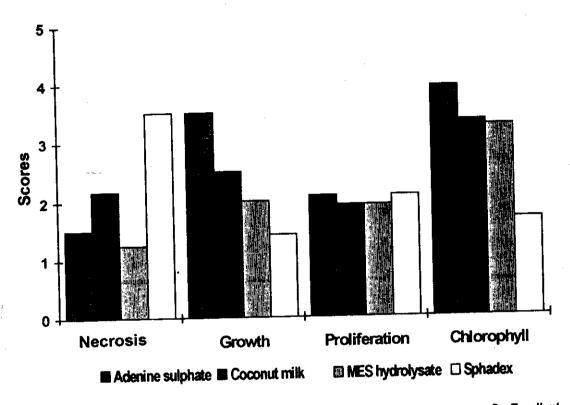


Fig. (6): Effect of different additives on growth and proliferation of cultured apricot explants.

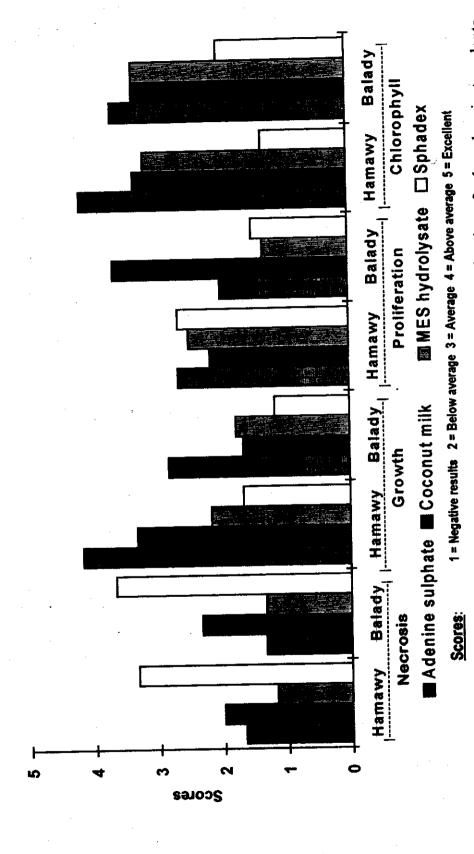


Fig. (7): Effect of interaction between additive and plant variety on growth and proliferation of cultured apricot explants.

4-1-2. Proliferation stage:

4-1-2-1. Effect of Cytokinins and thidiazuron:

Table (5) and Fig. (8-9) shows the effect of different cytokinins at the rate of 2 mg/liter on proliferation of apricot plants. It is found that, thiadiazuron caused significantly highest necrosis as compared with the other cytokinins used in this study. However, 6-benzylaminopurine, followed by kinetin and zeatin caused significantly less nicroses as compared with thidiazuron. On the other hand, growth and chlorophyll were increased significantly when the medium was supplemented by kinetin in comparison with zeatin, 6-benzylaminopurine and thidiazuron, in case of growth, and thidiazuron, only in case of chlorophyll. On the contrary, 6-benzylaminopurine significantly enhanced proliferation in relation to kinetin and thidiazuron. Moreover, 6-benzylaminopurine and zeatin enhanced proliferation with somewhat similar trend without any significant differences between them.

Table (5): Effect of different cytokinin types and thidiazuron on proliferation and growth characters of apricot plants.

Medium supplement	Necrosis	Growth	Proliferation	Chlorophyil
Kinetin	3.17	2.17	2.33	3.17
Zeatin	3.33	2.00	3.33	2.33
BAP	2.83	1.67	4.17	1.67
Thidiazuron	4.17	1.33	1.67	1.33
L.S.D. at 0.05	0.53	0.51	0.71	0.60
L.S.D. at 0.01	0.74	0.71	0,98	0.83

All supplements were applied at the rate of 2 mg/liter.

1-

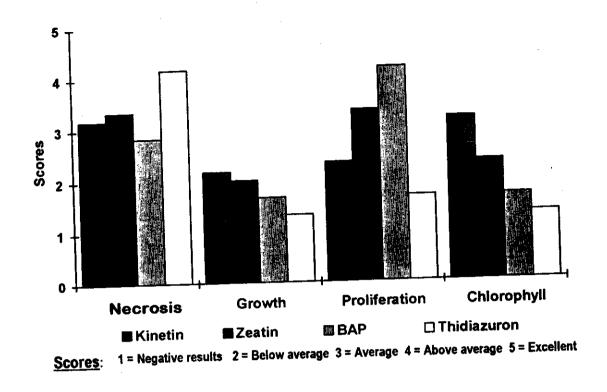
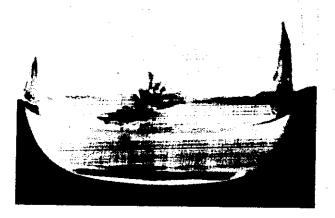


Fig. (8): Effect of different cytokinins and thidiazuron on proliferation and growth characters of apricot plants.

4-1-2-2. Effect of Cytokinin concentrations:

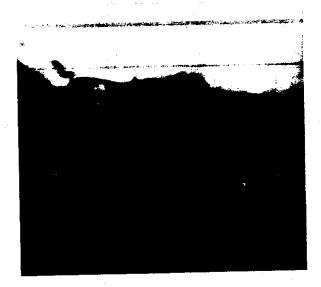
The effect of different concentrations of 6-benzylaminopurine (BAP) on proliferation of apricot plants is presented in **Table (6-A)** and **Fig. (10-11).** It is quite evident that, lower concentrations of 6-benzylaminopurine (0.5 mg/L.) significantly reduced both necrosis and proliferation. However, increasing the concentration from 2 to 4 mg/L. resulted in a significant increase in both proliferation and nicroses. On the other hand, growth and chlorophyll were significantly increased when 0.5 mg/L. BAP was used in comparison with higher concentrations. (2 & 4 mg/L.). In contrast, supplementing the medium with 2 & 4 mg/L. BAP significantly increased proliferation in relation to the other concentrations.



Kinetin



Zeatin



6-benzylaminopurine



thidiazuron

Fig. (9): Effect of different cytokinins and thidiazuron on proliferation of Hamawy plants.

Table (6): Effect of different concentrations of 6-benzylaminopurine (BAP) on proliferation and growth characters of apricot plants.

Necrosis	Growth	Proliferation	Chlorophyll
1.50	3.50	1.83	3.33
2.83	2.17	3.33	2.83
2.83	1.67	4.17	1.67
3.50	1.50	4.17	1.67
0.61	0.56	0.58	0.73
0.85	0.91	0.81	1.02
	1.50 2.83 2.83 3.50 0.61	1.50 3.50 2.83 2.17 2.83 1.67 3.50 1.50 0.61 0.56	Necrosis 3.50 1.50 3.50 2.83 2.17 3.33 2.83 1.67 4.17 3.50 1.50 4.17 0.61 0.56 0.58

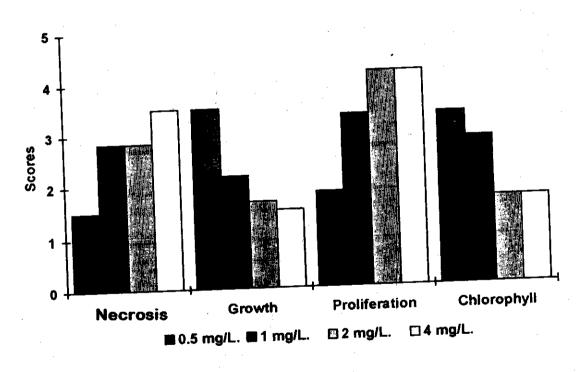


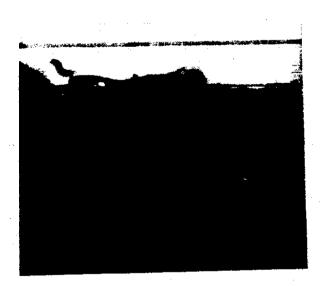
Fig. (10): Effect of different concentrations of 6-benzylaminopurine (BAP) on proliferation and growth characters of apricot plants.



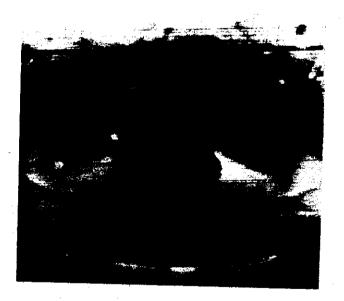
0.5 mg/L.



1.0 mg/L.



2.0 mg/L.



4.0 mg/L.

Fig. (11): Effect of different concentrations of 6-benzylaminopurine on proliferation of Hamawy plants.

Regarding the effect of thidiazuron with different concentrations on proliferation and growth characters of apricot plants. It is obvious from **Table** (7) and **Figs.** (12-13) that, necrosis increased significantly with increasing thidiazuron level. However, the use of 0.5 mg/L. significantly increased growth, while 0.5 and 1 mg/L. of thidiazuron significantly improved chlorophyll and enhanced proliferation in comparison with other concentrations used.

Table (7): Effect of different concentrations of thidiazuron on proliferation and growth characters of apricot plants.

Concentration	Necrosis	Growth	Proliferation	Chlorophyll
mg/L 0.5	1.50	2.50	4.33	2.17
1.0	2.17	2.00	4.50	1.83
2.0	4.17	1.33	1.83	1.33
4.0	5.00	1.00	1.00	1.00
L.S.D. at 0.05	0.42	0.51	0.49	0.47
L.S.D. at 0.01	0.57	0.70	0.69	0.66

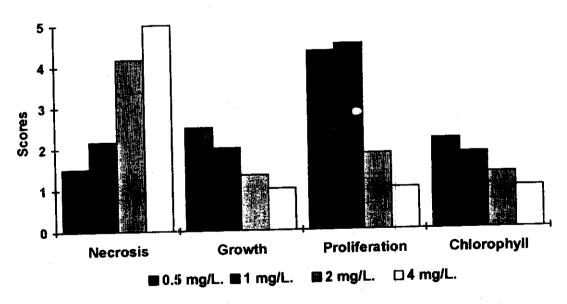
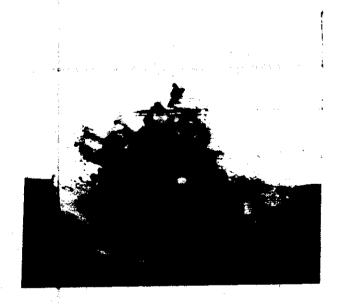


Fig. (12): Effect of different concentrations of thidiazuron on proliferation and growth characters of apricot plants.



0.5 mg/L.



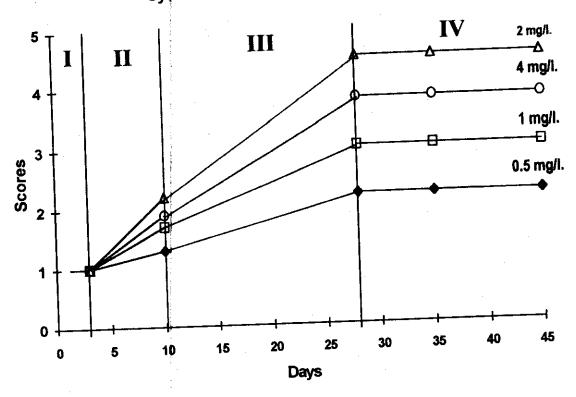
1.0 mg/L.

Fig. (13): Effect of different concentrations of thidiazuron on proliferation and growth characters of Hamawy plants.

4-1-2-3. Proliferation curve :

Figure (14) shows that, there is a direct relationship between proliferation of apricot and the increase in concentration of However, increasing different cytokinins up to 2 mg/l. concentration of cytokinins up to 4 mg/l. led to a decrease in proliferation as compared with 2 mg/l. level. Besides, different concentrations of cytokinins behaved somewhat similarly during different phases on the proliferation curve. Anyhow, the first phase of proliferation curve (lag phase) extended for about 3 days from culturing time where no signs of proliferation were noticed. In the second phase (log phase) proliferation started to increase and ended after 10 days from culturing time. Such increase was followed by a faster proliferation during the third phase (progressive phase) which continued up to 28 days from culturing time. Thus, subculturing process should be done at the end of this stage to increase the efficiency of the proliferation process. After stationary phase was appeared and it is not 28 recommended to let the explant to reach this phase. Accordingly, 2.0 mg/l. cytokinin level was the most promising concentration for increasing proliferation of apricot plants visually up to 30 days from subculturing.

Cytokinin concentrations (mg/L.)



I: Lag phase

III : Progressive phase

II : Log phase

IV: Stationary phase

Fig. (14): Proliferation curve of apricot plants under different concentrations of cytokinin.

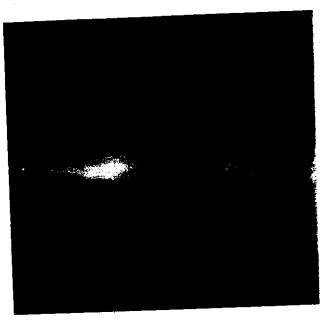
4-1-3. Rooting stage:

4-1-3-1. Effect of different concentrations of GA₃:

Table (8) and Fig. (15 & 16) show the effect of different concentrations of gibberellic acid (GA₃) on shoot elongation, chlorophyll and root primordia of apricot plants. It is clear that, shoot length was increased significantly with increasing GA₃ concentration up to 2.0 mg/liter as compared with the lower concentrations of GA₃ i.e. 0.0, 0.5, and 1.0 mg/liter. However, higher GA₃ concentrations in the media had a harmful effect on chlorophyll and root primordia of the explants.



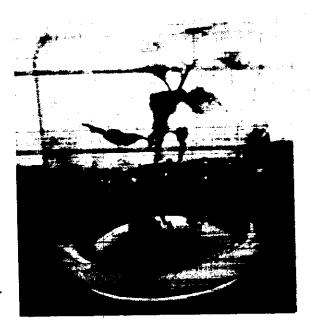
(A) 0.5 mg/L.



(B) 1.0 mg/L.



(C) 2.0 mg/L.



(D) 4.0 mg/L.

Fig. (16): Effect different concentrations of gibberellic acid (GA₃) on shoot elongation, chlorophyll and root primordia of Hamawy plants.

4-1-3-2. Effect of medium strength:

Table (9) and Fig. (17 & 18) show the effect of different Murashige and Skoog medium strengths on shoot elongation, chlorophyll and rooting of apricot plants. It is found that, one-fourth and one-eighth MS strengths encouraged significantly shoot length and rooting as compared with full MS strength. On the other hand, one-half strength surpassed significantly all other strengths used in its effect on chlorophyll.

Table (9): Effect of different Murashige and Skoog medium strengths on shoot length, chlorophyll and rooting of apricot plants.

Medium strength	Shoot length	Chlorophyll	Rooting	
Full -MS	1.33	2.00	1.00	
One-half	1.67	3.17	1.33	
One-Fourth	2.50	2.33	1.83	
One-eighth	2.33	1.83	2.17	
L.S.D. at 0.05	0.74	0.80	0.48	
L.S.D. at 0.01	1.03	1.11	0.66	

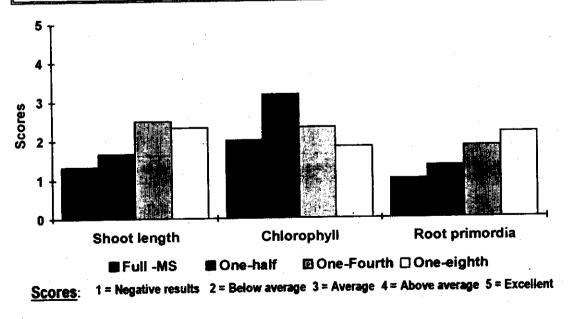


Fig. (17): Effect of different Murashige and Skoog medium strengths on shoot length, chlorophyll and rooting of apricot plants.



One-half medium strength



One-fourth medium strength

Fig (18): Effect of different Murashige and Skoog medium strengths on shoot length, chlorophyll and rooting of Hamawy plants.

4-1-3-3. Effect of auxin types and concentrations:

Table (10-A) shows the effect of different auxin types on rooting of apricot plants. It is obvious that, data slightly differed without any significancy in all criteria under study. Regarding, the effect of auxin concentration on rooting as shown in Table (10-B) and Figs. (19 & 20), it is obvious that, 2.0 and 4.0 mg/liter concentrations increased callus with high significancy as compared to the lower concentrations. Generally, lower auxin concentrations profoundly increased growth and chlorophyll (i.e. 0.5 and 1.0 mg/liter). Moreover, 1.0 and 2.0 mg/l levels increased rooting with high significancy.

Table (10): Effect of different auxin types and concentrations on growth and rooting of apricot plants.

10-A:	Effect	of	auxin:
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Auxin	Callus	Shoot Growth	Chlorophyll	Rooting
IBA	2.13	2.83	2.88	0.71
NAA	2.04	2.63	2.88	0.45
L.S.D. at 0.05	N.S	N.S	N.S	N.S
L.S.D. at 0.01	N.S	N.S	N.S	N.S

10-B: Effect of concentration:

Concentration mg/L	Callus	Shoot Growth	Chlorophyll	Rooting
0.5	1.00	3.33	3.25	1.08
1.0	1.42	3.17	3.25	3.42
2.0	2.33	2.50	2.75	3.58
4.0.	3.58	1.92	2.25	2.25
.S.D. at 0.05	0.39	0.39	0.44	0.43
.S.D. at 0.01	0.52	0.52	0.60	0.58

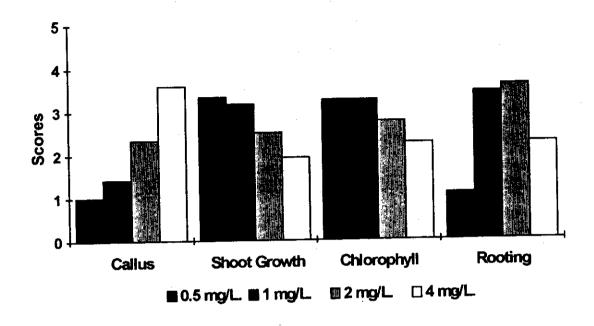


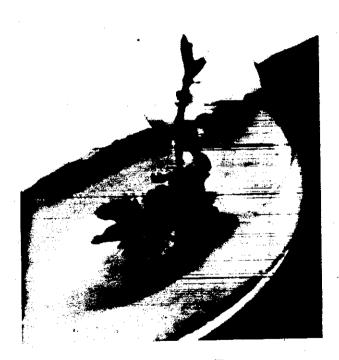
Fig. (19): Effect of different auxin types and concentrations on rooting and growth of apricot plants.



(A) 1.0 mg/L.



(B) 2.0 mg/L.



(C) 4.0 mg/L.

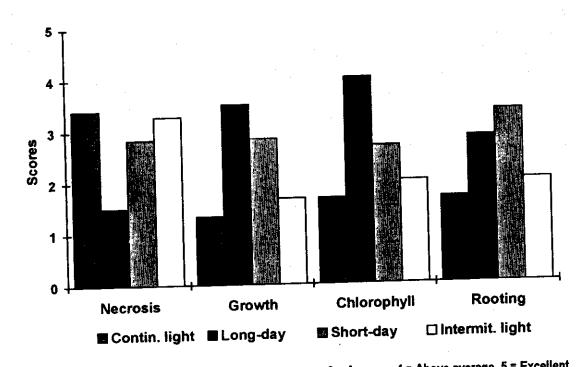
Fig (20): Effect of different auxin concentrations [1 mg/L. (A), 2 mg/L. (B) and 4 mg/L. (C)] on rooting and growth characters of Hamawy plants.

4-1-3-4. Effect of photoperiod:

Table (11) and Fig. (21) show the effect of different photoperiods on growth and rooting of apricot plants. It is found that, short days significantly increased rooting followed by long days and intermittent light in a descending order. However, continous light significantly increased necrosis followed by intermittent light as compared with the other photoperiods used. Furthermore, longdays induced less nicroses significantly and highest significant increase in shoot growth and chlorophyll in comparison with the other used photoperiods.

Table (11): Effect of different photoperiods on growth and rooting of apricot plants.

Photoperiod	Necrosis	Growth	Chlorophyll	Rooting
Continuous light	3.40	1.33	1.67	1.67
Long-day	1.50	3.50	4.00	2.83
Short-day	2.83	2.83	2.67	3.33
Intermittent light	3.27	1.67	2.00	2.00
L.S.D. at 0.05	0.67	0.78	0.41	0.67
L.S.D. at 0.01	1.01	1.18	0.62	1.01



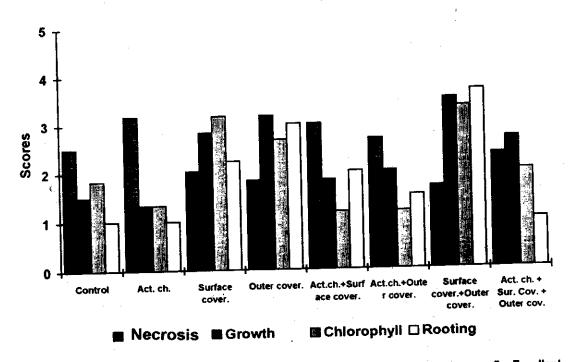
Scores: 1 = Negative results 2 = Below average 3 = Average 4 = Above average 5 = Excellent Fig. (21): Effect of different photoperiods on shoot growth and rooting of apricot plants.

4-1-3-5. Effect of etiolation:

The effect of etiolation treatments on growth and rooting of apricot plants is presented in **Table (12)** and **Figs. (22 & 23)**. It is quite evident that, necrosis was reduced with high significancy when combination of surface and outer coverage treatments were applied in comparison with the addition of activated charcoal treatment. However, using of outer coverage treatment or surface coverage or a combination of both treatments, resulted in highly significant increase of growth, chlorophyll and rooting in relation to the control and the addition of the activated charcoal treatment. On the other hand, addition of the activated charcoal (either alone or in combination with other treatments resulted in increased necrosis and decreased growth, chlorophyll and rooting.

Table (12): Effect of etiolation treatments on growth and rooting of apricot plants.

Etiolation	Necrosis	Growth	Chlorophyll	Rooting
Control	2.50	1.50	1.83	1.00
Active charcoal	3.17	1.33	1.33	1.00
Surface coverage	2.03	2.83	3.17	2.23
Outer coverage	1.83	3.17	2.67	3.00
Active charcoal + Surface coverage	3.00	1.83	1.17	2.00
Active charcoal + Outer coverage	2.67	2.00	1.17	1.50
Surface coverage + Outer coverage	1.67	3.50	3.33	3.67
Act. ch. + Sur. Cov. + Outer cov.	2.33	2.67	2.00	1.00
L.S.D. at 0.05	0.68	0.69	0.55	0.64
L.S.D. at 0.01	0.91	0.92	0.74	0.86



Scores: 1 = Negative results 2 = Below average 3 = Average 4 = Above average 5 = Excellent

Fig. (22): Effect of etiolation treatments on growth characters and rooting of apricot plants.



Active charcoal

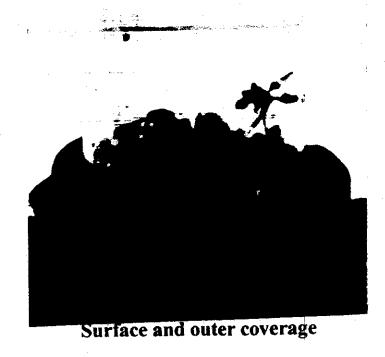


Fig (23): Effect of etiolation treatments on growth characters and rooting of Hamawy plants.



Root initiation



Complete root development

Fig (24): Development of rooting of Hamawy plants.

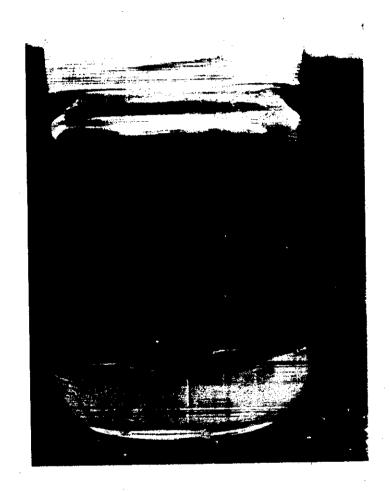


Fig (25): The final shape of Hamawy plants.

4-2. Micropropagation of peach plants:

4-2-1. Establishment stage:

4-2-1-1. Effect of nutrient medium:

Table (13-A) shows the effect of different nutrient media on development of peach explants. It is quite evident that Murashige and Skoog medium significantly developed the least amount of necrosis as compared with Nitsch and Nitsch and Anderson media. However, Murashige and Skoog medium induced highly significant increase in explant development, chlorophyll and medium efficiency as compared with other used media under study. Anderson medium had the poorest effects on all growth criteria of peach explant.

In addition, it is obvious from Table (13-B) that, explant development, chlorophyll and medium efficiency for Nemaguard peach were significantly enhanced more than Okinawa. However, significantly more necrosis was developed with Okinawa explants. Explant development of Nemaguard and Okinawa peach was presented in Table (13-C). It is clear that, shoot-tips had significantly better explant development, chlorophyll and medium efficiency in comparison with one-node cuttings. However, onenode cuttings had significantly higher necrosis in comparison with shoot-tip explants. The interaction between medium and explant as shown in Table (13-D) and Fig. (26) show a highly significant increase in explant development in relation to Murashige and Skoog medium followed by Anderson and Nitsch and Nitsch media in a descending order. Besides, the interaction between peach variety and explant type indicated a highly significant increase in Nemaguard explant development (Table, 13-E and Fig. 27). Generally, Murashige and Skoog medium for Nemaguard shoot-tip explants were superior in growth and survival parameters.

Table (13): Effect of different nutrient media and explants on explant development of peach plants.

13-A: Effect of nutrient medium.

Medium	Necrosis	Explant development	Chiorophyli	Medium efficiency
Murashige & Skoog	1.92	3.08	2.75	2.92
Nitsch & Nitsch	3.17	2.25	2.33	2.29
Anderson	3.83	1.92	1.83	1.87
L.S.D. at 0.05	0.33	0.34	0.43	0.35
L.S.D. at 0.01	0.44	0.46	0.59	0.48

13-B: Effect of variety.

Variety	Necrosis	Explant development	Chlorophyll	Medium efficiency
Nemaguard	2.22	2.67	2.50	2.59
Okinawa	3.72	2.17	1.83	2.00
L.S.D. at 0.05	0.27	0.28	0.35	0.29
L.S.D. at 0.01	0.36	0.33	0.42	0.34

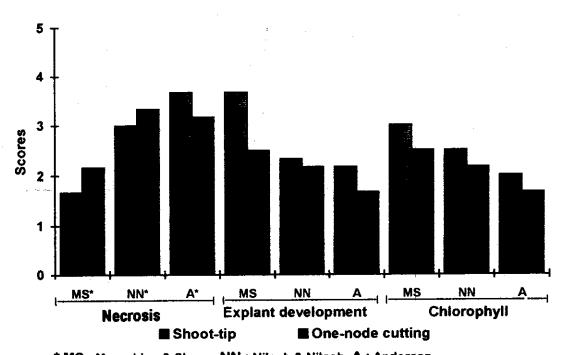
13-C: Effect of explant:

Explant	Necrosis	Explant development	Chlorophyll	Medium efficiency
Shoot-tip	2.78	2.72	2.56	2.64
One-node cutting	3.17	2.11	2.06	2.08
L.S.D. at 0.05	0.27	0.28	0.35	0.29
L.S.D. at 0.01	0.36	0.33	0.42	0.34

13-D	:	Effect	of	interaction	between	medium	and	explant.
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Medium	Necrosis		Explant development			Chlorophyll			
Explant	MS*	NN*	Α*	MS	NN	A	MS	NN	A
Shoot-tip	1.67	3.00	3.67	3.67	2.33	2.17	3.00	2.50	2.00
One-node cutting	2.17	3.33	3.17	2.50	2.17	1.67	2.50	2.17	1.67
L.S.D. at 0.05	N.S		0.48		N.S				
L.S.D. at 0.01	N.S		0.65		N.S				

* MS: Murashige & Skoog, NN: Nitsch & Nitsch, A: Anderson



* MS : Murashige & Skoog, NN : Nitsch & Nitsch, A : Anderson

Fig. (26): Effect of interaction between medium type and explant on explant development parameters of peach plants.

13-E: Effect of interaction between variety and explant.

Variety	Necrosis		Explant development		Chlorophyll		
Explant	Nemaguard	Okinawa	Nemaguard	Okinawa	Nemaguard	Okinawa	
Shoot-tip	2.00	3.55	3.11	2.33	3.11	2.00	
One-node cutting	2.44	3.89	2.12	2.00	2.44	1.67	
L.S.D. at 0.05	N	N.S		0.39		N.S	
L.S.D. at 0.01	N.S		0.46		N.S		

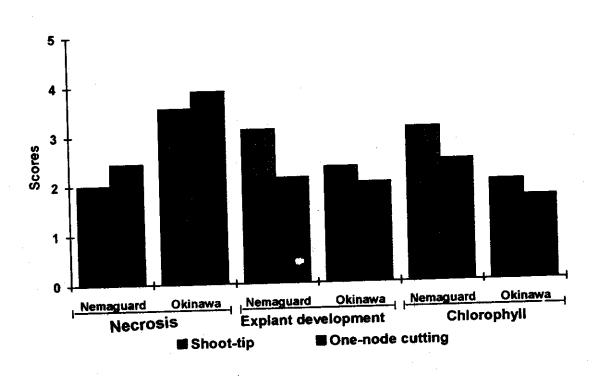


Fig. (27): Effect of interaction between variety and explant on explant development of peach plants.

4-2-1-2. Effect of Antioxidant treatments:

Dealing with the effect of antioxidant treatments, **Table (14-A)** and **Fig. (28)** explain that, necrosis was reduced with high significancy when the explants were pretreated with antioxidant solution as compared with the other treatments. Also, both antioxidant solution and antioxidant solution plus active charcoal significantly increased explant development and chlorophyll.

Considering the effect of variety, it is obvious from **Table** (14-B) that, Nemaguard peach showed slight differences than Okinawa in necrosis. While, Nemaguard gave the highest significant increase in explant development and chlorophyll in comparison with Okinawa.

Table (14): Effect of different antioxidant treatments on explant development parameters of peach plants.

14-A: Effect of antioxidant	treatments.
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Antioxidant	Necrosis	Explant development	Chlorophyll
Control	3.50	1.42	1.00
Antioxidant solution	1.25	3.50	1.83
Active charcoal	2.17	2.08	1.17
Ant. sol. + Act. char.	1.50	2.50	1.25
.S.D. at 0.05	0.44	0.49	0.35
L.S.D. at 0.01	0.66	0.66	0.47

14-B: Effect of variety.

Variety	Necrosis	Explant development	Chlorophyll
Nemaguard	2.64	2.88	1.50
Okinawa	2.17	1.88	1.12
L.S.D. at 0.05	N.S	0.35	0.25
L.S.D. at 0.01	N.S	0.54	0.33

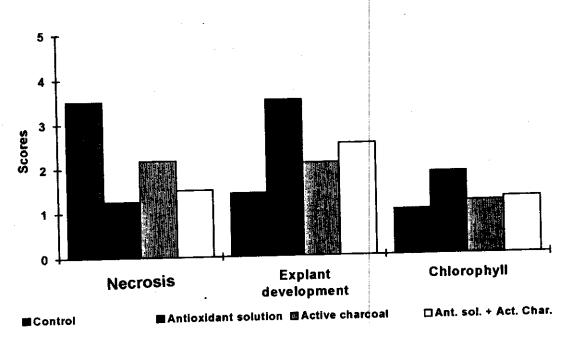


Fig. (28): Effect of different antioxidant treatments on explant development of peach plants.

4-2-1-3. Effect of medium strength:

It is found from Table (15-A) and Figs. (29 & 30) that reducing MS medium strength improved survival and growth parameters. In this sphere, one-fourth strength induced highly significant decrease in necrosis and highest increase in growth, chlorophyll and medium efficiency followed by half-strength as compared with the other strengths.

Table (15): Effect of different Murashige and Skoog medium strengths and explant type on growth of cultured of peach explants.

15-A: Effect of medium strength.

Medium strength	Necrosis	Growth	Chlorophyll	Medium efficiency
Full -MS	2.42	1.33	1.92	1.63
One-half	2.17	2.50	2.17	2.34
One-Fourth	1.92	3.83	2.42	2.12
One-eighth	2.33	1.83	2.33	2.08
L.S.D. at 0.05	0.34	0.43	0.34	0.36
L.S.D. at 0.01	0.78	0.57	0.78	0.49

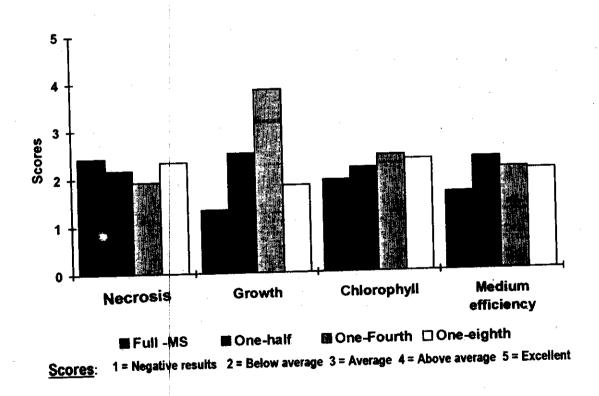


Fig. (29): Effect of different Murashige and Skoog medium strengths and explant type on growth and development of peach explants.



(A) One-half medium strength



(B) One-fourth medium strength

Fig. (30): Effect of medium strength [one-half medium strength (A) and one-fourth medium strength (B)] on growth of cultured Nemaguard explants.

In addition, **Table** (15-B) and **Fig.** (31) showed that, growth of cultured explants, chlorophyll and medium efficiency were enhanced significantly for Nemaguard more than Okinawa. On the contrary, necrosis was much higher for Okinawa than Nemaguard. Regarding the effect of explant type as shown in **Table** (15-C) and **Fig.** (32) it is clear that, shoot-tip explants shows slight differences than one-node cuttings in both growth and chlorophyll, while one-node cutting explants showed significantly higher necrosis than shoot-tip explants.

15-B: Effect of the variety.

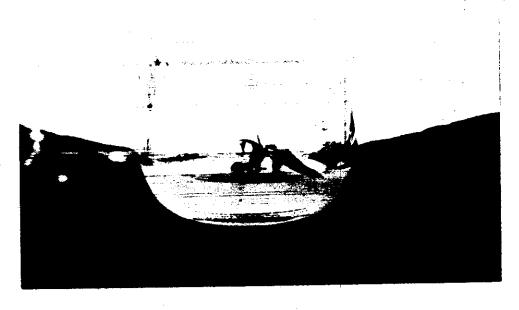
Variety	Necrosis Growth		Chiorophyli	Medium efficiency	
Nemaguard	1.91	2.67	2.50	2.56	
Okinawa	2.50	2.08	1.92	1.97	
L.S.D. at 0.05	0.24	0.30	0.24	0.26	
L.S.D. at 0.01	0.55	0.40	0.55	0.34	

15-C: Effect of the explant.

Explant	plant Necrosis Growth		Chlorophyli	Medium efficiency
Shoot-tip	1.88	2.63	2.54	2.26
one-node cutting	2.54	2.13	1.88	2.34
L.S.D. at 0.05	0.24	0.30	0.24	N.S
L.S.D. at 0.01	0.55	0.40	0.55	N.S



(A) Nemaguard

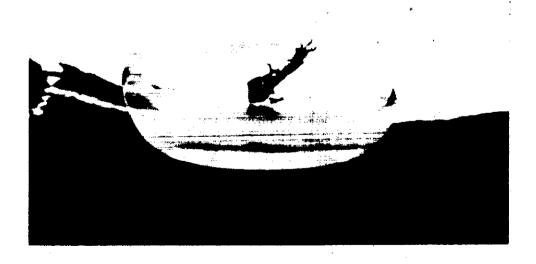


(B) Okinawa

Fig. (31): Effect of variety of peach Nemaguard (A) and Okinawa (B) on growth of cultured explants.



(A) Shoot-tip



(B) One-node cutting

Fig. (32): Effect of different explants [shoot-tip (A) and one-node cutting (B)] of Nemaguard peach plants on growth of cultured explants..

4-2-1-4. Effect of additives:

The effect of additive treatments on growth of cultured peach explants is presented in **Table (16-A)** and **Figs. (33, 34 & 35)**. It is obvious that, adenine sulphate induced higher significant decrease in necrosis, while plantlet regeneration and chlorophyll were promoted. Coconut milk and MES hydrolysate treatments followed adenine sulphate in a descending order. But, sphadex treatment failed to give good result in this respect.

Moreover, it is safe to notice from **Table (16-B)** that, Nemaguard showed significant increase in growth and chlorophyll in comparison with Okinawa.

Table (16): Effect of different additives on growth and proliferarion of cultured peach explants.

16-A: Effect of additives.

Additive	Necrosis	Growth	Proliferation	Chlorophyll
Adenine sulphate	1.83	3.83	1.33	3.83
Coconut milk	2.50	3.00	1.50	3.25
MES hydrolysate	2.25	2.33	1.25	2.58
Sphadex	2.58	1.50	1.17	2.33
L.S.D. at 0.05	0.40	0.40	N.S	0.42
L.S.D. at 0.01	0.54	0.54	N.S	0.56

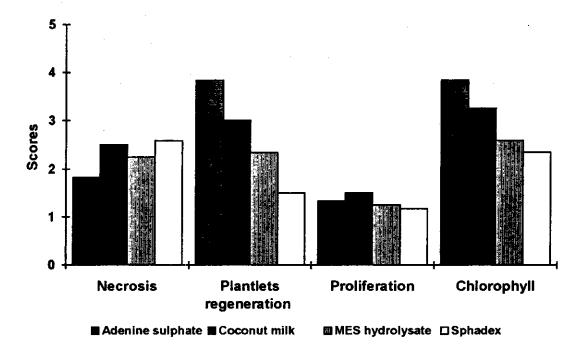


Fig. (33): Effect of different additives on growth proliferation of cultured peach explants.

16-B: Effect of the variety.

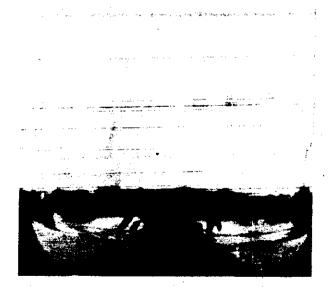
Variety	Necrosis	Growth	Proliferation	Chlorophyll
Nemaguard	2.21	2.88	1.42	3.17
Okinawa	2.42	2.46	1.21	2.83
L.S.D. at 0.05	N.S	0.28	N.S	0.29
L.S.D. at 0.01	N.S	0.38	N.S	0.40





MES hydrolysate

Coconut milk



Adenine sulphate

Fig. (34): Effect of different additives on growth of Nemaguard plants.

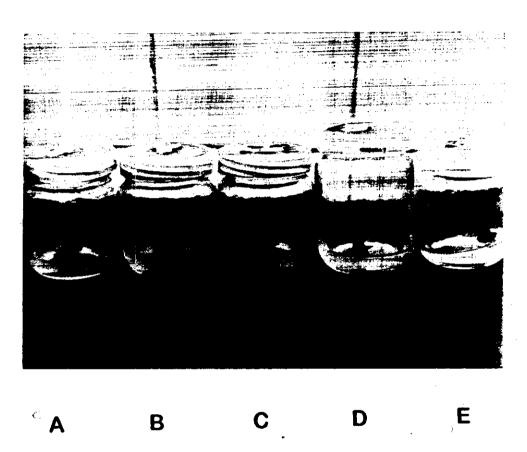


Fig. (35): Development of Nemaguard explant during establishment stage.

4-2-2. Proliferation stage:

4-2-2-1. Effect of Cytokinins and thidiazuron:

Table (17-A) and Figs. (36 & 37) show the effect of different cytokinins and thidiazuron at the rate of 2 mg/liter. It is clear that, thiadiazuron significantly increased necrosis as compared with the other cytokinins used in this study. Meanwhile, BAP induced a significant increase in proliferation among the other used cytokinins. Zeatin induced significant increase in growth and chlorophyll followed by kinetin, BAP and thidiazuron in a descending order. On the other hand, BAP was superior in its effect on proliferation. Regarding the response of variety as shown in Table (17-B) it is found that, Nemaguard surpassed significantly Okinawa in their response to cytokinin treatment.

Table (17): Effect of different cytokinins and thidiazuron on proliferation and growth characters of peach plants.

17-A:	Effect of	cytokinins	and	thidiazuron	:
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Medium supplement	Necrosis	Growth	Proliferation	Chlorophyll
Kinetin	2.17	1.84	2.67	2.33
Zeatin	2.00	2.50	2.83	3.17
BAP	1.00	1.67	4.33	2.50
Thidiazuron	4.00	1.00	1.83	1.17
L.S.D. at 0.05	0.24	0.25	0.22	0.22
L.S.D. at 0.01	0.55	0.33	0.52	0.30

All supplements were applied at the rate of 2 mg/liter.

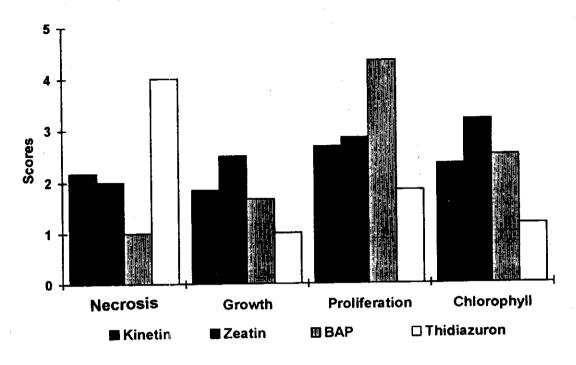
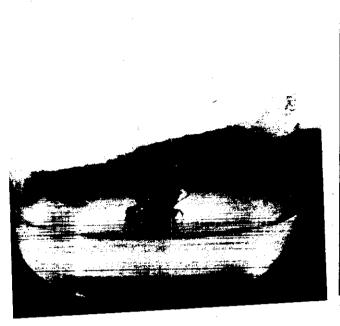


Fig. (36): Effect of different cytokinin types and thidiazuron on proliferation and growth characters of peach plants.

17-B: Effect of variety.

Variety	Necrosis	Growth	Proliferation	Chlorophyll
Nemaguard	1.98	3.06	2.92	2.92
Okinawa	2.04	2.81	2.67	2.46
L.S.D. at 0.05	N.S	0.20	0.24	0.19
L.S.D. at 0.01	N.S	0.34	0.56	0.31





Kinetin

Zeatin



6-benzylaminopurine



Thidiazuron

Fig. (37): Effect of different cytokinins and thidiazuron on proliferation of Nemaguard plants.

4-2-2. Effect of Cytokinin concentrations:

The effect of different concentrations of 6-benzylaminopurine (BAP) on proliferation of peach plants is presented in **Table (18-A)** and **Figs. (38 & 39).** It is quite evident that, necrosis was significantly increased with increasing BAP concentration from 0.5 mg/liter to 4 mg/liter. Thus, it is easy to conclude that, 1 and 2 mg/liter concentrations are superior in their effect on proliferation as compared with the other concentrations used. However, lower concentrations induced better growth and chlorophyll. Considering the effect of variety, it is cleat from **Table (18-B)** that, Nemaguard gave significantly better proliferation and chloropyll as compared with Okinawa.

Table (18): Effect of different concentrations of 6-benzylaminopurine (BAP) on proliferation and growth characters of peach plants.

18-A: Effect of concentration:

Concentration mg/L	Necrosis	Growth	Proliferation	Chlorophyll
0.5	1.75	3.50	1.75	3.92
1.0	2.17	2.17	3.50	3.50
2.0	2.92	1.67	3.92	1.75
4.0	3.50	1.50	3.17	2.17
L.S.D. at 0.05	0.63	0.66	0.63	0.63
L.S.D. at 0.01	0.85	0.91	0.85	0.96

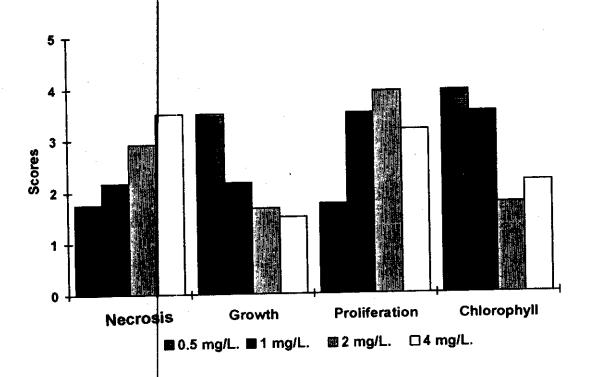


Fig. (38): Effect of different concentrations of 6-benzylaminopurine
(BAP) on proliferation and growth characters of peach plants.

18-B: Effect of variety.

Variety	Necrosis	Growth	Proliferation	Chlorophyll
Nemaguard	3.21	2.00	3.25	3.25
Okinawa	2.96	2.42	2.92	2.92
L.S.D. at 0.05	N.S	N.S	0.26	0.26
L.S.D. at 0.01	N.S	N.S	0.60	0.66



2 mg/L.



4 mg/L.

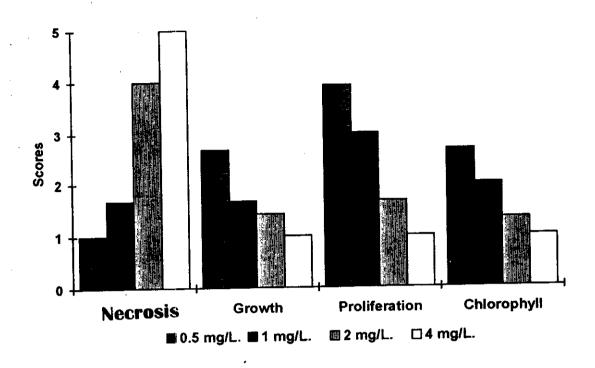
Fig. (39): Effect of different concentrations of 6-benzylaminopurine (BAP) on proliferation and growth characters of Nemaguard plants.

Moreover, it is obvious from **Table (19-A)** and **Figs. (40 & 41)** that less necrosis was significantly obtained with decreasing thidiazuron level. However, lower concentrations of thidiazuron greatly increased growth, proliferation and chlorophyll (i.e. 0.5 and 1.0 mg/liter) in comparison with the higher concentrations. Regarding the effect of variety, it is clear that Nemaguard gave significantly better proliferation as compared with Okinawa (**Table, 19-B**).

Table (19): Effect of different concentrations of thidiazuron on proliferation and growth characters of peach plants.

19-A: Effect of concentration.

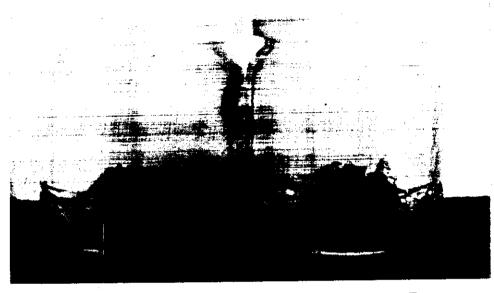
Concentration mg/L	Necrosis	Growth	Proliferation	Chlorophyll
0.5	1.00	2.67	3.92	2.67
1.0	1.67	1.67	3.00	2.00
2.0	4.00	1.42	1.67	1.33
4.0	5.00	1.00	1.00	1.00
L.S.D. at 0.05	0.42	0.44	0.37	0.47
L.S.D. at 0.01	0.57	0.60	0.51	0.66



Scores: 1 = Negative results 2 = Below average 3 = Average 4 = Above average 5 = Excellent
 Fig. (40): Effect of different concentrations of thidiazuron on proliferation and growth characters of peach plants.

19-B: Effect of variety.

Variety	Necrosis	Growth	Proliferation	Chlorophyll
Nemaguard	3.17	1.75	2.88	1.67
Okinawa	3.25	1.96	2.42	1.50
L.S.D. at 0.05	N.S	N.S	0.27	N.S
L.S.D. at 0.01	N.S	N.S	0.36	N.S



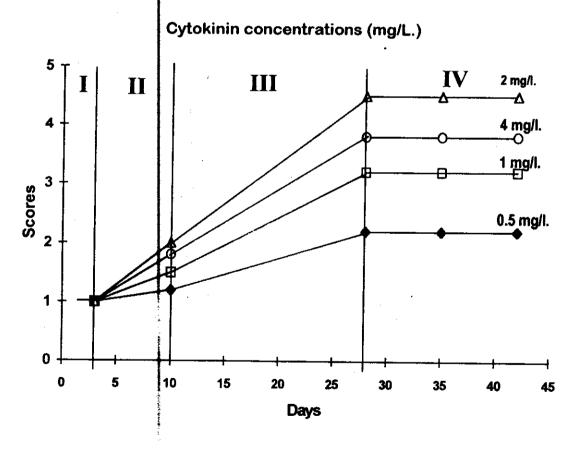
(A) 1 mg/L.

(B) 0.5 mg/L.

Fig. (41): Effect of different concentrations [1 mg/L. (A) and 0.5 mg/L. (B)] of thidiazuron on proliferation and growth characters of Nemaguard plants.

4-2-2-3. Proliferation curve

Figure (42) shows the different phases as distinguished on the proliferation curve of peach plants during the period from the begining of the culturing time to its end. During the first 3 days from subculturing (lag phase) no proliferation was found, followed by a period of increased proliferation (log phase) for 7 days. A rapid increase in proliferation curve (progressive phase) started and completed after 28 days from culturing time (third phase). The last phase, fourth one is the stationary phase and it is not recommended to let the explant to reach this phase. Therefore, the suitable time for subculturing should be done after 28 days. Meanwhile, proliferation increased by increasing the concentration of cytokinins up to 2 mg/l. Proliferation decreased by increasing the concentration to 4 mg/l. afterwards.



II: Log phase

IV: Stationary phase

Fig. (42): Proliferation curve of peach plants under different concentrations of cytokinin.

III: Progressive phase

I: Lag phase

4-2-3. Rooting stage:

4-2-3-1. Effect of different concentrations of GA₃:

The effect of different concentrations of gibberellic acid (GA₃) on shoot elongation, chlorophyll and root primordia of peach plants is shown in **Table (20)** and **Figs. (43 & 44)**. It is clear that, shoot length was increased significantly with increasing GA₃ concentrations up to 2.0 and 4.0 mg/liter as compared to the lower concentrations i.e. 0.5, and 1.0 mg/liter. However, chlorophyll and root primordia increased significantly with lower GA₃ concentrations (0.5 and 1.0 mg/L.).

Table (20): Effect of different concentrations of gibberellic acid (GA₃) on shoot elongation, chlorophyll and root primordia of peach plants.

GA ₃ Concentration mg/L	Shoot length	Chlorophyll	Root primordia
0.5	1.83	2.83	2.56
1.0	2.67	3.50	2.50
2.0	3.67	2.50	1.67
4.0	3.17	1.67	1.33
L.S.D. at 0.05	0.66	0.66	0.75
L.S.D. at 0.01	1.57	0.91	1.99

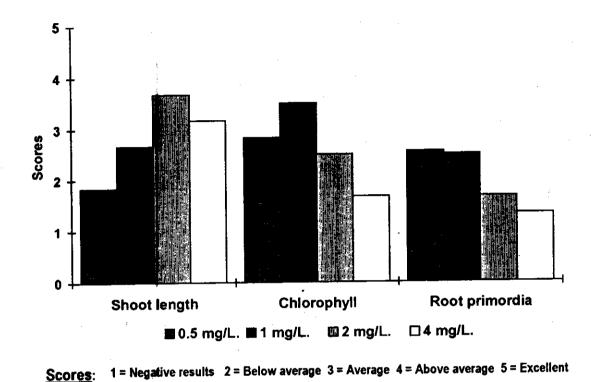


Fig. (43): Effect of different concentrations of gibberellic acid (GA₃) on shoot elongation, chlorophyll and root primordia of peach plants.

Scores:



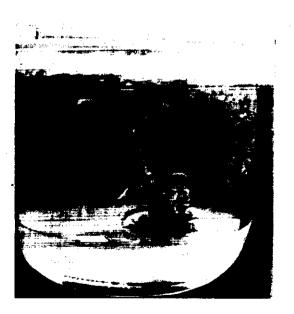
(A) 0.5 mg/L.



(B) 1.0 mg/L.



(C) 2.0 mg/L.



(D) 4.0 mg/L.

Fig. (44): Effect of different concentrations of gibberellic acid (GA3) on shoot elongation of Nemaguard plants.

4-2-3-2. Effect of medium strength:

Table (21) and Fig. (45) show the effect of different Murashige and Skoog strengths on shoot elongation, chlorophyll and rooting of peach plants. It is found that, one-fourth strength and one-eighth strength encouraged significantly shoot length and rooting as compared with other treatments. On the other hand, one-half strength surpassed significantly all other strengths used in their effect on chlorophyll followed by one-fourth strength.

Table (21): Effect of different Murashige and Skoog medium strengths on shoot length, chlorophyll and rooting of peach plants.

Medium strength	Shoot length	Chlorophyll	Rooting
Full -MS	1.33	1.50	1.67
One-half	1.67	3.17	2.50
One-Fourth	2.56	2.67	2.83
One-eighth	2.50	1.83	3.50
L.S.D. at 0.05	0.75	0.61	0.66
L.S.D. at 0.01	1.79	0.85	0.91

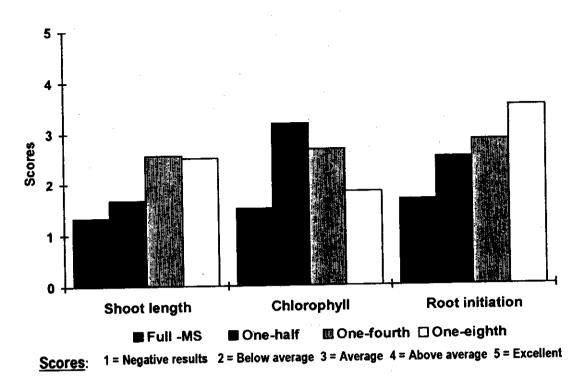


Fig. (45): Effect of different Murashige and Skoog medium strengths on shoot length, chlorophyll and rooting of peach plants.

4-2-3-3. Effect of auxin types and concentrations:

Table (22-A) shows the effect of different auxins on rooting of peach plants. It is found that, callus increased significantly by IBA application. However, (IBA plus NAA) induced highly significant increase in shoot growth and rooting over the other treatments used.

Regarding, the effect of auxin concentration on rooting as shown in **Table (22-B)** and **Figs. (46 & 47)**, it is obvious that, 2.0 and 4.0 mg/L. increased callus with high significancy as compared with the lower concentrations. Generally, lower auxin concentrations profoundly increased growth and chlorophyll (i.e. 0.5 and 1.0 mg/liter). Moreover, 1.0 and 2.0 mg/L. increased rooting with high significancy.

Table (22): Effect of different auxin types and concentrations on growth and rooting of peach plants.

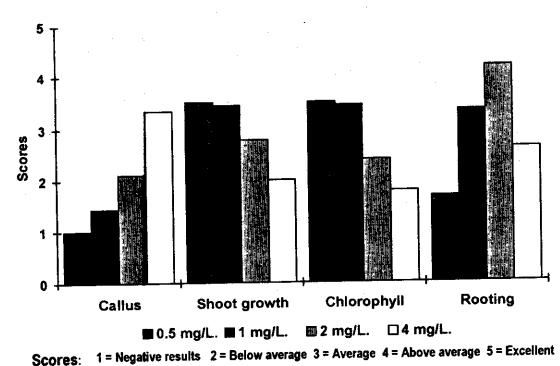
22-A: Effect of auxin type:

Auxin	Callus	Shoot Growth	Chlorophyll	Rooting
IBA	2.13	2.84	2.71	2.58
NAA	2.09	2.79	2.67	2.79
IBA + NAA	1.84	3.17	3.00	2.96
L.S.D. at 0.05	0.25	0.25	N.S	0.23
L.S.D. at 0.01	0.59	0.34	N.S	0.54

22-B: Effect of concentration:

Auxin concentration mg/L	Callus	Shoot Growth	Chlorophyll	Rooting
0.5	1.00	3.50	3.50	1.67
1.0	1.44	3.44	3.44	3.33
2.0	2.11	2.78	2.39	4.17
4.0	3.33	2.00	1.78	2.61
L.S.D. at 0.05	0.29	0.29	0.33	0.26
L.S.D. at 0.01	0.69	0.47	0.79	0.62

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Scores: 1 = Negative results 2 = Below average 3 = Average 4 = Above average 3 = Excention.

Fig. (46): Effect of different auxin concentrations on growth and rooting of peach plants.

Regarding the response of variety, as shown in **Table** (22-C) it is clear that Nemaguard significantly surpassed Okinawa in its response to auxin treatments.

22-C: Effect of variety:

Variety	Callus	Shoot Growth	Chlorophyil	Rooting
Nemaguard	1.89	3.06	2.92	2.92
Okinawa	2.04	2.81	2.67	2.64
L.S.D. at 0.05	N.S	0.20	0.24	0.19
L.S.D. at 0.01	N.S	0.34	0.56	0.31





(A) 1.0 mg/L.

(B) 2.0 mg/L.



(C) 4.0 mg/L.

Fig. (47): Effect of different auxin concentrations [1 mg/L. (A), 2 mg/L. (B) and 4 mg/L. (C)] on growth characters of Nemaguard plants.

4-2-3-4. Effect of photoperiod:

Table (23-A) and Fig. (48) show the effect of different photoperiods on growth and rooting of peach plants. It is found that, continous light significantly increased necrosis followed by intermittent light as compared with the other photoperiods used. However, long photoperiod treatment was preferable a sit significantly reduced necrosis. While, best growth and chlorophyll occurred when either continuous light or intermittent light were applied. Furthermore, short photoperiod significantly increased rooting followed by long-days and intermittent light in a descending order. Regarding the response of variety to photoperiod as shown in Table (23-B), it is easy to conclude that, Nemaguard showed significantly less necrosis along with better shoot growth and rooting than Okinawa.

Table (23): Effect of different photoperiods on growth characters and rooting of peach plants.

23-A: Effect of different photoperiods:

Photoperiod	Necrosis	Shoot Growth	Chlorophyll	Rooting
Continuous light	3.33	1.33	1.67	1.67
Long-day	1.67	3.17	4.17	3.83
Short-day	2.00	2.83	1.83	4.33
Intermittent light	2.33	1.67	2.00	2.00
L.S.D. at 0.05	0.53	0.25	0.53	0.47
L.S.D. at 0.01	0.80	0.57	0.80	0.71

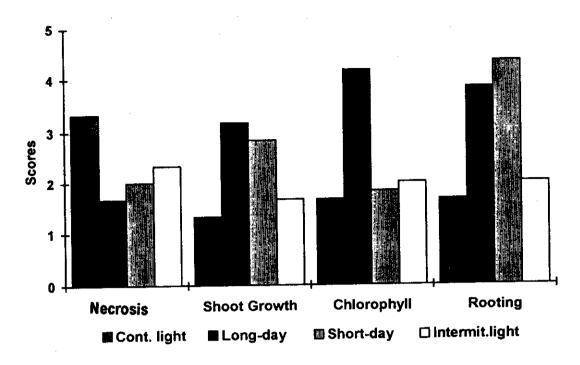


Fig. (48): Effect of different photoperiod treatments on shoot growth and rooting of peach plants.

23-B: Effect of variety:

Necrosis	Nicroses	Growth	Chlorophyil	Rooting
Nemaguard	1.67	3.50	3.17	4.33
Okinawa	2.33	2.50	3.17	3.83
L.S.D. at 0.05	0.53	0.85	N.S	0.47
L.S.D. at 0.01	0.80	0.91	N.S	0.71

4-2-3-5. Effect of etiolation:

The effect of etiolation treatments on growth and rooting of peach varieties is presented in Table (24-A) and Figs. (49 & 50). It is found that, necrosis increased with high significancy when active charcoal treatment was used as compared with the addition of surface coverage or outer coverage. On the other hand, using either the combination of both surface and outer coverage treatment, outer coverage or surface coverage resulted in highly significant increase in growth, chlorophyll and rooting in relation to the control and the addition of the activated charcoal treatment. However, rooting was increased with high significancy when the combination of both surface and outer coverage treatment was used in comparison with the addition of activated charcoal treatment.

Table (24): Effect of etiolation treatments on growth and rooting of peach plants.

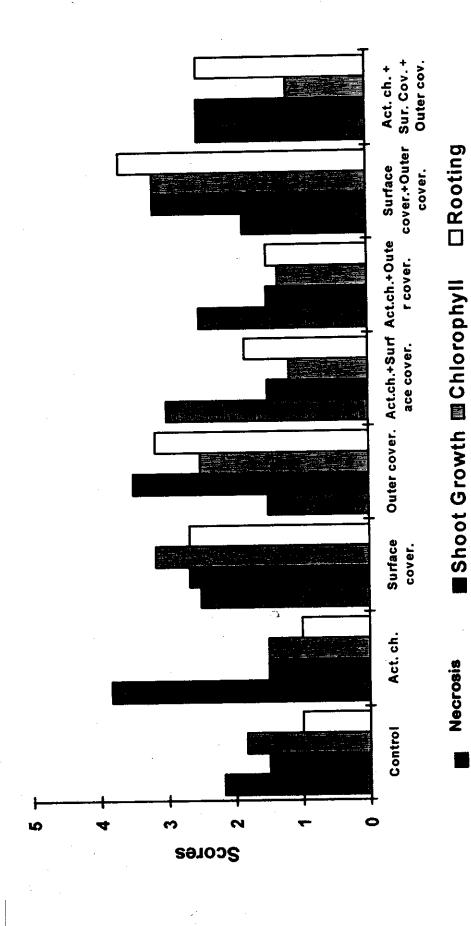
24-A: Effect of etiolation treatments:

Treatment	Necrosis	Growth	Chiorophyll	Rooting
Control	2.17	1.50	1.83	1.00
Active charcoal	3.83	1.50	1.50	1.00
Surface coverage	2.50	2.67	3.17	2.67
Outer coverage	1.50	3.50	2.50	3.17
Active charcoal + Surface coverage	3.00	1.50	1.17	1.83
Active charcoal + Outer coverage	2.50	1.50	1.33	1.50
Surface coverage + Outer coverage	1.84	3.17	3.17	3.67
Act. ch. + Sur. Cov. + Outer cov.	2.50	2.50	1.17	2.50
L.S.D. at 0.05	0.62	0.63	0.63	0.45
L.S.D. at 0.01	1.45	0.85	0.84	0.61

Furthermore, the effect of variety is presented in **Table (24-B)**. It is clear that, growth and rooting responses of Nemaguard were significantly better than Okinawa.

24-B: Effect of variety:

Variety	Necrosis	Shoot Growth	Chlorophyll	Rooting
Nemaguard	2.14	2.33	2.33	3.67
Okinawa	1.91	2.00	2.13	2.67
L.S.D. at 0.05	N.S	0.32	N.S	0.31
L.S.D. at 0.01	N.S	0.43	N.S	0.73



1 = Negative results 2 = Below average 3 = Average 4 = Above average 5 = Excellent Fig. (49): Effect of etiolation treatments on growth characters and rooting of peach plants. Scores



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Fig. (50): Comparison between the addition of activated charcoal (A) and the control (B) on growth characters of Nemaguard plants.



Root initiation



Complete root development

Fig. (51): Development of rooting of Nemaguard plants.

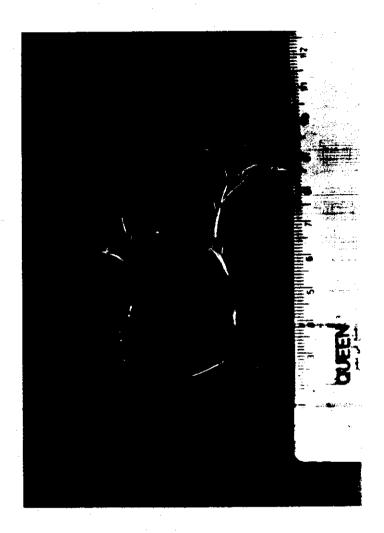


Fig. (52): The final shape of Nemaguard plants.

4-3. Micropropagation of almond plants:

4-3-1. Establishment stage:

4-3-1-1. Effect of nutrient medium:

Table (25-A) shows the effect of different nutrient media on development of almond explants. It is clear that necrosis was significantly at its lowest level as Murashige and Skoog medium was used followed by Anderson and Nitsch and Nitsch media in an ascending order.

In addition, Murashige and Skoog medium induced highly significant increase in explant development, chlorophyll and medium efficiency as compared with the other used media. On the other hand, Nitsch and Nitsch medium had the poorest effect on all growth criteria of almond explant.

Table (25): Effect of different nutrient media and explants on explant development parameters of almond plants.

25-A	:	Effect	0	f nutrient	medium.
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Nutrient medium	Necrosis	Explant development	Chlorophyll	Medium efficiency
Murashige & Skoog	2.33	3,33	1.83	2.58
Nitsch & Nitsch	3.33	1.75	1.25	1.50
Anderson	2.50	2.25	1.75	2.00
L.S.D. at 0.05	0.41	0.40	0.48	0.49
L.S.D. at 0.01	0.56	0.55	0.64	0.66

Moreover, it is found that Bitter almond showed significantly better explant development, chlorophyll and medium efficiency in comparison with Ne Plus Ultra (Table, 25-B).

However, the interaction between medium and variety as shown in **Table (25-C)** and **Fig. (53)** indicated that, Bitter almond gave significant better explant development on MS medium.

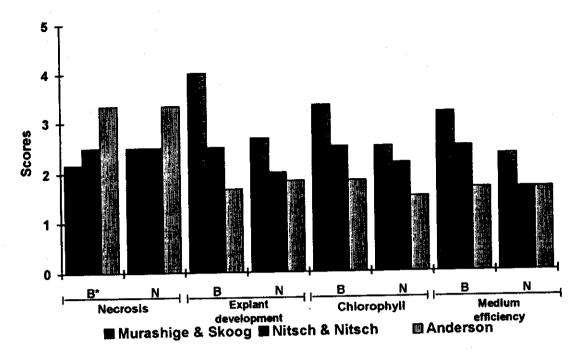
25-B: Effect of variety.

Variety	Necrosis	Explant development	Chlorophyll	Medium efficiency
Bitter almond	2.67	2.72	2.56	2.66
Ne Plus Ultra	2.78	2.17	2.06	2.12
L.S.D. at 0.05	N.S	0.33	0.37	0.40
L.S.D. at 0.01	N.S	0.45	0.51	0.47

25-C: Effect of interaction between medium and variety.

Variety	Necrosis		Explant development		Chlorophyll		Medium efficiency	
Medium	B*	N*	В	N	В	М	В	N
Murashige & Skoog	2.17	2.50	4.00	2.67	3.33	2.50	3.17	2.33
Nitsch & Nitsch	2.50	2.50	2.50	2.00	2.50	2.17	2.50	1.67
Anderson	3.33	3.33	1.67	1.83	1.83	1.50	1.67	1.67
L.S.D. at 0.05	N	.s	0.	57	N	.s	N	. S
L.S.D. at 0.01	N	.s	0.	77	N	.s	. N	l.S

^{*} B : Bitter almond, N : Ne Plus Ultra



* B : Bitter almond, N : Ne Plus Pitra

Scores: 1 = Negative results 2 = Below average 3 = Average 4 = Above average 5 = Excellent

Fig. (53): Effect of interaction between medium and variety on explant development of almond plants.

4-3-1-2. Effect of Antioxidant treatments:

Table (26-A) and Fig. (54) show the effect of antioxidant treatments on explant development of some almond plants. It is quite evident that, antioxidant solution induced less necrosis significantly, while explant development and chlorophyll were obviously increased followed by the combination of antioxidant solution plus active charcoal and active charcoal treatments. Considering the effect of variety, it is clear from Table (26-B) that, Bitter almond gave significantly better explant development and chlorophyll as compared with Ne Plus Ultra.

Table (26): Effect of different antioxidant treatments on explant development parameters of almond plants.

26-A: Effect of antioxidant treatments.

Antioxidant	Necrosis	Explant development	Chlorophyll
Control	2.17	2.75	3.13
Antioxidant solution	1.75	3.67	3.80
Active charcoal	3.92	1.67	1.71
Ant. sol. + Act. char.	3.50	2.58	2.88
L.S.D. at 0.05	0.36	0.43	0.46
L.S.D. at 0.01	0.85	0.58	0.62

26-B: Effect of variety.

Necrosis	Explant development	Chlorophyll
3.25	2.96	3.11
2.92	2.38	2.65
0.26	0.30	0.32
0.66	0.41	0.44
	3.25 2.92 0.26	Necrosis development 3.25 2.96 2.92 2.38 0.26 0.30

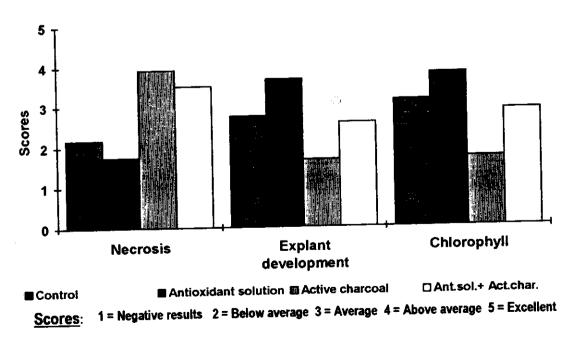


Fig. (54): Effect of different antioxidant treatments on explant development of almond plants.

4-3-1-3. Effect of medium strength:

It is found from **Table (27-A)** and **Fig. (55)** that reducing MS medium strength improved survival and growth parameters. In this sphere, one-fourth strength induced the least in necrosis, while caused highest increase in growth, chlorophyll and medium efficiency followed by one-half strength in a descending order.

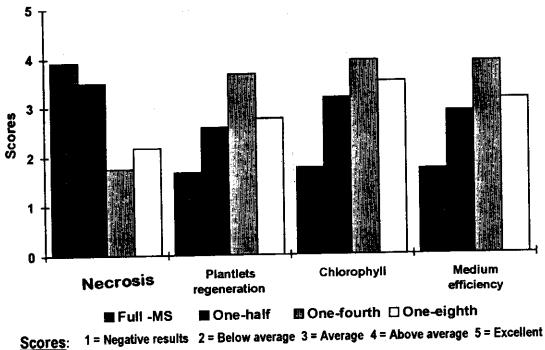
Regarding the effect of variety as shown in **Table (27-B)** and **Fig. (56)**, it is clear that, Bitter almond gave significantly better growth of cultured explants and higher medium efficiency as compared with Ne Plus Ultra.

Considering the effect of explant, it is clear from **Table** (27-C) and **Fig.** (57) that, shoot-tips surpassed significantly one-node cuttings.

Table (27): Effect of different Murashige and Skoog medium strengths and explant type on growth and development of almond explants.

27-A: Effect of medium strength.

Medium strength	Necrosis Growth		Chlorophyll	Medium efficiency	
Full -MS	3.92	1.67	1.75	1.71	
One-half	3.50	2.58	3.17	2.88	
One-Fourth	1.75	3.67	3.92	3.88	
One-eighth	2.17	2.75	3.50	3.13	
L.S.D. at 0.05	0.36	0.43	0.36	0.46	
L.S.D. at 0.01	0.85	0.58	0.85	0.62	



Scores: 1 = Negative results 2 = Below average 3 = Average 3

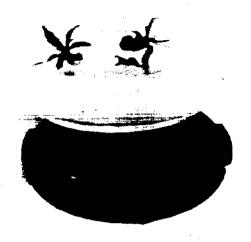
Fig. (55): Effect of different Murashige and Skoog medium strengths and explant type on growth and development of almond explants.

27-B: Effect of variety.

Variety	Necrosis	Growth	Chlorophyll	Medium efficiency
Bitter almond	3.21	2.96	3.21	3.09
Ne Plus Ultra	2.96	2.38	2.86	2.62
L.S.D. at 0.05	N.S	0.30	[⊃] N.S	0.32
L.S.D. at 0.01	N.S	0.41	N.S	0.44

27-C: Effect of explant.

Explant	Necrosis	Growth	Chlorophyli	Medium efficiency
Shoot-tip	3.25	2.96	3.25	2.96
One-node cutting	2.92	2.38	2.92	2.58
L.S.D. at 0.05	0.26	0.30	0.26	0.32
L.S.D. at 0.01	0.60	0.41	0.60	0.44

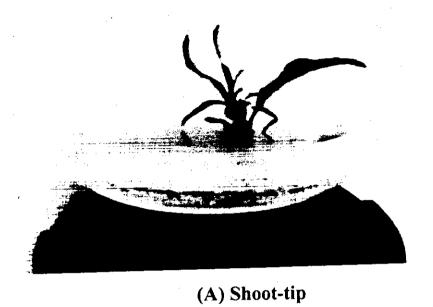


(A) Ne Plus Ultra



(B) Bitter almond

Fig. (56): Effect of variety of almond [Ne Plus Ultra (A) and Bitter almond (B)] on growth of cultured explants.



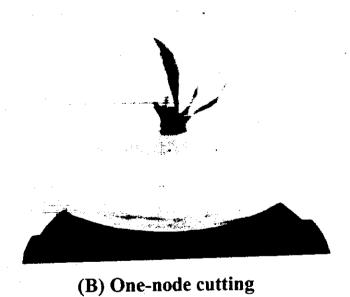


Fig. (57): Effect of different explants [shoot-tip (A) and one-node cutting (B)] of Bitter almond plants on growth of cultured explants.

4-3-1-4. Effect of additives:

Table (28-A) and Fig. (58) shows the effect of additive treatments on growth of cultured of almond explants. It is obvious that, adenine sulphate induced significantly less necrosis and in the same time caused highest significant increase in plantlet regeneration and chlorophyll followed by sphadex and coconut-milk in a descending order. However, MES hydrolysate treatments was not promising in this respect. Regarding the effect of variety as shown in Table (28-B) it is clear that, Bitter almond performance surpassed significantly, Ne Plus Ultra.

Table (28): Effect of different additives on growth and development of cultured almond explants.

28_	Δ		Effect	αf	additives	
40-	\boldsymbol{H}	-	CHIECL	OI (auuiuvus	

Additives	Necrosis	Growth	Chlorophyll	Medium efficiency
Adenine	1.42	3.92	1.42	3.25
Coconut milk	1.67	2.00	1.92	2.58
MES hydrolysate	2.67	1.67	1.58	2.25
Sphadex	1.67	3.00	1.83	3.08
L.S.D. at 0.05	0.44	0.37	N.S	0.39
L.S.D. at 0.01	0.60	0.51	N.S	0.52

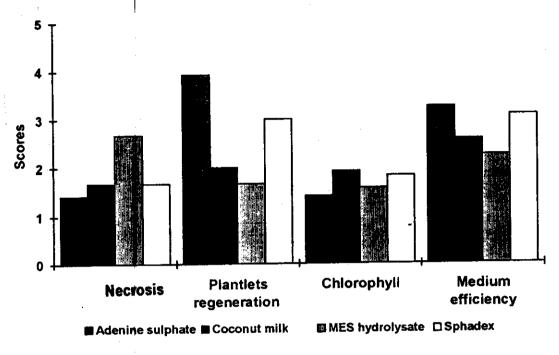


Fig. (58): Effect of different additives on growth and development of cultured almond explants.

28-B: Effect of variety.

Variety	Necrosis	Growth	Chlorophyll	Medium efficiency
Bitter almond	1.75	2.84	1.88	2.83
Ne Plus Ultra	1.96	2.46	1.50	2.75
L.S.D. at 0.05	N.S	0.27	0.30	N.S
L.S.D. at 0.01	N.S	0.36	0.57	N.S

4-3-2. Proliferation stage:

4-3-2-1. Effect of Cytokinin type and thidiazuron:

Table (29-A) shows the effect of different cytokinins at the rate of 2 mg/liter on proliferation of almond plants. It is clear that, BAP significantly increased proliferation. The response to BAP was followed by zeatin and kinetin in a descending order.

However, thidiazuron treatment at the used concentration was not promising in this respect. However, kinetin induced highly significant increase in growth as compared with the other used cytokinin.

A glance to **Table (29-B)** show that, Bitter almond had significantly better growth and chlorophyll in comparison with Ne Plus Ultra.

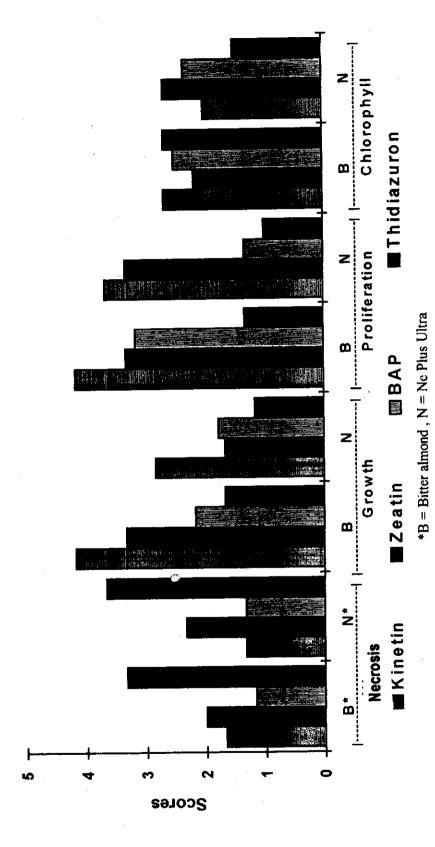
Regarding the effect of the interaction between different cytokinins and variety, it is clear from **Table (29-C)** and **Fig., (59)** that growth and chlorophyll increased significantly when different cytokinins were used.

Table (29): Effect of different cytokinins and thidiazuron on proliferation and growth characters of almond plants.

29-A	•	Effect	of	different	cytokinins	
	•	LIIOU	CI	CHILLOI GIZE	- J	

Medium supplement	Necrosis	Growth	Proliferation	Chlorophyll	
Kinetin	1.50	3.50	1.67	2.08	
Zeatin	2.17	2.50	3.25	2.08	
BAP	1.25	2.00	3.33	1.92	
Thidiazuron	3.50	1.00	1.33	1.00	
L.S.D. at 0.05	0.44	0.49	0.40	N.S	
L.S.D. at 0.01	0.60	0.66	0.54	N.S	

All supplements were applied at the rate of 2 mg/liter.



Scores: 1 = Negative results; 2 = Below average; 3 = Average; 4 = Above average 5 = Excellent

Fig. (59): Effect of interaction between different cytokinins and variety on proliferation and growth characters of almond plants.

4-3-2-2. Effect of Cytokinin concentrations:

The effect of different concentrations of 6-benzylaminopurine (BAP) on proliferation of almond plants is presented in **Table (30-A)** and **Fig. (60)**. It is clear that both necrosis and proliferation were significantly increased with increasing BAP concentration from 0.5 mg/liter to 4 mg/liter. The picture was changed to the reverse when both growth and chlorophyll parameters were concerned.

Moreover, it is safe to notice from **Table (30-B)** that, Bitter almond had significantly higher proliferation in comparison with Ne plus ultra.

Besides, the interaction between concentration and variety indicated that 1.0 mg/L. BAP gave best proliferation for Better almond, while higher concentration of BAP (2.0 mg/L.) were needed to achieve similar effect on Ne Plus Ultra (**Table, 30-C**) and **Fig. (61).**

Table (30): Effect of different concentrations of 6-benzylaminopurine (BAP) on proliferation and growth characters of almond plants.

30-A: Effect of concentration.

Concentration mg/L	Necrosis	Growth	Proliferation	Chlorophyll
0.5	1.67	3.67	2.33	2.33
1.0	2.33	2.67	3.33	3.00
2.0	1.67	1.67	3.67	2.00
4.0	3.00	1.33	4.33	1.00
L.S.D. at 0.05	0.25	0.25	0.25	0.24
L.S.D. at 0.01	0.57	0.33	0.57	0.32

30-B: Effect of variety.

Variety	Necrosis	Growth	Proliferation	Chlorophyll
Bitter almond	2.57	2.33	2.92	2.59
Ne Plus Ultra	2.69	2.08	2.63	2.65
L.S.D. at 0.05	N.S	N.S	0.18	N.S
L.S.D. at 0.01	N.S	N.S	0.40	N.S

30-C: Effect of interaction between different concentrations and variety.

BAP	Necrosis		Explant development		Proliferation		Chlorophyll	
Concentration mg/L	Bitter almond	Neplus ultra	Bitter almond	Neplus ultra	Bitter almond	Neplus ultra	Bitter almond	Neplus ultra
0.5	1.50	1.67	3.42	3.00	2.17	2.25	3.17	3.08
1.0	1.92	2.33	2.50	2.25	3.25	2.92	2.83	2.92
2.0	3.17	3.25	1.67	1.83	3.14	3.42	2.17	2.42
4.0	3.67	3.50	1.33	1.25	2.83	1.92	2.17	2.17
L.S.D. at 0.05	N.S		N.S		0.25		N.S	
L.S.D. at 0.01	N	.s	N.S		0.57		N.S	

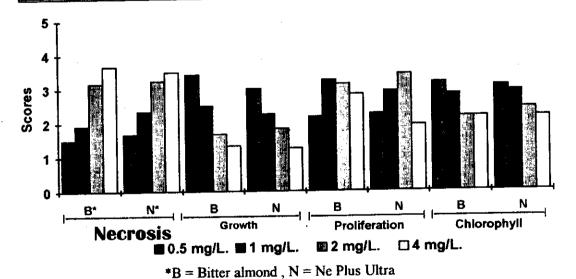
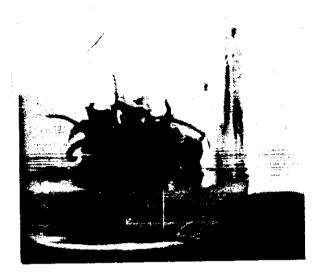


Fig. (60): Effect of interaction between different concentration of BAP and variety on proliferation and growth characters of almond plants.



(A) 0.5 mg/L.



(B) 1.0 mg/L.



(C) 2.0 mg/L.



(D) 4.0 mg/L.

Fig. (61): Effect of different concentrations of 6-benzylaminopurine (BAP) on proliferation and growth characters of Bitter almond plants.

Regarding the effect of different concentrations of thidiazuron on proliferation of almond plants, it is quite evident from **Table** (31-A) and **Fig.** (62) that necrosis increased significantly with increasing thidiazuron level. Meanwhile, growth, proliferation and chlorophyll were best at the lowest thidiazuron level (0.5 mg/L.).

Regarding the effect of variety, as shown in **Table (31-B)**, it is clear that Bitter almond surpassed significantly Ne Plus Ultra in growth and proliferation.

Referring to the effect of interaction between concentration and variety, it is clear from **Table (31-C)** and **Fig. (63)** that, growth and proliferation increased significantly when 0.5 mg/L. concentration was used with Bitter almond over that of Ne Plus Ultra.

Generally, it is easy to conclude that 0.5 mg/liter of thidiazuron enhanced growth and proliferation, while 4 mg/L. BAP induced best proliferation of almond varieties.

Table (31): Effect of different concentrations of thidiazuron on proliferation and growth characters of almond plants.

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31-A:	Effect	of	concentration	,
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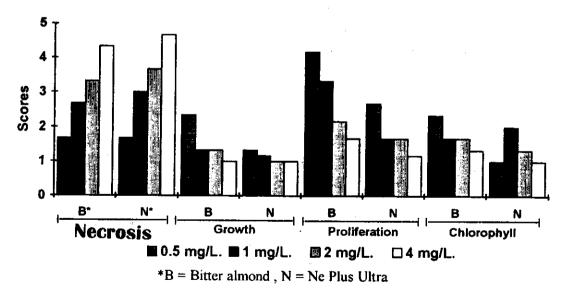
Concentration mg/L	Necrosis	Growth	Proliferation	Chlorophyll
0.5	1.67	1.83	3.50	2.17
1.0	2.83	1.25	2.50	1.83
2.0	3.50	1.17	2.00	1.33
4.0	5.00	1.00	1.00	1.00
L.S.D. at 0.05	0.25	0.25	0.25	0.24
L.S.D. at 0.01	0.57	0.33	0.57	0.32

31-B: Effect of variety.

Variety	Necrosis	Growth	Proliferation	Chlorophyll
Bitter almond	2.75	1.50	2.83	0.67
Ne Plus Ultra	3.08	1.12	1.87	1.50
L.S.D. at 0.05	N.S	0.25	0.35	N.S
L.S.D. at 0.01	N.S	0.33	0.47	N.S

31-C: Effect of interaction between different concentrations and variety.

TDZ	Necrosis		Growth		Proliferation		Chlorophyll	
concentration mg/L	Bitter almond	Neplus ultra	Bitter almond	Neplus ultra	Bitter almond	Neplus ultra	Bitter almond	Neplus ultra
0.5	1.67	1.67	2.34	1.33	4.17	2.67	2.33	1.00
1.0	2.67	3.00	1.33	1.17	3.33	1.67	1.67	2.00
2.0	3.33	3.67	1.33	1.00	2.17	1.67	1.67	1.33
4.0	4.33	4.67	1.00	1.00	1.67	1.17	1.33	1.00
L.S.D. at 0.05	N.	.s	0.4	19	0.	70	N.	S
L.S.D. at 0.01	N.S		0.0	66 0.94		94	N.S	



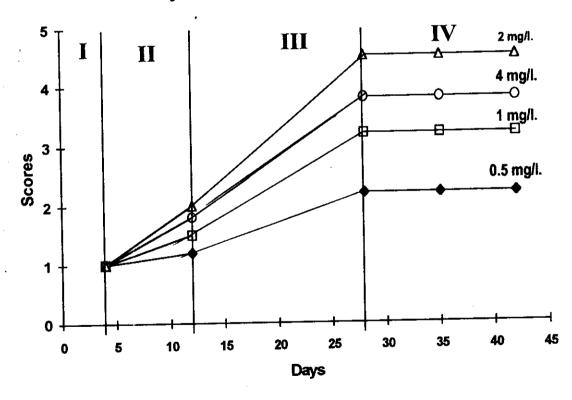
Scores: 1 = Negative results 2 = Below average 3 = Average 4 = Above average 5 = Excellent

Fig. (62): Effect of interaction between concentration of thidiazuron and variety on proliferation and growth characters of almond plants.

4-3-2-3. Proliferation curve :

Figure (64) reflects that during the first four days from culturing time (lag phase) proliferation of almond was nearly steady since the explant acclimatized with the medium. During the next ten dayes, proliferation started to increase (log phase) followed by a fast increase in proliferation (progressive phase) for 28 days from culturing time. Thus, subculturing should take place in almond after 28 days from culturing and before the start of stationary phase. Meanwhile, there is a direct relationship between concentration of different cytokinins and proliferation. The best proliferation was acieved when 2 mg/l. was used. However, increasing the concentration up to 4 mg/l. led to a decrease in proliferation as compared with 2 mg/l.

Cytokinin concentrations (mg/L.)



I: Lag phase

III : Progressive phase

II: Log phase

IV: Stationary phase

Fig. (64): Proliferation curve of almond plants under different concentrations of cytokinin.

4-3-3. Rooting stage:

4-3-3-1. Effect of different concentrations of gibberellic acid:

Considering the effect of different concentrations of gibberellic acid (GA₃) on shoot elongation of almond plants, it is found from **Table (32)** and **Figs. (65 & 66)** that shoot length increased significantly with increasing GA₃ concentration up to 2 and 4 mg/liter as compared with the lower concentrations of GA₃ (i.e. 0.0, 0.5 and 1.0 mg/liter). On the contrary, chlorophyll and root primordia increased significantly with decreasing GA₃ concentrations to 0.5 mg/liter as compared with the higher concentrations of GA₃ (i.e. 1.0, 2.0 and 4.0 mg/liter).

Table (32): Effect of different concentrations of gibberellic acid (GA₃) on shoot elongation, chlorophyll and root primordia of almond plants.

GA ₃ Concentration mg/L	Shoot length	Chlorophyll	Root primordia
0.0	1.67	2.67	2.33
0.5	1.83	3.50	1.67
1.0	2.50	2.50	1.67
2.0	3.50	2.50	1.33
4.0	3.17**	1.83	1.00
L.S.D. at 0.05	0.66	0.69	0.51
L.S.D. at 0.01	1.59	0.96	0.79

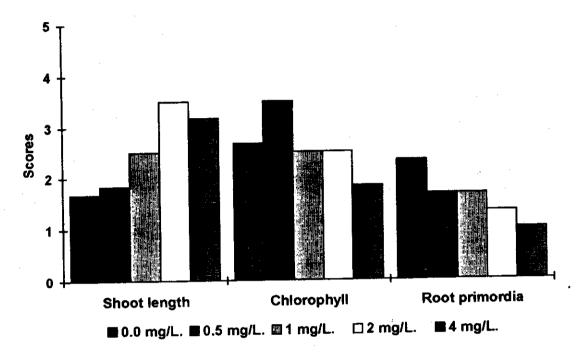


Fig. (65): Effect of different concentrations of gibberellic acid (GA₃) on shoot elongation, chlorophyll and root primordia of almond plants.

4-3-3-2. Effect of medium strength:

Table (33) and Fig. (67) show the effect of different Murashige and Skoog strengths on shoot elongation, chlorophyll and rooting. It is clear that one-fourth strength encouraged significantly shoot elongation and chlorophyll as compared with the other used medium strengths. On the other hand, one-eighth strength increased significantly root primordia as compared with the other used medium strengths.

Table (33): Effect of different Murashige and Skoog medium strengths on shoot length, chlorophyll and root primordia of almond plants.

Medium strength	Shoot length	Chlorophyll	Rooting
Full -MS	1.33	2.00	1.00
One-half	1.50	3.17	1.33
One-fourth	2.50	2.67	1.83
One-eighth	2.33	2.17	2.17
L.S.D. at 0.05	0.72	0.46	0.48
L.S.D. at 0.01	1.72	0.64	0.66

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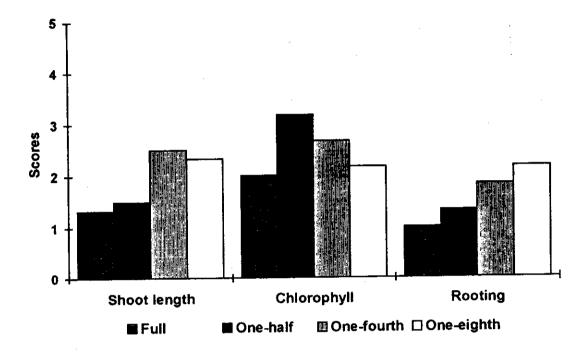


Fig. (67): Effect of different Murashige and Skoog medium strengths on shoot length, chlorophyll and rooting of almond plants.

4-3-3-3. Effect of Auxin types and concentrations:

Referring to the effect of different auxins on rooting of almond plants. It is clear that, data of auxin types slightly differed without any significancy in all criteria under study **Table (34-A)**.

Concerning the effect of auxin concentration Table (34-B) and Figs. (68 & 69) indicated that callus production was significantly increased as concentration of auxin increased. Meanwhile, higher concentration of 2 and 4 mg/L. significantly enhanced root formation.

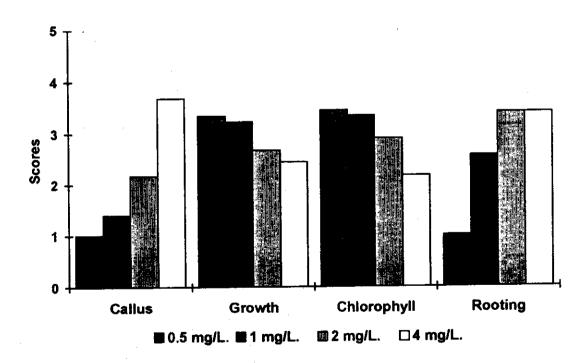
Table (34): Effect of different auxin types and concentrations on growth and rooting of almond plants.

34-A: Effect of different auxin types:

Auxin	Callus	Growth	Chlorophyll	Rooting
IBA	2.08	2.67	2.96	2.58
NAA	2.12	2.16	2.70	2.71
IBA + NAA	1.79	2.50	3.12	2.88
L.S.D. at 0.05	N.S	N.S	N.S	N.S
L.S.D. at 0.01	N.S	N.S	N.S	N.S

34-B: Effect of different concentrations:

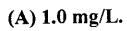
Concentration mg/L	Callus	Growth	Chiorophyll	Rooting
0.5	1.00	3.33	3.44	1.00
1.0	1.39	3.22	3.33	2.56
2.0	2.17	2.67	2.89	3.39
4.0	3.67	2.44	2.17	3.39
L.S.D. at 0.05	0.29	0.33	0.33	0.34
L.S.D. at 0.01	0.68	0.45	0.78	0.45



Scores: 1 = Negative results 2 = Below average 3 = Average 4 = Above average 5 = Excellent

Fig. (68): Effect of different auxin concentrations on growth and rooting of almond plants.







(B) 2.0 mg/L.



(C) 4.0 mg/L.

Fig. (69): Effect of different auxin concentrations [1.0 mg/L. (A), 2.0 mg/L. (B) and 4.0 mg/L. (C)] on growth characters of Bitter almond plants.

4-3-3-4. Effect of photoperiod:

Table (35-A) and Fig. (70) show the effect of different photoperiods on growth and rooting of almond plants. It is obvious that long-day induced highly significant increase in necrosis and in the same time caused highest significant increase in chlorophyll followed by short-day and intermittent light in a descending order. However, short-day significantly increased rooting followed by long-day and intermittent light in a descending order.

Regarding the effect of variety as shown in **Table (35-B)**, it is obvious that, Bitter almond surpassed significantly Ne Plus Ultra.

Table (35): Effect of different photoperiods on growth and rooting of almond plants.

35-A: Effect of different photoperiods:

Photoperiod	Necrosis	Growth	Chlorophyll	Rooting
Continuous light	3.20	1.33	1.67	1.20
Long-day	1.83	3.17	4.33	3.83
Short-day	2.17	2.67	3.83	4.17
Intermitant light	2.67	1.80	2.00	2.00
L.S.D. at 0.05	0.36	0.43	0.47	0.53
L.S.D. at 0.01	0.49	0.57	0.71	0.80

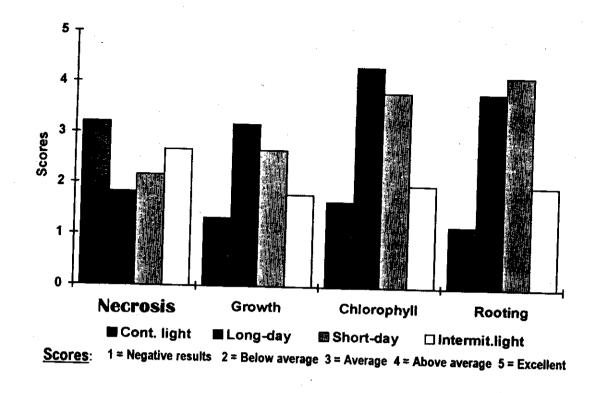


Fig. (70): Effect of different photoperiods on growth characters and rooting of almond plants.

35-B: Effect of variety:

Variety	Necrosis	Growth	Chlorophyli	Rooting
Bitter almond	1.83	3.50	3.17	4.33
Ne Plus Ultra	2.17	2.33	3.17	3.83
L.S.D. at 0.05	N.S	0.67	N.S	0.47
L.S.D. at 0.01	N.S	1.01	N.S	0.71

4-3-3-5. Effect of etiolation:

The effect of different etiolation treatments on growth and rooting of almond plants as shown in **Table (36-A)** and **Fig. (71)**, indicated that necrosis was reduced with high significancy when a combination of both surface and outer covarge was used followed by the outer coverage treatment in comparison with the addition of activated charcoal treatment. However, using of either combined surface and outer coverage treatment, outer coverage treatment or surface coverage resulted in highly significant increase of growth, chlorophyll and rooting in relation to the control and the addition of the activated charcoal treatment. On the other hand, addition of the activated charcoal either alone or in combination with other treatments resulted in a harmful effect on the cultured explant as indicated by increased nicroses and decreased growth, chlorophyll and rooting.

However, surface coverage plus outer coverage treatment induced highly significant increase in rooting followed by outer coverage as compared with the other used treatments. Generally, surface coverage plus outer coverage treatments gave the best rooting survival. Moreover, the effect of variety on rooting and growth was presented in **Table (36-B)**. It is clear that Bitter almond gave statistically better rooting than Ne Plus Ultra.

Table (36): Effect of etiolation treatments on growth and rooting of almond plants.

36-A: Effect of etiolation treatments:

Treatment	Necrosis	Growth	Chlorophyll	Rooting
Control	2.67	1.50	1.83	1.00
Active charcoal	3.17	1.50	1.00	1.33
Surface coverage	2.83	2.67	2.83	2.17
Outer coverage	1.83	2.83	3.17	3.17
Active charcoal + Surface coverage	3.00	1.33	1.67	2.17
Active charcoal + Cluter coverage	2.33	2.50	1.50	1.17
Surface coverage + Outer coverage	1.50	3.17	3.00	3.33
Act. ch. + Sur. Cov. + Outer cov.	2.50	2.67	2.00	1.00
L.S.D. at 0.05	0.68	0.69	0.64	0.55
L.S.D. at 0.01	0.91	0.93	0.86	0.75

36-B: Effect of variety:

Variety	Necrosis	Growth	Chlorophyli	Rooting
Bitter almond	2.75	2.29	2.08	2.29
Ne Plus Ultra	3.08	1.96	1.79	2.00
L.S.D. at 0.05	N.S	N.S	N.S	0.28
L.S.D. at 0.01	N.S	N.S	N.S	0.37

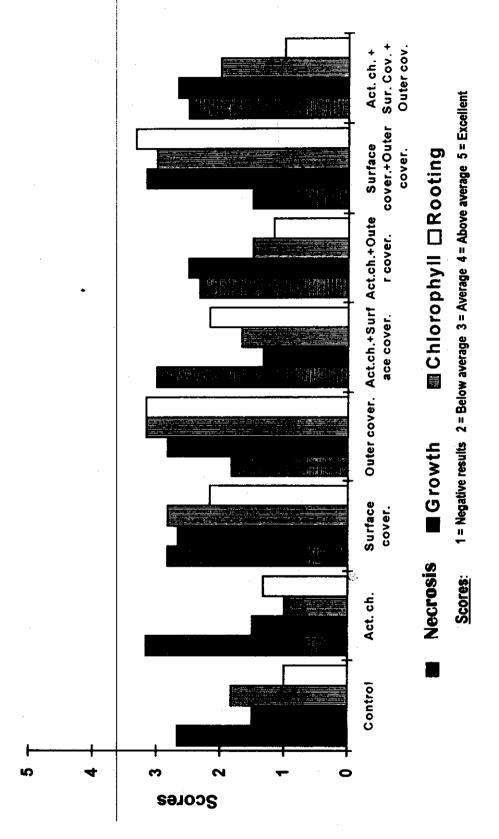


Fig. (71): Effect of etiolation treatments on growth and rooting of almond plants.