IV- RESULTS AND DISCUSSION

4.1. Marjoram (direct regeneration):

4.1.1. Establishment stage:

4.1.1.a. Effect of medium type:

Data in Table (1) and Photo (1) reflect the effect of different medium types on explant development, necrosis, browning, growth and greening parameters. It is clear that Murashige and Skoog medium induced a significant increase in explant development, growth and greening compared with the other two media under study. However, Nitsch and Nitsch medium recorded the second rate then followed by Woody plant medium which showed the lowest level in improving all parameters.

Generally, the beforementioned results reflect that Murashige and Skoog was preferred in increasing the explant development, growth and greening. These results are in agreement with the finding of **Arafeh (2003)** on marjoram and **Echeverrigaray *et al.* (2005)** on *Lavandule dentate.* They recommended Murashige and Skoog medium in enhancing explant development.

Table (1): Effect of medium type on growth and explant development parameters of Marjoram.

Parameters / Medium type	Necrosis	Browning	Growth	Explant development	Greening
MS	1.93 B	1.10 C	4.07 A	3.70 A	3.53 A
N.N	2.40 B	2.63 B	1.80 B	1.83 B	1.93 B
W.P.M	3.73 A	4.50 A	1.60 B	1.67 B	1.80 B

Means of different medium types followed with the same letter withen each column are not significantly different from each other at 1% level.

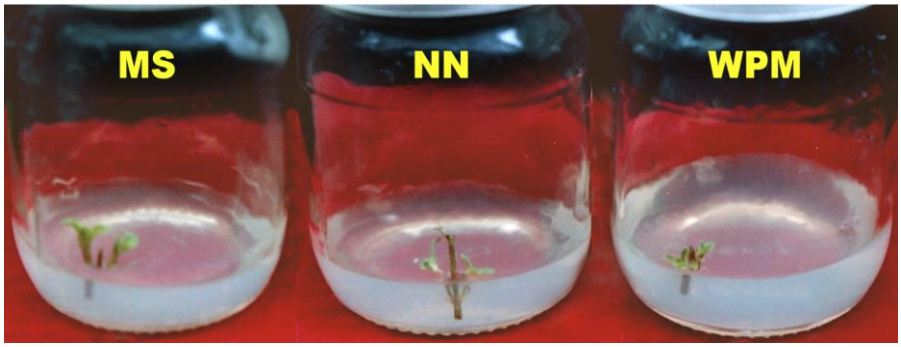


Photo (1): Effect of medium type on explant development parameters of Marjoram.

4.1.1.b. Effect of explant type:

Table (2) and Photo (2) deal with the effect of different explants on necrosis, browning, explant development, growth and greening parameters. It is noticed that shoot tip explant significantly surpassed one-node cuttings in having less necrosis, while maximized explant development. However, significant differences between different explants used were disappeared when browning, growth and greening parameters were involved.

The above results assured the superiority of shoot tip explant on the one node cuttings. These results confirm the findings of **Youssef (2003)** on Yucca and **Abd El-Kader (2004)** on Cupressus. They found that shoot tips surpassed one node cuttings in increasing explant development.

Table (2): Effect of explant type on growth and explant development parameters of Marjoram.

Parameters / Explant type	Necrosis	Browning	Growth	Explant development	Greening
Shoot tip	2.17 B	1.93 A	3.33 A	3.70 A	3.47 A
One node cutting	3.27 A	2.70 A	2.10 A	2.10 B	2.73 A

Means of different explant types followed with the same letter withen each column are not significantly different from each other at 1% level.

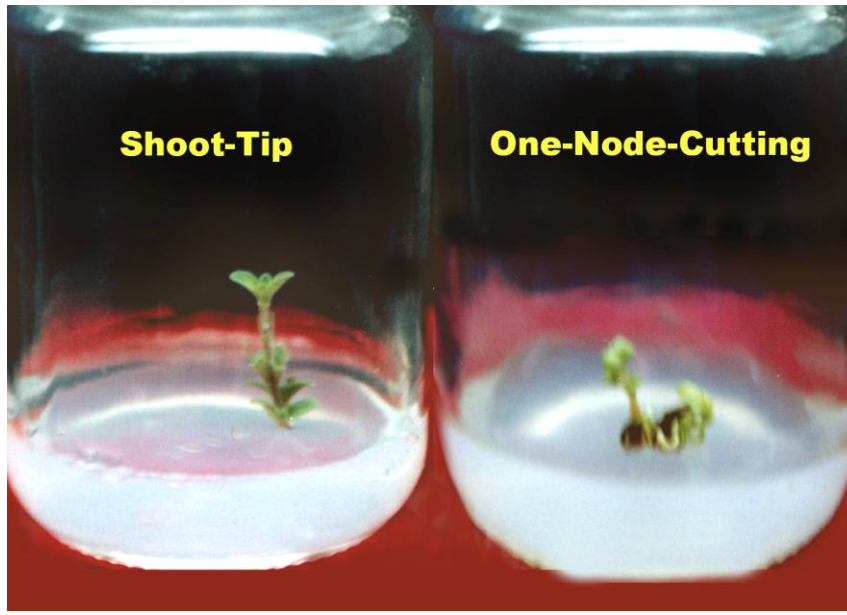


Photo (2): Effect of explant type on growth and explant development parameters of Marjoram.

4.1.1.c. Effect of anti-oxidant treatment:

Results in Table (3) and Fig. (1) verify the effect of different anti-oxidant treatments on necrosis, browning, explant development, growth and greening parameters. It is obvious that anti-oxidant solution as a pretreatment either alone or in combination with PVP resulted in a significant decrease of both necrosis and browning parameters as compared with the control and PVP treatments. Meanwhile, explant development and greening were significantly maximized as the explant pretreated with antioxidant solution and cultured on medium containing PVP.

The above results reflected the importance of combined treatment (anti-oxidant solution and PVP) in improving all parameters under study. This may be due to the combined treatment successively prohibited oxidation of phenolic compounds which resulted in decreasing the formation and discharging of free phenolic compounds to the medium and the cultured explants and in turn improved all parameters of explant development. These results are in general agreement with **Abd El-Kader (2004)** who reported that the best explant development was occurred when the explants pretreated with antioxidant solution before culturing on medium containing PVP.

Table (3): Effect of antioxidant treatment on explant development parameters of Marjoram.

Parameters / Antioxidant treatment	Necrosis	Browning	Explant development	Greening
Cont.	2.93 A	2.80 A	2.10 C	2.31 C
Anti	1.12 C	1.04 C	3.18 B	3.10 B
PVP	1.83 B	1.46 B	3.12 B	3.18 B
Anti + PVP	1.013 C	1.06 C	4.30 A	4.26 A

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

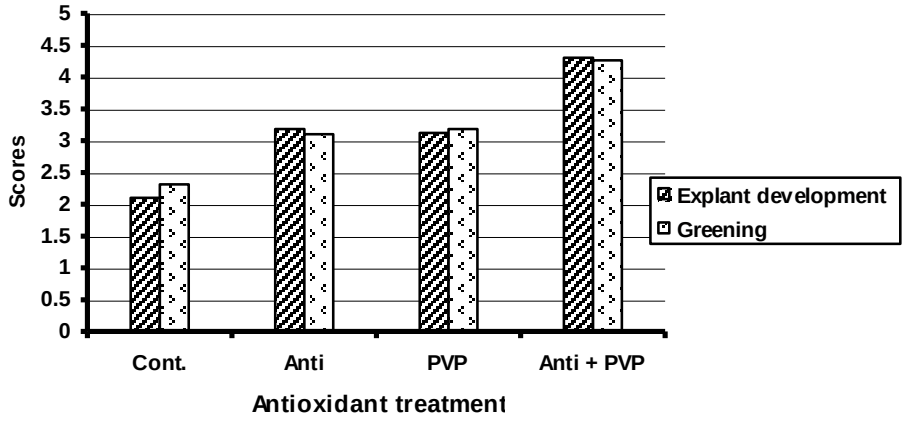


Fig. (1): Effect of antioxidant treatment on explant development and greening parameters of Marjoram.

4.1.1.d. Effect of cold pretreatment:

The data outlined in the Table (4) and Fig. (2) showed the effect of different preculture cold periods on necrosis, browning, explant development and greening parameters.

It is appeared that keeping of the explant for 3 days in refrigerator (5°C) enhanced a significant improvement of the explant as it significantly increased explant development and greening parameters in relation to the other periods and the control. However, keeping the explants in the refrigerator for 3 days succeeded in reducing both necrosis and browning significantly as compared with both control and one day treatment. On the other hand, keeping the explants for 7 days in the refrigerator resulted in harmful effect on most parameters under study i.e. explant development and greening as compared with the other studied cold periods.

Generally, cold treatments succeeded in improving all parameters under study compared with those untreated with cold (control). Also, increasing the period of cold pretreatment induced a positive effect on all parameters up to 3 days which reach the peak of improvement then declined slowly but still better than untreated (control). This may be due to the effect of cold treatment on reducing the discharge of free phenolic compounds and in turn improved explant development parameters. These results are in harmony with the findings of **Gardi *et al.* (2001)** on blackberry and kiwi fruit and **Kumar *et al.* (2001)** on lily. They preferred pre-treating the explant with cold treatment.

Table (4): Effect of cold pretreatment on explant development parameters of Marjoram.

Parameters / Cold periods (days)	Necrosis	Browning	Explant development	Greening
0.0 (Cont.)	3.73 A	4.10 A	1.17 D	1.20 D
1	2.83 B	3.47 A	2.07 C	1.97 C
3	1.97 C	2.03 B	3.67 A	3.23 A
5	2.40 BC	2.50 B	3.03 B	2.67 B
7	2.80 B	2.57 B	2.50 BC	2.0 C

Means of different cold treatment followed with the same letter withen each column are not significantly different from each other at 1% level.

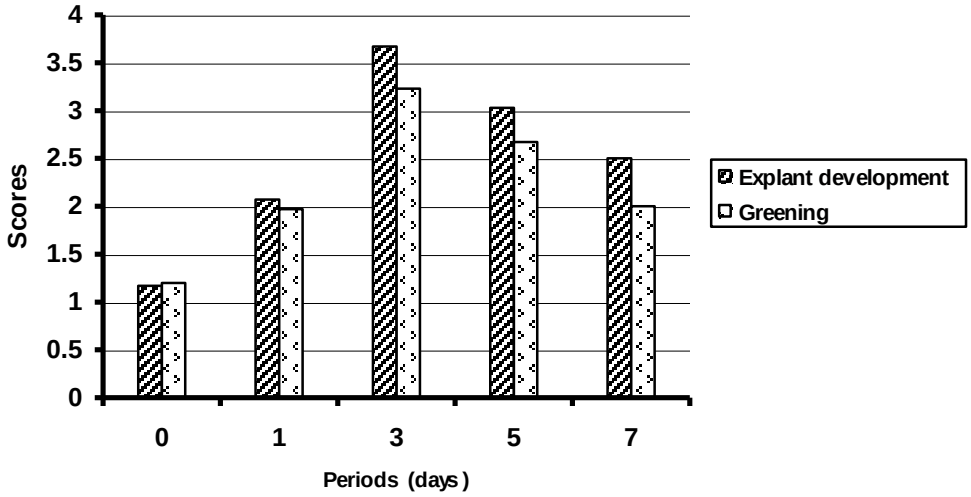


Fig. (2): Effect of cold pretreatment on explant development and greening parameters of Marjoram.

4.1.1.e. Effect of additives:

Table (5), Fig. (3) and photo (3) describes the effect of different additives to the culture medium on the necrosis, browning explant development, growth and greening parameters. It is noticed that supplementing the culture medium with adenine sulphate was significantly effective in reducing necrosis and browning while significantly improved explant development, growth and greening parameters in relation to the other additives. However, adding coconut oil to the culture medium induced an adverse effect on all parameters studied as it maximized necrosis and browning, while reduced growth, explant development, and greening parameters. Meanwhile, addition of casein hydrolysate took the second rank in improving the parameters.

The aforementioned results clarify that addition of adenine sulphate to the culture medium was more effective in improving explant development, greening and growth parameters. The positive effect of adenine sulphate may be due its stimulatory actions on growth and development. These results go in line with the findings of **Miller and Skoog (1953).** They reported that adenine sulphate has a stimulative effect on the adventions shoot formation of tobacco plants. Also, in harmony with the findings of **Dodds and Roberts (1982).** They indicated that some of morphogenic responses can be initiated with the addition of adenine sulphate.

Table (5): Effect of different additives on explant development parameters of Marjoram.

Parameters / Additives	Necrosis	Browning	Growth	Explant development	Greening
Adenine sulfate	1.22 C	1.10 C	3.10 A	3.80 A	3.10 A
Casein hydrolysate	2.50 B	2.03 B	2.03 B	2.17 B	1.87 B
Coconut oil	3.47 A	2.63 A	1.20 C	1.30 C	1.40 C

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

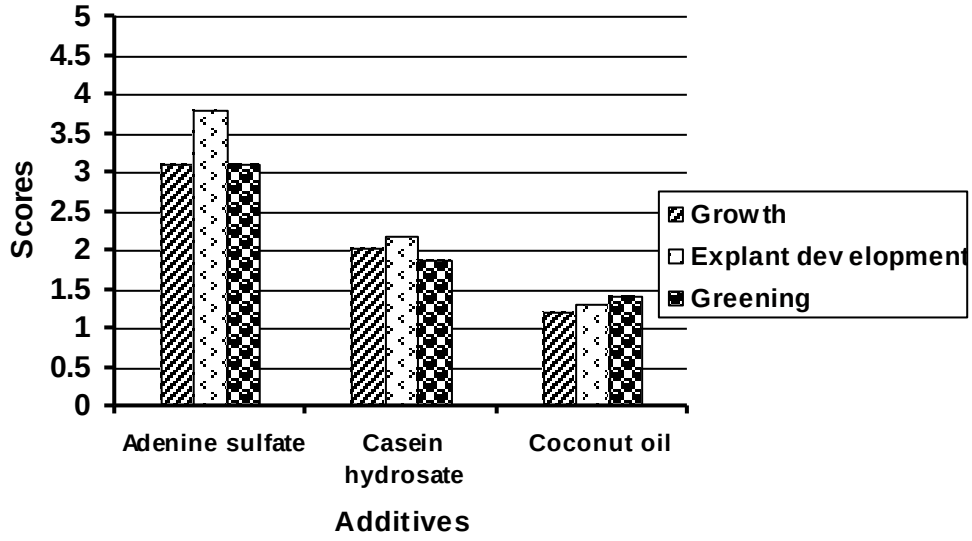


Fig. (3): Effect of different additives on explant development, growth and greening parameters of Marjoram.

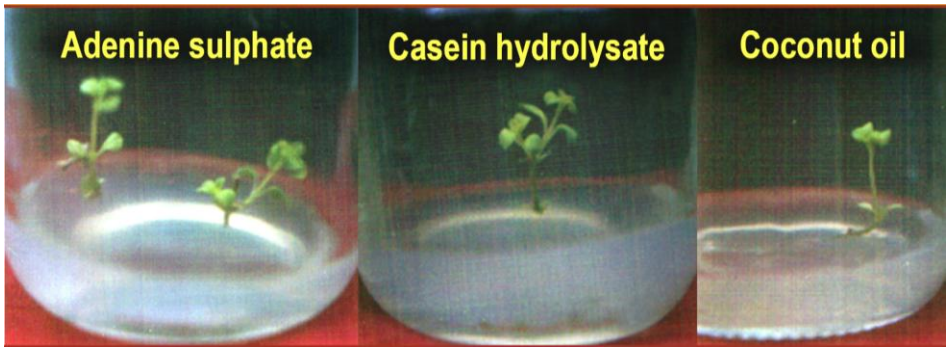


Photo (3): Effect of different additives on explant development parameters of Marjoram.

4.1.2. Proliferation stage:

4.1.2.a. Effect of cytokinin type:

Table (6), Fig. (4) and Photo (4) explains the effect of different cytokinin types i.e. kinetin, 2-ip (2-isopentenyl-adenine), and BAP (6-benzylaminopurine) on necrosis, growth, proliferation and greening. It is obvious that a significant increase in proliferation and necrosis was appeared when BAP was used instead of 2-ip and kinetin. While both growth and greening criteria were significantly maximized when kinetin was supplemented to the culture medium as compared with either 2-ip or BAP.

The aforementioned results conclude that BAP was superior in enhancing proliferation while kinetin improved growth and greening parameters.

These results confirm the findings of **Abd El-Kader (2004)** who reported that proliferation of the taxodium responded well to using of BAP.

Table (6): Effect of cytokinin type on growth and proliferation parameters of Marjoram.

Parameters / Cytokinin type	Necrosis	Growth	Proliferation	Greening
Ki	1.09 C	4.17 A	1.87 C	3.96 A
2-ip	1.46 B	2.83 B	2.60 B	2.87 B
Bap	2.00 A	2.73 B	3.90 A	2.67 B

Means of different cytokinin types followed with the same letter withen each column are not significantly different from each other at 1% level.

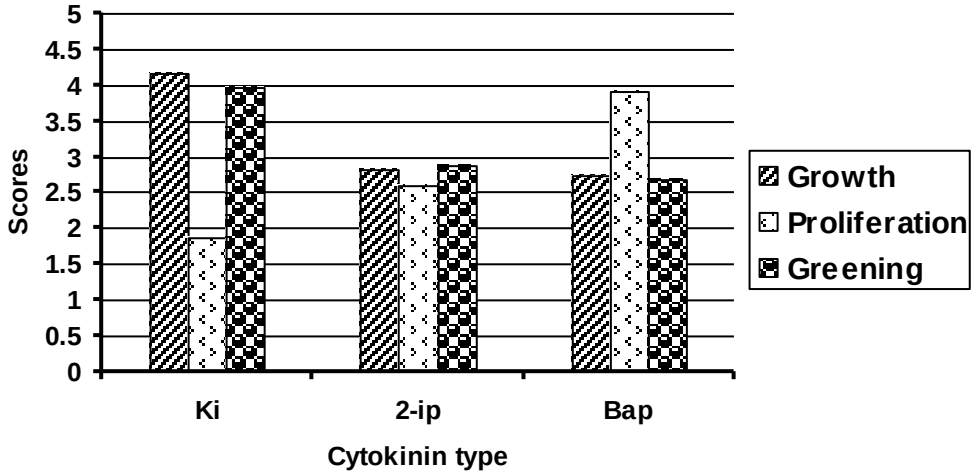


Fig. (4): Effect of cytokinin type on growth, proliferation and greening parameters of Marjoram.

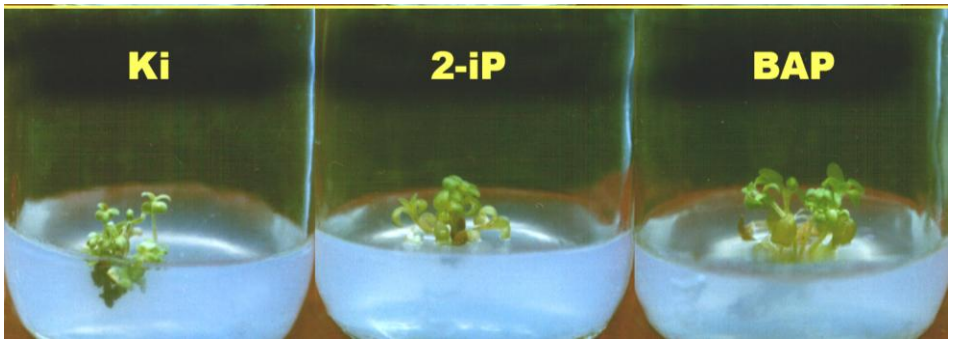


Photo (4): Effect of cytokinine type on growth and proliferation parameters of Marjoram.

4.1.2.b. Effect of BAP concentration:

Table (7), Fig. (5) and photo (5) verifies the growth, greening, proliferation and necrosis parameters as affected by different BAP concentrations. It is quite evident that most parameters i.e. growth, greening and necrosis were significantly improved with lower concentrations of BAP (0.0, 1.0 and 2.0 mg/L) as compared with higher concentrations (3.0 mg/L). On the other hand, supplementation of the culture medium with either 1.0 or 2.0 mg/L BAP enhanced significant increase of proliferation in comparison with the other concentrations. However, higher BAP concentration (3.0 mg/L) induced an adverse effect on all parameters under study.

The results conclude the addition of 1.0 or 2.0 mg/L BAP improved all parameters under study. These results go in line with the finding of **Abd El-Kader (2004)** on taxodium who found that 2.0 mg/L BAP enhanced the highest proliferation.

Table (7): Effect of BAP concentration on growth and proliferation parameters of Marjoram.

Parameters / BAP concentration	Necrosis	Growth	Proliferation	Greening
Control	1.23 C	2.57 AB	1.13 D	2.60 A
1.00	1.31 BC	2.80 A	2.80 A	2.63 A
2.00	1.55 B	2.43 AB	3.67 A	2.50 A
3.00	2.40 A	2.30 B	2.23 C	2.10 B

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

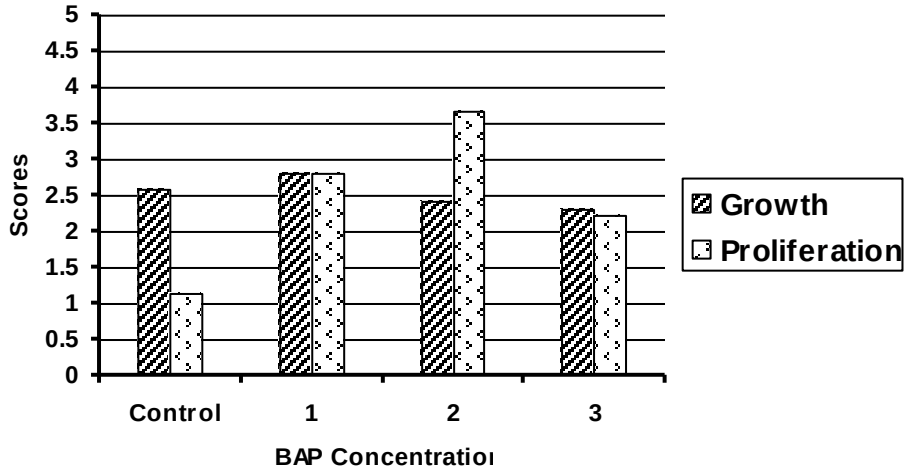


Fig. (5): Effect of BAP concentration on growth and proliferation parameters of Marjoram.

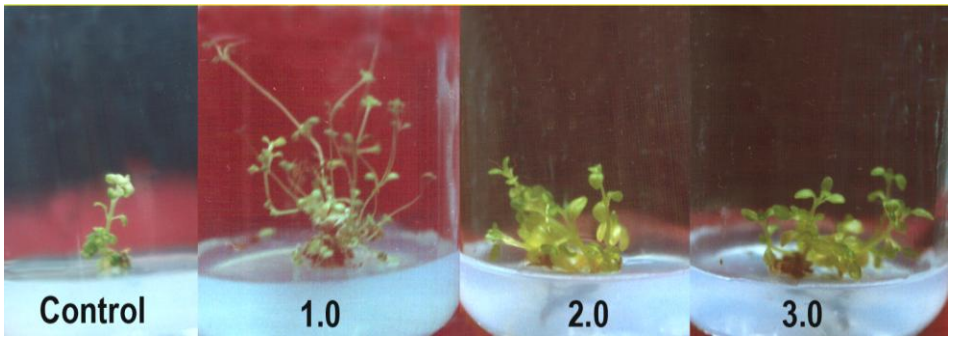


Photo (5): Effect of BAP concentration (mg/L) on growth and proliferation parameters of Marjoram.

4.1.3. Rooting stage:

4.1.3.1. Shoot elongation:

4.1.3.1.a. Effect of medium strength:

The results in Table (8), Fig. (6) and Photo (6) explain the effect of medium strength on necrosis, shoot elongation, proliferation and greening. It is appear that half strength MS medium resulted in significant increase of shoot elongation, proliferation and greening parameters as compared with the other medium strengths. However, continuous diluting of the medium strength caused a significant reduction in necrosis, shoot elongation, proliferation and greening parameters. Meanwhile, one-fourth and one-eighth medium strengths showed no statistical differences between both of them and with either full or one half medium strength when proliferation took in consideration.

In general, it is shown that half medium strength enhanced shoot elongation, greening and proliferation parameters. This was may be due to half medium strength have large quantities of free

water compared with full medium strength and more nutritional status than diluted other medium strengths. Thus, the plant under study (Marjoram) requires large free water with suitable nutritional status which available in half medium strength and in turn maximized shoot elongation, proliferation, and greening parameters. These results confirm the findings of **Xing *et al.* (1997),** they declared that shoot elongation of American chestnut was obtained on half strength MS and powdered carbon.

Table (8): Effect of medium strength on shoot elongation and proliferation parameters of Marjoram.

Parameters / Medium strength	Necrosis	Shoot elongation	Proliferation	Greening
Full	4.68 A	1.54 D	1.28 B	1.26 D
One-half	1.91 B	3.15 A	2.05 A	3.58 A
One-quarter	1.63 B	2.11 B	1.78 AB	3.13 B
One-eighth	1.23 C	1.75 C	1.79 AB	2.76 C

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

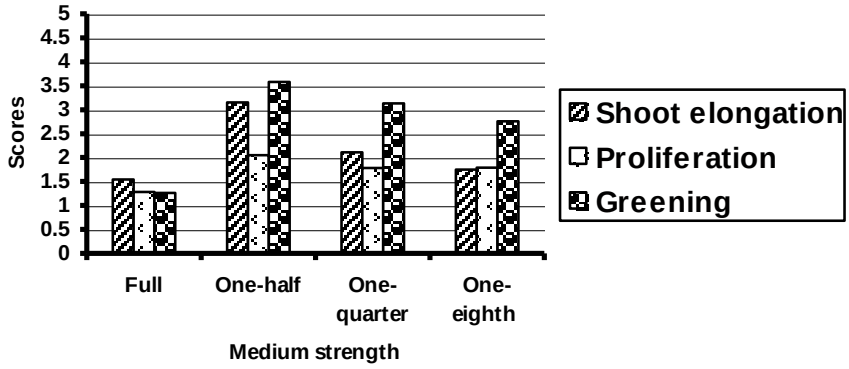


Fig. (6): Effect of medium strength on shoot elongation and proliferation parameters of Marjoram.

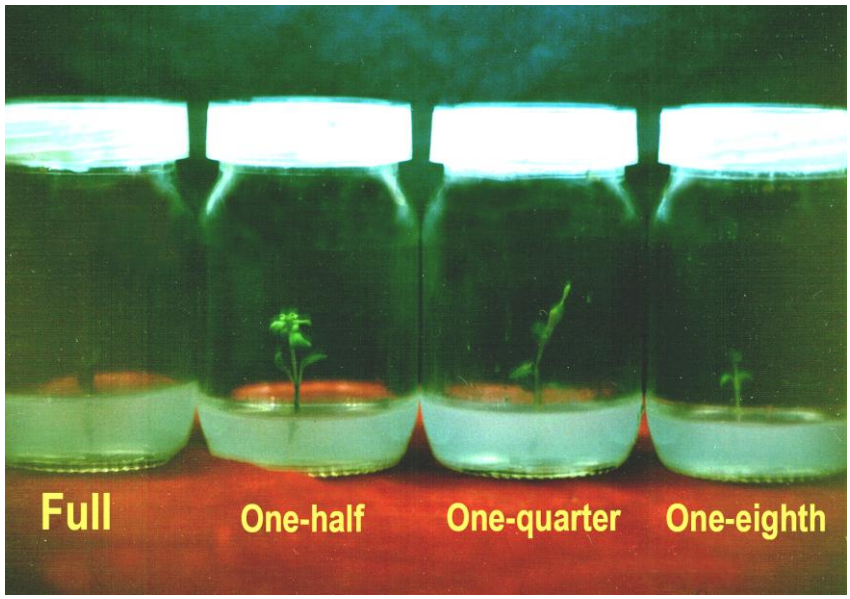


Photo (6): Effect of medium strength on shoot elongation and proliferation parameters of Marjoram.

4.1.3.1.b. Effect of GA_3 concentration:

Table (9), Fig. (7) and photo (7) reflects the effect of different GA_3 concentrations on necrosis, shoot elongation, greening and rooting. It is clear that supplementation of the culture medium with 4.0 mg/L GA_3 succeeded in inducing significant increase of shoot elongation and rooting in relation to the other GA_3 concentrations. Meanwhile, both 2.0 and 4.0 mg/L enhanced a significant increase in greening parameter in comparison with the other GA_3 concentrations. However, supplementation of culture medium with 1.0 and 2.0 mg/L GA_3 encouraged significant reduction of necrosis as compared with the others.

The above results revealed that high concentration of GA_3 (4.0 mg/L) improved shoot elongation, greening and rooting. These results in general agreement with the findings of **Bayomy (1998)** on communis pear and **Hassan (2004)** on apple rootstocks. They declared that shoot length was maximized by adding 4.0 mg/L GA_3 to the culture medium.

Table (9): Effect of different GA_3 concentrations on shoot elongation and rooting parameters of Marjoram.

Parameters / GA_3 concentrations	Necrosis	Shoot elongation	Rooting	Greening
Control	3.80 A	2.00 D	1.10 B	1.83 C
0.5	2.93 B	1.60 D	1.00 B	1.80 C
1.0	1.40 C	2.60 C	1.20 B	2.93 B
2.0	1.33 C	3.43 B	1.67 B	4.10 A
4.0	2.57 B	4.83 A	2.30 A	3.80 A

Means of different GA_3 concentrations followed with the same letter withen each column are not significantly different from each other at 1% level.

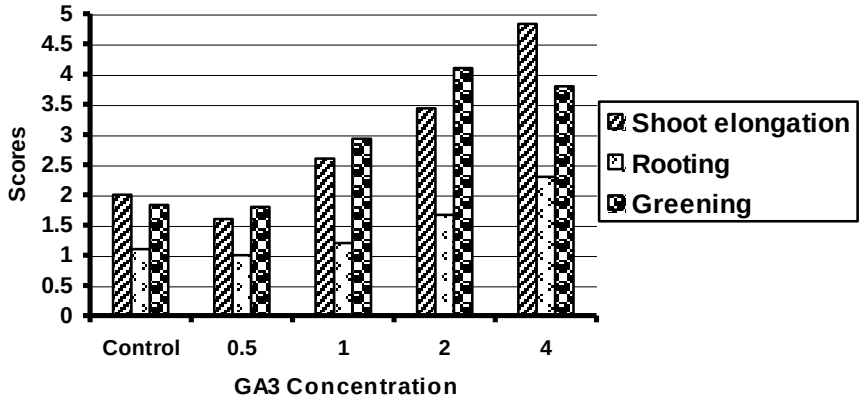


Fig. (7): Effect of different GA_3 concentrations on shoot elongation and rooting parameters of Marjoram.

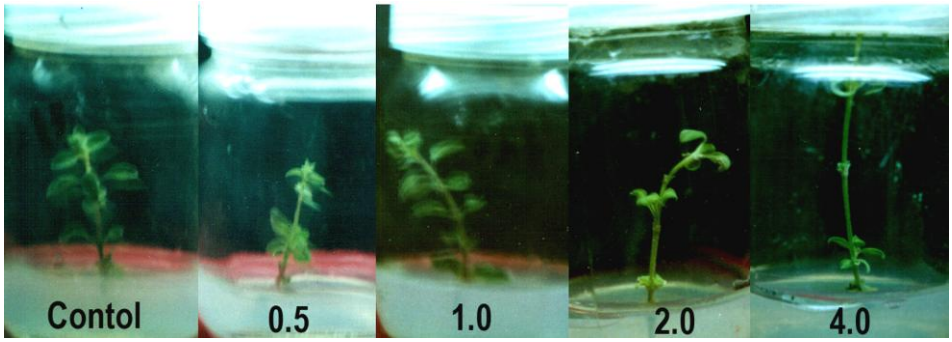


Photo (7): Effect of different GA_3 concentration (mg/L) on shoot elongation and rooting parameters of Marjoram.

4.1.3.2. Root formation:

4.1.3.2.a. Effect of auxin type:

Data tabulated in Table (10) and photo (8) showed the effect of different auxin types (IAA, NAA, IBA) on growth and rooting parameters. It is quite evident that IAA had a positive significant effect in improving greening parameter while reduced necrosis. Meanwhile, supplementation of the culture medium with either IAA or NAA resulted in significant maximizing growth parameter. However, addition of IBA to the culture medium induced significant increase of rooting in comparison with the other auxin types.

The abovementioned results summarized that IBA is preferred for encouraging the best rooting of marjoram plants. These results are in general agreement with the findings of

Woodward and Thomson (1996) on Silk tassel bush. They reported that they obtained the highest rooting when Woody plant medium supplemented with 2.5 µM indole butyric acid.

Table (10): Effect of auxin type on growth and rooting parameters of Marjoram.

Parameters / Auxin type	Necrosis	Growth	Greening	Rooting
IAA	1.10 B	3.40 A	3.17 A	1.00 B
NAA	1.83 A	2.93 A	2.60 B	1.20 B
IBA	2.27 A	2.03 B	2.10 B	3.70 A

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

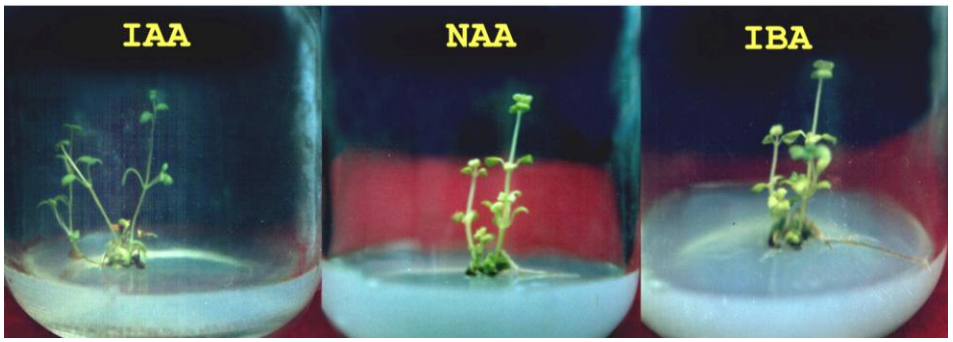


Photo (8): Effect of auxin type on growth and rooting parameters of Marjoram.

4.1.3.2.B. Effect of auxin concentration:

Table (11) clarifies the effect of different auxin concentrations on necrosis, growth, greening, and rooting parameters. It is clear that addition of IBA to the culture medium at level 2.0 mg/L succeeded in encouraging a significant increase of rooting in comparison with the other IBA concentrations. Moreover, using of lower concentration of IBA (1.0 mg/L) and control resulted in significant improvement of growth while greening was significantly improved when control was used. On the other hand, using of IBA concentrations (1.0 and 2.0 mg/L) resulted in significant increase of necrosis in relation to control.

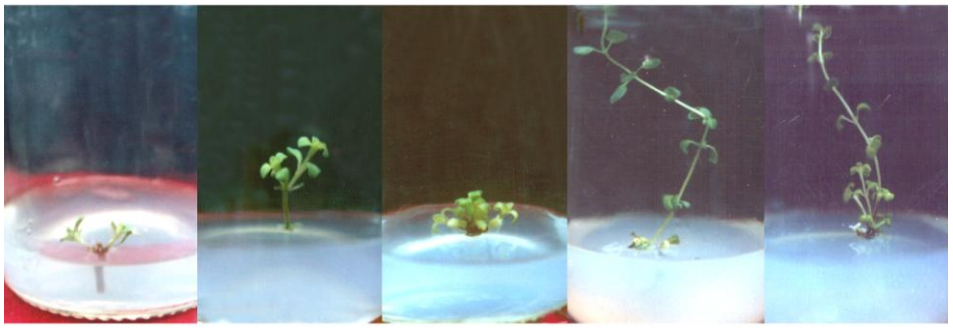
The before mentioned results concluded that using of 2.0 mg/L IBA in the culture medium maximized rooting. These results assured the findings of **Youssef (2003)** who reported that the maximum rooting of *Yucca elephantips* occurred on medium supplemented with 2.0 mg/L IBA.

Photo (9) showed the developmental phases of Marjoram explants which started by establishment stage (A & B) proliferation stage (C), shoot elongation stage, and root formation (E).

Table (11): Effect of different IBA concentration on growth and rooting parameters of Marjoram.

Parameters / IBA concentration	Necrosis	Growth	Greening	Rooting
Control	1.20 B	3.37 A	3.10 A	0.0001 C
1.00	1.73 A	3.13 A	2.57 B	1.10 B
2.00	1.90 A	2.33 B	2.10 B	2.10 A

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



(A) **(B)** **(C)** **(D)** **(E)**

Photo (9): Developmental phases of in vitro propagation of Marjoram.

(A & B) Establishment stage.

(C) Proliferation stage.

(D) Shoot elongation stage.

(E) Root formation.

4.2. Meadow saffron (Indirect regeneration):

4.2.1. Callus production:

4.2.1.a. Effect of medium type:

Data in Table (12) and Fig. (8) deals with the effect of different medium types on necrosis, browning, explant development and callus production. It is clear that Murashige and Skoog medium was significantly superior than either Woody plant medium or Nitsch and Nitsch medium in reducing necrosis and browning while increased both explant development and callus production parameters. Meanwhile, Nitsch and Nitsch medium took the second rank after MS medium in reducing necrosis while increased, explant development and callus production.

These results are in general agreement with the findings of **Jevremovic and Radojevic (2002)** on *Iris pumila* and **Chen-Jentsung and Chang-Weichin (2000)** on *Cidium orchid*. They recommended Murashige and Skoog medium for callus production.

Table (12): Effect of medium type on explant development and parameters callus production of Meadow saffron explant.

Parameters / Medium type	Necrosis	Browning	Explant development	Callus production
MS	3.05 B	2.08 B	3.14 A	3.40 A
N.N	2.80 B	4.50 A	2.14 B	2.15 B
W.P.M	4.86 A	4.92 A	1.00 C	1.00 C

Means of different medium types followed with the same letter withen each column are not significantly different from each other at 1% level.

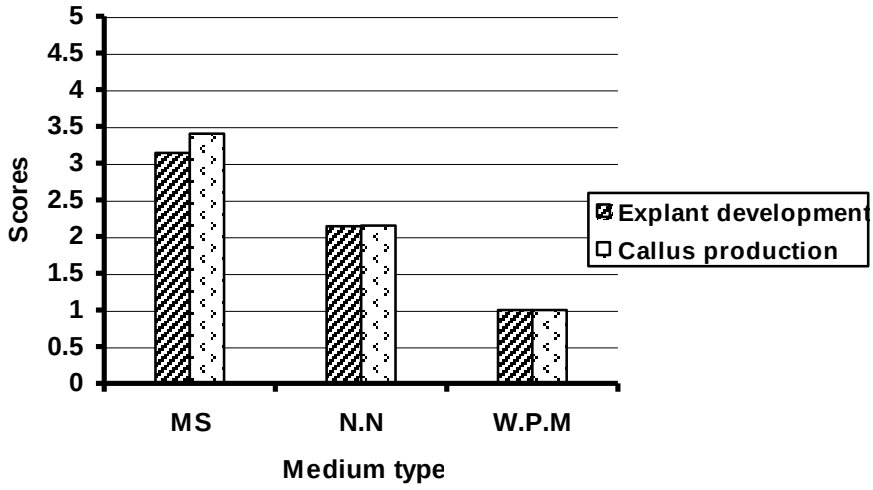


Fig. (8): Effect of medium type on explant development and parameters callus production of Meadow saffron explant.

4.2.1.b. Effect of explant:

Data in Table (13) and photo (10) show the effect of different explants on necrosis, browning, explant development and callus production. It is obvious that apical bud segments were significantly surpassed other explant type in improving all studied parameters under study i.e., necrosis, browning, explant development and callus production. Meanwhile, corm pieces explant followed apical bud segments explant in enhancing callus production, explant development and browning. However, necrosis was not significantly affected by using all explant types.

The above results showed the importance of apical bud segments in increasing callus production. This may be due to that

apical bud segments contain a lot of meristemic tissues which divided more faster than other tissues and in turn increase size of the explant. These results confirm the findings of **Youssef (2003)** on Yucca who shoot tips surpassed nodal cuttings in explant development.

Table (13): Effect of explant type on callus production of Meadow saffron.

Parameters / Explant type	Necrosis	Browning	Explant development	Callus production
Leaf disc	2.55 A	2.10 B	9.27 B	2.27 B
Corm pieces	2.22 A	1.81 B	3.07 A	2.57 AB
Apical bud segments	2.39 A	3.55 A	3.28 A	3.30 A

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

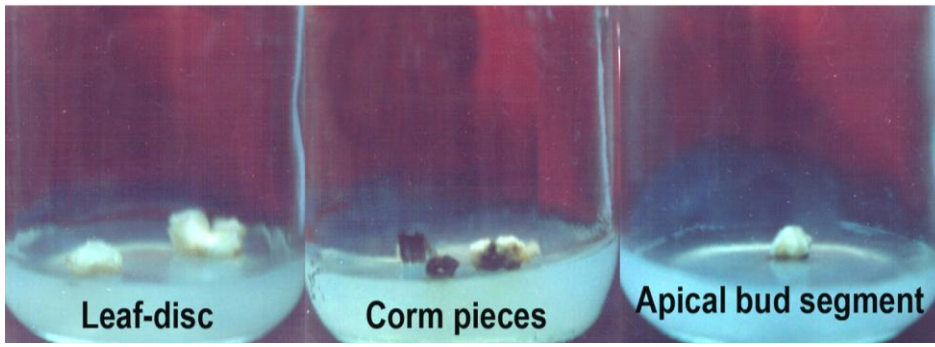


Photo (10): Effect of explant type on callus production of Meadow saffron.

4.2.1.c. Effect of anti-oxidant treatment:

Data in Table (14) and Fig. (9) show the effect of different anti-oxidant treatments on necrosis, browning, explant development and callus production. It is appear that combination treatment consisted of anti-oxidatn solution and addition of PVP to the culture medium resulted in significant decrease of both necrosis and browning parameters as compared with the other treatments. However, significancy between explant development and callus production parameters were disappeared when antioxidant solution and PVP in the medium treatments were used. On the other hand, antioxidant treatments showed a significant improvement of all parameters under study as compared with control (untreated with antioxidant).

The above results reflected the importance of combined treatment (anti-oxidant solution and PVP) in improving all parameters under study. These results are in general agreement with the findings of **Abd El-Kader (2004)** on taxodium. He recommended using the combination (antioxidant solution plus PVP) treatment for enhancing the explant development.

Table (14): Effect of antioxidant treatments on callus production parameters of Meadow saffron.

Parameters / Antioxidant treatment	Necrosis	Browning	Explant development	Callus production
Control	4.70 A	4.63 A	1.00 C	1.12 C
Anti	2.37 B	2.50 B	3.10 B	2.62 B
PVP	1.60 C	1.57 C	3.08 B	2.46 B
Anti + PVP	1.13 C	1.00 C	4.43 A	4.40 A

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

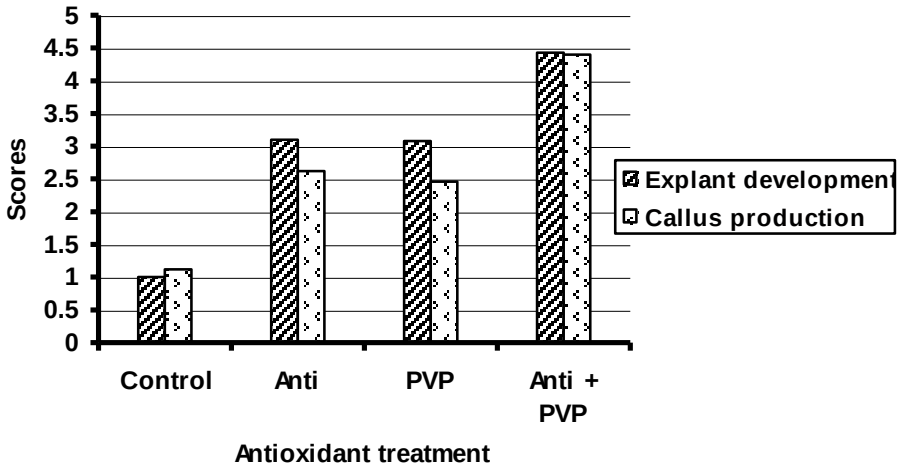


Fig. (9): Effect of antioxidant treatments on callus production parameters of Meadow saffron.

4.2.1.d. Effect of cold pretreatment:

The outlined data in the Table (15) and Fig. (10) describe the effect of different preculture cold periods on necrosis, browning, explant development and callus production. It is reflected that increasing keeping period of the explant in the refrigerator (5°C) up to 5 days before culturing encouraged the best results as it significantly reduced necrosis and browning and significantly increased the other parameters used under study (explant development and callus production) in comparison with the other cold periods. However, continuous increase of cold period to 7 days resulted in significant reduction of the most parameters under study as compared with 5 days add treatment.

The above results conclude that cold pre-treatment of the explants for 5 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 **Anderson *et al.* (2002)** on peach seed embryo. They preferred cold pretreatment for the best callus production and explant development.

Table (15): Effect of cold pretreatment on callus production parameters of Meadow saffron.

Parameters / Periods (days)	Necrosis	Browning	Explant development	Callus production
1	4.58 A	4.53 A	1.00 C	1.00 C
3	4.42 A	4.14 A	1.66 B	1.29 C
5	3.24 C	1.11 B	2.15 A	3.27 A
7	3.57 B	1.03 B	1.52 B	2.20 B

Means of different cold pretreatments followed with the same letter withen each column are not significantly different from each other at 1% level.

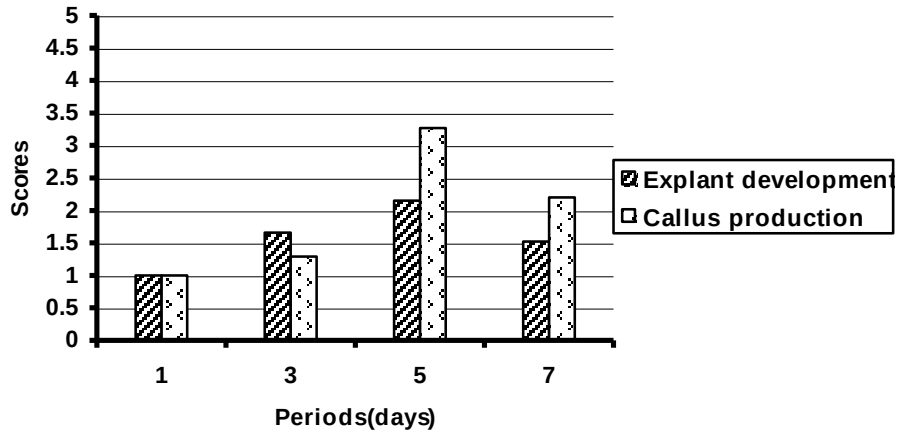


Fig. (10): Effect of cold pretreatment on callus production parameters of Meadow saffron.

4.2.1.e. Effect of 2,4-D concentration:

Table (16) and Fig. (11) reveals the effect of different 2,4-D concentration on necrosis, browning, explant development and callus production. It is noticed that there was a direct relationship between 2,4-D concentration and browning, explant development and callus production parameters. Thus, increasing of 2, 4-D concentration resulted in improving callus production parameters up to 6.0 mg/L which reached to the peak and increasing explant development and callus production followed by 4.0 mg/L then 2.0 mg/L in a descending order as compared with the control. However, increasing the concentration resulted in declining of all parameters under study.

The aforementioned results verify that culturing medium supplemented with 6.0 mg/L 2,4-D is the most effective concentration for explant development and callus production parameters. These results are in harmony with the findings of **Singh and Suresh-chand (2003)** on Leguminous tree; **Martin (2004)** on *Ardrographis paniculata*; **Thakara *et al.* (2004)** on chlorophytum and **Iyer-PV and Pai-JS (2000)** on Marjoram. They reported that addition of 2,4-D encouraged the highest callus production parameters.

Table (16): Effect of 2,4-D concentration on callus production parameters of Meadow saffron.

Parameters / 2,4-D Conc.	Necrosis	Browning	Explant development	Callus production
0.0	1.42 E	1.00 C	1.00 D	1.00 D
2.0	2.10 D	2.20 B	1.80 C	1.93 C
4.0	2.50 C	3.20 A	2.43 B	3.32 B
6.0	3.32 B	3.20 A	3.67 A	4.14 A
8.0	3.71 A	3.60 A	2.20 BC	2.03 C

Means of different 2,4-D concentrations followed with the same letter withen each column are not significantly different from each other at 1% level.

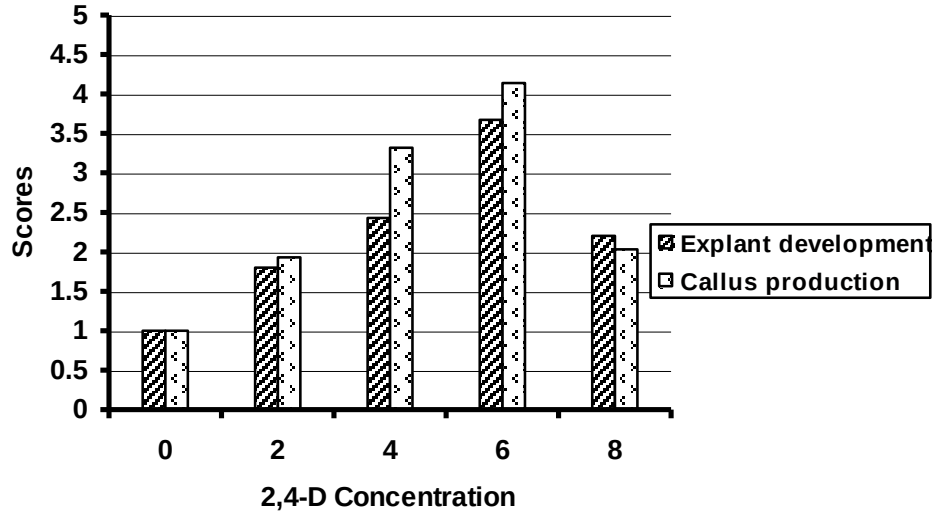


Fig. (11): Effect of 2,4-D concentration on callus production parameters of Meadow saffron.

4.2.1.f. Effect of BAP concentration:

Data tabulated in Table (17) and Fig. (12) explains the effect of different BAP (6-benzylaminopurine) on necrosis, browning, explant development and callus production. It is obvious that increasing BAP (6-benzylaminopurine) concentration resulted in improving all parameters under study as it reduced necrosis, while increased explant development and callus production. Meanwhile, supplementing the culture medium with 4.0 mg/L BAP (6-benzylaminopurine) was effective in encouraging an increase of explants development and callus production as compared with 2.0 mg/L BAP and 6.0 mg/L. On the other hand, necrosis and browning parameters were directly responsed with increasing of BAP concentrations.

Table (17): Effect of BAP concentration on callus development of Meadow saffron.

Parameters / BAP Conc.	Necrosis	Browning	Explant development	Callus production
0.0	1.41 C	4.42 A	1.11 D	1.00 D
1.0	1.47 C	4.22 A	1.80 C	1.63 C
2.0	2.19 BC	3.06 B	2.07 B	1.87 BC
4.0	2.55 B	2.09 C	3.10 A	2.70 A
6.0	3.51 A	1.80 C	2.00 B	2.03 B

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

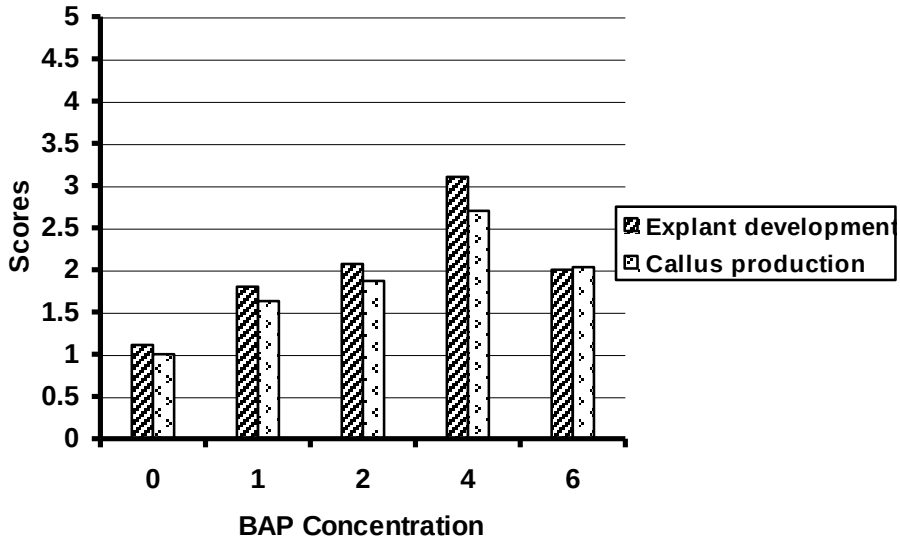


Fig. (12): Effect of BAP concentration on callus development of Meadow saffron.

The beforementioned results showed that addition of 4.0 mg/L BAP to the cultured medium maximized explant development and callus production. These results go in line with the findings of **Ritika *et al.* (2002)** on (*Rosa scented geraninum*) *Pelargonium graveolens* and **Depkuniene *et al.* (2000)** on lily. They preferred using of BAP for the highest callus production.

4.2.2.a. Effect of organic additives:

Data in Table (16) clarify that the effect of different organic additives to the culture medium on the necrosis, callus maturation, No. of lopes / callus and No. of somatic embryos / callus parameters. It is quite evident that adding yeast extract to culture medium was significantly valuable in maximizing callus

maturation, number of lobes / callus and number of somatic embryos callus developed as compared with other used additives followed by malt extract and casein hydrolysate.

Table (18): Effect of organic additives on callus maturation, No. of lopes / callus and No. of somatic embryos / callus of Meadow saffron.

Parameters / Additives	Necrosis	Callus maturation	No. of lopes / callus	No. of somatic embryos / callus
Casein hydrolysate	3.20 A	1.23 C	2.17 B	2.23 B
Malt extract	3.19 A	2.27 B	2.13 B	2.33 B
Yeast extract	2.51 B	3.86 A	10.97 A	10.82 A

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

The aforementioned results clarify that addition of yeast extract was more effective in improving both number of lobes and number of somatic embryos. These results are in harmony with the findings of **Inada *et al.* (1993)** on Cupressus; **Mahmoud (2001)** on Magnolia and **Kim-Hy Kyong *et al.* (2001)** on *Agastache rugosa.* They reported that adding of yeast extract to culture medium improved callus development.

4.2.2.b. Effect of hormonal balance:

Data tabulated in Table (19) explain the effect of hormonal balance 6-benzylaminopurine and indole-3-butyric acid (BAP and IBA ratio) on necrosis, callus maturation, number of lobes / callus

and number of somatic embryos parameters. It is appear from Table (19-A) that there is a direct relationship between BAP concentrations and improving the parameters under study as addition of BAP with high levels 2.0 mg/L resulted in improving all parameters under study with significant levels i.e. callus maturation, number of lobes / callus, and number of somatic embryos / callus. However, negative responses in all parameters were appeared when lower concentrations were used. Moreover, Table (19-B) clatifies that necrosis, callus maturation, number of lobes / callus, and number of somatic embryos / callus were significantly increased by increasing IBA concentrations up to 1.0 mg/L which induced the highest response. Furthermore, Table (19-C) reflected the interaction between 6-benzylaminepurine (BAP) and indole-3-butyric acid (IBA) concentrations (hormonal balance). It is clear that combination of 2.0 mg/L BAP and 1.0 mg/L IBA was significantly increased callus maturation, number of lobes / callus, and number of somatic embryos / callus as compared with the other interactions under study.

The abovementioned results concluded that using of 2.0 mg/L BAP and 1.0 mg/L IBA as hormonal balance induced the highest levels of all parameters studied. These results go in line with the findings of **Baccheta *et al.* (2003)**. They reported that culturing of *in vitro* leaves of four Lily hybrids on MS medium supplemented with BA and NAA at different concentrations induced the highest plantlets regeneration.

Photo (11) explain the phases of callus production development, and plantlet regeneration of Meadow saffron.

Table (19): Effect of hormonal balance on callus maturation and callus development parameters of Meadow saffron.

Table (19-A): Effect of BAP concentration.

Parameters / BAP Conc.	Necrosis	Callus maturation	No. of lopes / callus	No. of somatic embryos / callus
0.0	2.31 C	1.30 D	2.08 C	2.160 C
0.5	2.38 B	1.57 C	4.27 A	4.470 B
1.0	2.54 A	2.27 B	3.81 B	4.362 B
2.0	2.58 A	3.11 A	3.80 B	4.872 A

Means of different BAP concentration followed with the same letter withen each column are not significantly different from each other at 1% level.

Table (19-B): Effect of IBA concentration.

Parameters / IBA Conc.	Necrosis	Callus maturation	No. of lopes / callus	No. of somatic embryos / callus
0.0	2.79 A	1.77 C	2.99 C	3.36 D
0.1	2.66 B	2.02 B	3.40 B	3.85 C
0.5	2.12 D	2.14 B	3.57 B	4.08 B
1.0	2.24 C	2.31 A	3.98 A	4.57 A

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

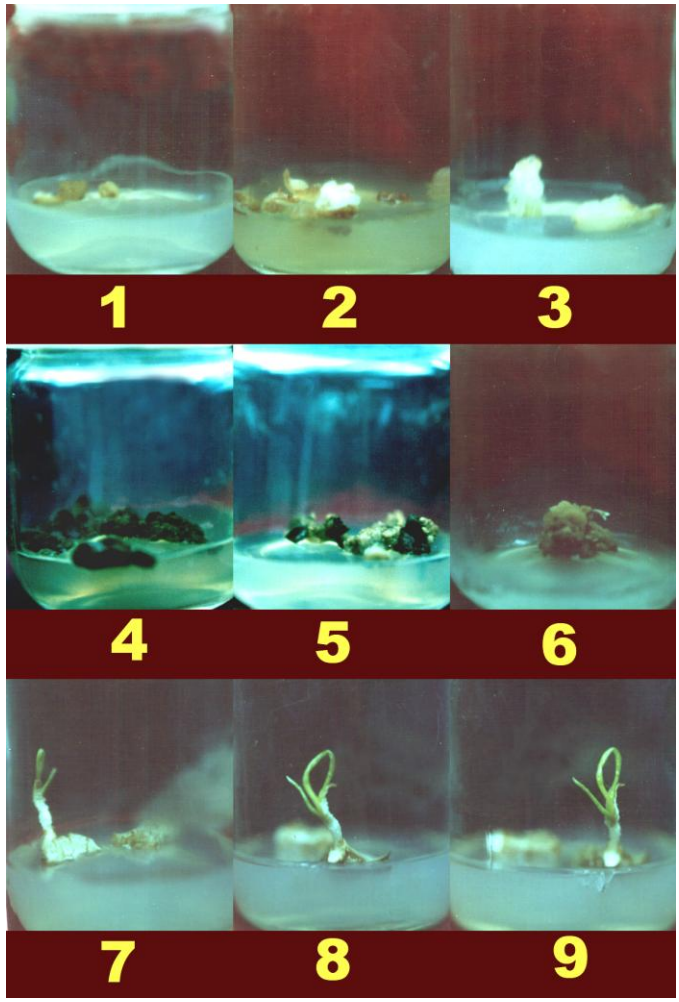


Photo (11): Developmental stage of Meadow saffron explant.

1- Starting of callus.
2- Callus formation
3- Increase of callus production.
4- Callus maturation.
5- Formation of somatic embryos.
6- Starting of regeneration.
7- Development of shoots from somatic embryos.
8- Further development of shoot.
9- Complete plantlet regeneration.