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# In vitro PROPAGATION OF COMMUNIS Pear rootstock

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#### **ABSTRACT**

Shoot tips and one node (nodal cuttings) of communis pear were cultured on either solid, semi-solid, or liquid Murashige and Skoog, Lepoivre, or Anderson media. The explants were collected at different dates and cultured on different medium strengths. Also, solid, semi-solid, and liquid Murashige and Skoog medium and different cytokinin types with different concentrations were evaluated during proliferation stage. Meanwhile, medium strengths, GA<sub>3</sub> concentrations, auxin type with different levels, and darkening treatments were concerned in this study.

It was found that shoot tips cultured on solid or semi solid Murashige and Skoog with full or one-half medium strength produced the best results. Also, collection of the explant during the period from April to June gave excellent explant development and greening. However, semi-solid medium and BAP at 4mg/L induced the highest proliferation. Moreover, using full or half medium strength as well as 4mg/L GA<sub>3</sub> gave the highest shoot elongation and root initiation. Furthermore, liquid medium supplemented with 1.0mg/L IBA and applying outer or surface coverage or combination of both induced the highest root formation.

Key words: In vitro, communis pear.

## INTRODUCTION

Communis pear is the most suitable rootstock for pear in Egypt. It is mainly propagated by seeds imported from abroad. Consequently, high costs of transportation, agriculture quarantine, and relatively low viability are the main problems facing the use of communis seeds. Hence, micropropagation is the basic alternative to overcome all these problems. Tissue culture industry is preferred for the production of large number of homogenous plants similar to the mother trees and free from most spreading diseases.

The ultimate goal of this study was to find out the best posibilities of establishing communis pear plantlets in vitro through identifying suitable medium type, state, and strength as well as the best collection date and additives. Also,

determing the best tools of multiplying and rooting of these plantlets for getting large numbers of homogenous and healthy plants.

#### MATERIALS AND METHODS

This study was conducted in the Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture, Moshtohor, Zagazig University during the period from 1995 to 1997.

New growing shoots of communis pear (*Pyrus communis L*.) rootstock were taken monthly around the year. The collected new shoots were subjected to the running water for 15 minutes then immersed in 10% Clorox solution (0.5% NaOCl ) with two drops of Tween-20 for 15 minutes, then immersed in sterilized distilled water 3 times, for 5 minutes each.

Shoot tips of communis pear were excised from the terminal parts with 5 mm long and the rest of the shoot parts were divided into one-node cuttings (nodal cuttings). These parts (shoot tips and nodal cuttings) were aseptically dissected and termed as explants.

Accordingly, these explants were cultured on different nutrient media supplemented with 0.5mg/L BAP (6-benzylaminopurine), 0.1mg/L IBA (Indole-3-butyric acid), 30gm/L sucrose and 7gm/L Difico Bacto agar. The pH of the media was adjusted to 5.7 and autoclaved at 121 $^{\circ}$ C and 15 lb/in² for 15 minutes. The cultured explants were incubated under 16 hours of artificial light (fluorescent light at 30  $\mu$ M/m³/sec) and 8 hours of dark at average temperature of 28-30 $^{\circ}$ C. Subculturing was done regularly at 4 weeks intervals in all stages and experiments.

The following experiments were carried out:

## I- Establishment stage:

#### I-a- Effect of medium type and state as well as explant type:

Shoot tips and nodal cuttings were taken at the begining of the growing season and cultured either on solid (7.0g/L agar), semi-solid (5.0g/L agar), or liquid medium (free from agar) of Murashige and Skoog (MS; 1962), Lepoivre (Lepoivre, et al 1978), and Anderson (Anderson, 1978) media to find out the best explant type developed on suitable medium type and state.

#### I-b- Effect of explant collection date:

Shoot tips were taken monthly around the year and cultured on solidified MS medium to determine the suitable date for explant collection during the lowest level of phenolic compounds in tissues. On this concern, the year was divided into 4 quarters as follow:

- The first quarter: samples collected during April, May and June.
- The second quarter: samples collected during July, August, and September.
- The third quarter: samples collected during October, November, and December.
- · The fourth quarter: samples obtained during January, February, and March.

I-c- Effect of medium strength:

Shoot tips were cultured on either full, one-half, one-fourth, and oneeighth MS medium to detect the most suitable medium strength to encourage healthy shoots regeneration.

**II- Proliferation stage:** 

The regenerated shoots from establishment stage were considered as explants for proliferation stage. The used culture medium was the same basal medium used in establishment stage with some modifications in growth regulators.

II-a- Effect of medium state:

Different medium states (solid, semi-solid, and liquid) of MS medium were used to investigate the best state to induce the highest proliferation.

II-b- Effect of cytokinin type and concentration:

Kinetin and BAP were added to the MS medium with different levels i.e. 2,4,6mg/L to identify the best cytokinin type and concentration.

III- Rooting stage:

III-1- Shoot elongation:

III-1-a- Effect of medium strength:

Full, one-half, one-fourth, and one-eighth medium strengths were concerned to select the most suitable medium strength to enhance shoot elongation and root initiation.

III-1-b- Effect of GA3 concentration:

Different GA<sub>3</sub> concentrations i.e. 0.0, 2.0, 4.0, 6.0, and 8.0mg/L were tested to induce the highest shoot elongation.

III-2- Root formation:

III-2-a- Effect of auxin type and concentration:

Indole acetic acid (IAA), indole-3-butyric acid (IBA), and naphthalene acetic acid (NAA) were supplemented to MS medium at levels of 0.0, 0.5, 1.0, 2.0, and 4.0mg/L to select the best auxin type and concentration to enhance root formation.

III-2-b- Effect of medium state:

Solid, semi-solid, and liquid MS medium states were evaluated to obtain sufficient root formation.

III-2-c- Effect of darkening treatment:

Different darkening treatments were formulated to encourage root formation. Thus, the following treatments were tested:

1- Control: no darkening.

2- Activated charcoal: added to the medium at the rate of 300mg/L.

- 3- Surface coverage: dark polyethylene coverage on the surface of the medium.
- 4- Outer coverage: covering the medium portion in the jar with black sheet.
- 5- Combination of surface and outer coverage.

#### Data and Calculations:

Scores were given for necrosis, explant development, shoots regeneration, shoots elongation, browning, plantlets regeneration, proliferation, greening, root initiation, root formation as follow:

Negative results = 1; below average = 2; average = 3; above average = 4; and excellent = 5 according to (Pottino, 1981).

Generally, all treatments used in this study were arranged in a complete randomized block design with 6 replicates for each treatment. The obtained data were statistically analysed according to Snedecor and Cochron, (1980) and the means were differentiated using Duncan's multiple range test at 5% level as described by (Duncan, 1955).

#### RESULTS AND DISCUSSION

#### I- Establishment stage:

I-a- Effect of medium type and state as well as explant type:

Data in Table (1-A) indicates that Murashige and Skoog medium significantly reduced necrosis and increased both explant development and greening compared with the other used medium types. Meanwhile, Table (1-B) reflects that solid and liquid medium states reduced necrosis significantly in comparison with the semi-solid one. On the other hand, solid medium was superior statistically in improving explant development and greening compared with other medium states. In contrast, semi-solid medium had significant adverse effect on all parameters studied during establishment stage. Furthermore, Table (1-C) and Photo (1) clarify that shoot tips were the best explant type as it significantly reduced necrosis, increased explant development and greening as compared with nodal cuttings explant. With respect to the different interactions, Table (1-D) pointed out that Murashige and Skoog in either solid or liquid states as well as liquid Lepoivre medium reduced necrosis statistically. However, significant increase in both explant development and greening occurred when solid Murashige and Skoog medium was used. Moreover, it is obvious from Table (1-E) that significant differences between different combinations of medium and explant types were lacking when necrosis and greening parameters were taken into consideration. On the other hand, culturing shoot tips on Murashige and Skoog medium induced significant increase in explant development compared with the other combinations in this respect. Besides, Table (1-F) shows that culturing shoot-tips on solid medium is preferable since it significantly reduced necrosis and consequently increased explant development. However, different combinations gave the same effect statistically with respect to greening. Meanwhile, Table (1-G) indicates that combinations of medium type, medium state and explant type failed to induce any statistical differences among them when both necrosis and explant development parameters were concerned. On contrast, culturing shoot-tips explant on solid Murashige and Skoog medium significantly surpassed other combinations in improving greening parameter.

Generally, the aforementioned results reflect that shoot-tips explants were better than nodal cutting ones. Such result agree with the findings of James and

Thurbon, (1978) on apple M9 & M26 rootstook; and Jones et al (1982) on apple and plum. They recommended apical parts of shoots with 5mm long as explants. Also, the results indicate that solid medium is preferred which go in line with the findings of Vinterhalter and Neskovic, (1992) who found that cultures of quince were initiated on solidified medium. In-addition, the results revealed that Murashige and Skoog medium is the best medium type which was in line with the findings of Singha, (1984) who recommended Murashige and Skoog medium as a basal medium either for Seckel pear or quince.

Table (1): Effect of different medium types, states and explants on explant development parameters of communis pear.

1-A: Effect of medium type:

| Growth parameters   | Nonnain  | E                   | · · ·    |  |
|---------------------|----------|---------------------|----------|--|
| Medium type         | Necrosis | Explant development | Greening |  |
| Murashige and Skoog | 1.96 C   | 2.20 A              | 2.20 A   |  |
| Lepoivre            | 2.24 B   | 1.76 B              | 1.76 B   |  |
| Anderson            | 3.95 A   | 1.35 C              | 1.54 C   |  |

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

## 1-B: Effect of medium state:

| Growth parameters | Necrosis | Evalent             | Croning  |  |
|-------------------|----------|---------------------|----------|--|
| Medium state      | Necrosis | Explant development | Greening |  |
| Solid             | 2.44 B   | 2.39 A              | 2.44 A   |  |
| Semi-solid        | 3.15 A   | 1.24 C              | 1.35 C   |  |
| Liquid            | 2.56 B   | 1.69 B              | 1.70 B   |  |

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

1-C: Effect of explant type:

| Growth parameters  Explant type | Necrosis | Explant development | Greening |  |
|---------------------------------|----------|---------------------|----------|--|
| Shoot-tips                      | 2.43 B   | 1.96 A              | 1.98 A   |  |
| One-node cuttings               | 3.00 A   | 1.58 B              | 1.69 B   |  |

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

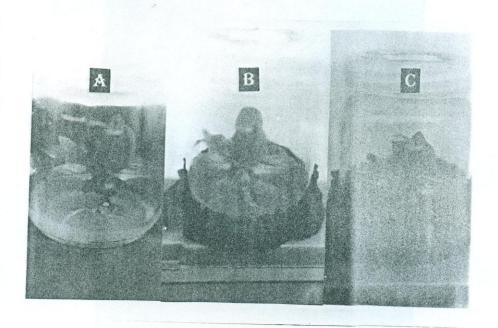


Photo (3): Effect of different different darkening treatments on growth and rooting of communis pear.

A = Control.

B = Outer coverage.

C = Surface + outer coverage.

1-D: Effect of the interaction between medium state and medium type:

| Growth<br>para-<br>meters | Necrosis |                | Explant<br>development |       |                | Greening |        |                |        |
|---------------------------|----------|----------------|------------------------|-------|----------------|----------|--------|----------------|--------|
| Medium<br>state           | Solid    | Semi-<br>solid | Liquid                 | Solid | Semi-<br>solid | Liquid   | Solid  | Semi-<br>solid | Liquid |
| Medium<br>type            |          |                |                        |       |                |          |        |                |        |
| Murashige<br>and Skoog    | 1.72DE   | 2.78B          | 1.39E                  | 3.00A | 1.28E          | 2.33B    | 2.78A  | 1.39DE         | 2.45B  |
| Lepoivre                  | 1.83D    | 2.67B          | 2.22C                  | 2.33B | 1.33DE         | 1.61CD   | 2.39BC | 1.28EF         | 1.61D  |
| Anderson                  | 3.78A    | 4.00A          | 4.06A                  | 1.83C | 1.11E          | 1.11E    | 2.17C  | 1.39DE         | 1.06F  |

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

1-E: Effect of the interaction between medium type and explant type:

| Growth parameters   | Necrosis      |              | Explant<br>development |              | Greening      |              |  |
|---------------------|---------------|--------------|------------------------|--------------|---------------|--------------|--|
| Explant type        | Shoot-<br>tip | One-<br>node | Shoot-                 | One-<br>node | Shoot-<br>tip | One-<br>node |  |
| Medium type         |               | cutting      |                        | cutting      |               | cutting      |  |
| Murashige and Skoog | 1.70 A        | 2.22 A       | 2.56 A                 | 1.85 BC      | 2.37 A        | 2.04 A       |  |
| Lepoivre            | 2.00 A        | 2.48 A       | 1.89 B                 | 1.63 CD      | 1.85 A        | 1.67 A       |  |
| Anderson            | 3.59 A        | 4.30 A       | 1.44 DE                | 1.26 E       | 1.70 A        | 1.37 A       |  |

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

1-F: Effect of the interaction between medium state and explant type:

| Growth parameters          | Necrosis      |                         | Explant development |                         | Greening      |                         |
|----------------------------|---------------|-------------------------|---------------------|-------------------------|---------------|-------------------------|
| Explant type  Medium state | Shoot-<br>tip | One-<br>node<br>cutting | Shoot-<br>tip       | One-<br>node<br>cutting | Shoot-<br>tip | One-<br>node<br>cutting |
| Solid                      | 2.04 D        | 2.85 B                  | 2.74 A              | 2.04 B                  | 2.63 A        | 2.26 A                  |
| Semi-solid                 | 2.82 B        | 3.48 A                  | 1.33 CD             | 1.15 D                  | 1.52 A        | 1.18 A                  |
| Liquid                     | 2.44 C        | 2.67 BC                 | 1.82 B              | 1.55 C                  | 1.78 A        | 1.63 A                  |

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

1-G: Effect of the interaction between medium type, medium state and

| Growth                 | Medium     |                  | M                      | ledium typ | e        |
|------------------------|------------|------------------|------------------------|------------|----------|
| parameters state       |            | Explant type     | Murashige<br>and Skoog | Lepoivre   | Anderson |
|                        | Soild      | Shoot-tip        | 1.33 A                 | 1.45 A     | 3.33 A   |
|                        |            | One-node cutting | 2.11 A                 | 2.22 A     | 4.22 A   |
| Necrosis               | Semi-solid | Shoot-tip        | 2.45 A                 | 2.44 A     | 3.56 A   |
| Liquid                 |            | One-node cutting | 3.11 A                 | 2.89 A     | 4.45 A   |
|                        | Liquid     | Shoot-tip        | 1.33 A                 | 2.11 A     | 3.89 A   |
|                        |            | One-node cutting | 1.45 A                 | 2.33 A     | 4.22 A   |
|                        | Soild      | Shoot-tip        | 3.67 A                 | 2.56 A     | 2.00 A   |
|                        |            | One-node cutting | 2.33 A                 | 2.11 A     | 1.67 A   |
| Explant<br>development | Semi-solid | Shoot-tip        | 1.44 A                 | 1.33 A     | 1.22 A   |
| development            |            | One-node cutting | 1.11 A                 | 1.33 A     | 1.00 A   |
|                        | Liquid     | Shoot-tip        | 2.56 A                 | 1.78 A     | 1.11 A   |
|                        |            | One-node cutting | 2.11 A                 | 1.44 A     | 1.11 A   |
| 10                     | Soild      | Shoot-tip        | 3.00 A                 | 2.56 B     | 2.33 BC  |
|                        |            | One-node cutting | 2.56 B                 | 2.22 BC    | 2.00 CI  |
| Greening               | Semi-solid | Shoot-tip        | 1.56 EF                | 1.22 FGH   | 1.78 DE  |
|                        |            | One-node cutting | 1.22 FGH               | 1.33 FGH   | 1.00 H   |
|                        | Liquid     | Shoot-tip        | 2.56 B                 | 1.78 DE    | 1.00 H   |
|                        |            | One-node cutting | 2.33 BC                | 1.44 EFG   | 1.11 GF  |

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

## I-b- Effect of explant collection date:

Table (2) shows that the first quarter, (April, May and June) is the best time for explant collection. This period is usually characterized with lower accumulation of phenolic compounds, which decrease necrosis and simultaneously increase explant development and greening. However, the reverse was true when the explants were collected during the fourth quarter, (January, February, and March).

The previous results showed that necrosis decreased sharply at the beginning of the growing season then increased with the advance in growing season. However, the opposite was true for both explant development and greening. These results might have relation with the accumulation of the phenolic compounds in the tissues mainly the free from which decreased as the growing season go by and this greatly affect on growth development. These results are in general agreement with the findings of Kim, et al., (1982); and Joung & Ko (1983) who preferred spring for shoot collection in some apple cvs.

#### I-c- Effect of medium strength:

It is quite evident from Table (3) that diluting medium strength to onequarter or one-eighth succeeded significantly in reducing necrosis. However, full and one-half medium strengths were valuable in improving growth by increasing shoots regeneration and greening as compared with the other medium strengths except one-quarter medium strength in case of greening parameter. Meanwhile, differences between full and one-half medium strengths from one side and from one-quarter and one-eighth medium strengths from the other were insignificant.

Table (2): Effect of explant collection date on explant development

parameters of communis pear.

| The state of the s |           |                     |          |  |  |  |  |
|--|-----------|---------------------|----------|--|--|--|--|
| Growth parameters  | Necrosis  | Explant development | Greening |  |  |  |  |
| Explant collection date  | 110010313 | development         |          |  |  |  |  |
| The first quarter  | 1.56 C    | 4.00 A              | 2.78 A   |  |  |  |  |
| The second quarter   | 1.67 C    | 3.22 B              | 2.33 AB  |  |  |  |  |
| The third quarter  | 2.67 B    | 2.33 C              | 2.11 B   |  |  |  |  |
| The fourth quarter   | 3.33 A    | 1.56 D              | 1.56 C   |  |  |  |  |

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

Concisely, the above results show that full and one-half medium strengths increased shoots regeneration and greening. These results partially agreed with the findings of Snir & Erez, (1980); Barghchi & Alderson (1985); and Oh. et al., (1991). They recommended half-medium strength for the best shoots regeneration.

Table (3): Effect of different MS medium strengths on shoots regeneration

parameters of communis pear

| Growth parameters | Necrosis | Shoots regeneration | Greening |  |
|-------------------|----------|---------------------|----------|--|
| Medium strength   |          |                     |          |  |
| Full              | 2.33 A   | 3.56 A              | 2.45 A   |  |
| One-half          | 2.22 A   | 3.33 A              | 2.33 A   |  |
| One-quarter       | 1.33 B   | 2.33 B              | 2.11 AB  |  |
| One-eighth        | 1.33 B   | 1.56 C              | 1.56 B   |  |

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

#### II- Proliferation stage:

## II-a- Effect of medium state:

Table (4) indicates that semi-solid medium induced significant increases in necrosis and proliferation as compared with other medium states. However, greening was significantly improved by liquid medium. Meanwhile, slight differences were noticed in growth when solid, semi-solid or liquid medium was involved.

Table (4): Effect of medium state on growth and proliferation parameters of

| communis pea      | communis pear. |        |            |          |  |  |  |  |
|-------------------|----------------|--------|------------|----------|--|--|--|--|
| Growth parameters | Necrosis       | Growth | Prolifera- | Greening |  |  |  |  |
| Medium state      |                |        | tion       |          |  |  |  |  |
| Solid             | 1.56 B         | 2.33 A | 3.33 B     | 2.44 B   |  |  |  |  |
| Semi-solid        | 2.56 A         | 1.78 A | 4.11 A     | 1.78 C   |  |  |  |  |
| Liquid            | 1.44 B         | 2.67 A | 1.56 C     | 3.44 A   |  |  |  |  |

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

#### II-b- Effect of cytokinin type and concentration:

It is clear from Table (5-A) that kinetin surpassed BAP significantly in reducing necrosis and increasing both growth and greening parameters, while proliferation was significantly increased by BAP as compared with kinetin. Meanwhile, Table (5-B) and Photo (2) show that the lower concentration i.e. 2mg/L enhanced a significant increase in growth and greening while necrosis was significantly decreased. However, supplementation the medium with 4mg/L gave a significant increase in proliferation as compared with the other concentrations used. On the other hand, the highest concentration (6mg/L) caused an adverse effect on all parameters under investigation. Furthermore, Table (5-C) reveals that supplementation the medium with either 2 or 4mg/L kinetin or 2mg/L BAP resulted in reducing necrosis. Besides, 2mg/L BAP was valuable in increasing proliferation as compared with the other combinations. However, all combinations failed to induce any statistical differences when either growth or greening parameters were concerned.

In general, the results point out that kinetin was superior to BAP in improving growth and greening as well as reducing necrosis. However, 4mg/L BAP was the best for proliferation, which might indicate that kinetin is less efficient than BAP and consequently its encouraged growth and greening while BAP encouraged proliferation on account of growth. These results are in general agreement with the findings of Emam, (1997) on grape and strawberry and Zaied, (1997) on stone fruits. They found that kinetin or zeatin at 2mg/L enhanced growth and improved greening, while BAP at the same level encouraged proliferation.

#### III- Rooting stage:

#### III-1- Shoot elongation:

## III-1-a- Effect of medium strength:

Table (6) reveals that full and one-half medium strengths enhanced significant increase in shoot elongation, root initiation, and greening in relation to the others. However, the reverse was true in case of necrosis. These results go in line partially with the findings of Standardi & Romani (1990) on apple and Dumanogla et al., (1994) on quince. They recommended half-medium strength for enhancing root initiation and shoot elongation.

Table (5): Effect of different cytokinin types and concentrations on growth and prolifertion parameters of communis pear.

5-A: Effect of cytokinin type:

| Growth parameters Cytokinin type | Necrosis | Growth | Proliferation | Greening |
|----------------------------------|----------|--------|---------------|----------|
| Kinetin                          | 1.43 B   | 3.19 A | 2.37 B        | 3.22 A   |
| BAP                              | 3.15 A   | 2.26 B | 3.15 A        | 2.30 B   |

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

#### 5-B: Effect of concentration:

| Growth parameters     | Necrosis | Growth   | Proliferation | Greening |  |
|-----------------------|----------|----------|---------------|----------|--|
| Concentrations (mg/L) |          | dedel ma |               | o.coming |  |
| 2                     | 1.22 C   | 3.22 A   | 2.83 B        | 3.56 A   |  |
| 4                     | 2.30 B   | 2.83 A   | 3.33 A        | 2.56 B   |  |
| 6                     | 3.33 A   | 2.11 B   | 2.11 C        | 2.17 B   |  |

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

## 5-C: Effect of the interaction between cytokinin type and concentration:

| Growth<br>parameters                          | N     | ecros | is    | (     | Growt | h     | Pro   | lifera | tion  | G     | reeni | ng   |
|---|-------|-------|-------|-------|-------|-------|-------|--------|-------|-------|-------|------|
| Concentra-<br>tion(mg/L)<br>Cytokinin<br>type | 2     | 4     | 6     | 2     | 4     | 6     | 2     | 4      | 6     | 2     | 4     | 6    |
| Kinetin                                       | 1.00D | 1.28D | 2.00C | 3.45A | 3.33A | 2.78A | 1.56D | 3.22B  | 2.33C | 3.78A | 3.11A | 2.78 |
| BAP   | 1.45D | 3.33B | 4.67A | 3.00A | 2.33A | 1.45A | 4.11A | 3.44B  | .89D  | 3.33A | 2.00A | 1.6  |

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

#### III-1-b- Effect of GA<sub>3</sub> concentration:

It is clear from Table (7) that shoots elongation significantly increased when 4mg/L  $GA_3$  was added to the medium in relation to other  $GA_3$  concentrations. Meanwhile, greening and root primordia were significantly increased when the medium was supplemented with either 2 or 4mg/L  $GA_3$ .

## III-2- Root formation:

## III-2-a- Effect of auxin type and concentration:

Table (8-A) shows that IBA surpassed other auxin types in increasing callus and necrosis. However, rooting was significantly increased when either IBA or NAA was added to the medium. On the other hand, different auxin types

failed to induce any statistical differences when growth and greening parameters were taken into consideration.

Table (6): Effect of different medium strengths on necrosis, greening, shoots elongation, and root initiation of communis pear.

| Growth parameters | Necrosis | Greening | Shoot elongation | Root initiation |
|-------------------|----------|----------|------------------|-----------------|
| Medium strength   |          |          |                  |                 |
| Fuil              | 1.00 B   | 2.56 A   | 3.33 A           | 2.45 A          |
| One-half          | 1.00 B   | 2.67 A   | 3.67 A           | 2.45 A          |
| One-quarter       | 2.22 A   | 2.11 AB  | 1.67 B           | 1.55 B          |
| One-eighth        | 2.44 A   | 1.56 B   | 1.22 C           | 1.11 B          |

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

Table (7): Effect of different concentrations of gibbrelic acid (GA<sub>3</sub>) on shoot elongation, greening and rooting parameters of communis pear.

| Growth parameters     | Shoot elongation | Greening | Root-<br>premordia |
|-----------------------|------------------|----------|--------------------|
| Concentrations (mg/L) | - Conganion      | g        |                    |
| 2.0                   | 2.89 B           | 3.56 A   | 3.78 A             |
| 4.0                   | 4.22 A           | 4.00 A   | 4.00 A             |
| 6.0                   | 3.00 B           | 2.22 B   | 2.89 B             |
| 8.0                   | 1.00 C           | 1.89 B   | 1.78 C             |

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

Table (8-B) indicated the least auxin concentration (0.5mg/L) reduced both callus and necrosis statistically in comparison with the other used concentrations. However, the addition of either 0.5 or 1.0mg/L of auxins to the medium gave the best significant growth and rooting while greening was significantly improved when only 1.0mg/L was used.

Table (8-C) reflects that different combinations of auxin types and concentrations induced slight differences without significancy as callus, growth, and greening parameters were considered. However, necrosis was significantly decreased when either 0.5, 1.0, or 2.0mg/L of IAA or 0.5mg/L of IBA or NAA were used. On the other hand, supplementation the medium with 1mg/L IBA enhanced root formation.

The above results give basis to concluded that 1.0mg/L of IBA induced the best rooting. Moreover, IBA surpassed IAA in inducing the best rooting parameters. These results confirmed the findings of Liu, et al., (1978) on some apple rootstocks and Dumanoglu et al., (1994) on quince. They found that the best rooting occurred by using 1.0mg/L IBA. Also, Wiesman, et al., (1988)

declared that IBA induced better rooting parameters than IAA because IBA is more resistant to oxidation due to the presence of side chain in IBA.

Table (8): Effect of different auxin types and concentrations on growth and rooting parameters of communis pear.

8-A: Effect of auxin type:

| Growth parameters Auxin types | Callus | Necrosis | Growth | Greening | Rooting |
|-------------------------------|--------|----------|--------|----------|---------|
| IAA                           | 2.61 C | 1.45 C   | 2.37 A | 3.22 A   | 2.22 B  |
| IBA                           | 3.39 A | 2.31 A   | 2.14 A | 3.06 A   | 3.08 A  |
| NAA                           | 2.95 B | 1.83 B   | 2.56 A | 3.00 A   | 3.08 A  |

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

#### 8-B: Effect of concentration:

| Growth parameters          | Callus | Necrosis | Growth | Greening | Rooting |
|----------------------------|--------|----------|--------|----------|---------|
| Auxin concentration (mg/L) |        |          |        |          |         |
| 0.5                        | 2.00 D | 1.00 D   | 2.76 A | 3.11 B   | 3.11 A  |
| 1.0                        | 2.78 C | 1.48 C   | 2.99 A | 3.70 A   | 3.44 A  |
| 2.0                        | 3.30 B | 2.00 B   | 2.15 B | 3.07 B   | 2.41 B  |
| 4.0                        | 3.85 A | 2.96 A   | 1.52 C | 2.48 C   | 2.22 B  |

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

#### III-2-b- Effect of medium state:

Table (9) indicates that solid and liquid medium states enhanced significantly growth and greening while significantly decreased necroses as compared to semi-solid medium. Meanwhile, liquid medium was significantly effective in maximizing rooting in comparison with the others.

The previous results indicate that liquid medium was valuable in rooting, which was in general agreement with the findings of Farid, (1997) on pecan and pistachio.

III-2-c- Effect of darkening treatment:

Table (10) and Photo (3) show that all-darkening treatments significantly increased callus production as compared with the control. Meanwhile, necrosis, growth and greening parameters didn't show any statistical responses to any darkening treatment. On the other hand, surface and outer coverage as well as their combination encouraged root formation significantly.

8-C:Effect of interaction between auxin type and concentartion:

| Growth parameters     |       | Callus | 4           | -      | Necrosis                |       |       | Growth   |       |        | Greening | Ď      |                                | Rooting  |        |
|-----------------------|-------|--------|-------------|--------|-------------------------|-------|-------|--|-------|--------|----------|--------|--------------------------------|----------|--------|
| Auxin types           |       |        |             |        |                         |       |       |  |       |        |          | 0      |                                | THOO THE |        |
| Concentrations (mg/L) | IAA   | IBA    | NAA         | IAA    | IBA                     | NAA   | IAA   | IBA NAA IAA IBA NAA IBA NAA IAA IBA NAA IBA NAA IBA NAA                              | NAA   | IAA    | IBA      | NAA    | IAA                            | IBA      | NAA    |
|                       |       |        |             |        |                         |       |       |  |       |        |          |        |                                |          |        |
| 0.5                   | 1.67A | 2.11A  | 2.11A 2.22A | 1.00E  | 1.00E 1.00E 1.00E 2.95A | 1.00E | 2.95A | 2.67A 2.67A 2.78A  | 2.67A | 2.78A  | 3.22.A   | 3.33A  | 3.22A 3.33A 1.89C 3.44B 4.00AB | 3.418    | 1.00AB |
|                       |       |        |             |        |                         |       |       |  |       |        |          |        |                                |          |        |
| 1.0                   | 2.56A | 3.22A  | 2.56A       | 1.11E  | 1.11E 2.00D 1.33E       |       | 2.87A | 2.78A 3.33A 4.00A 3.56A 3.56A  | 3.33A | 4.00.4 | 3.56A    | 3.56A  | 2.00C 1,14,A                   | 4,44,4   | 3.89AB |
| 2.0                   | 2.78A | 3.78A  | 3.33A       | 1.33E  | 1.33E 2.67BC            | 2.00D | 2.00A | 2.00A 1.67A 2.78A 3.22.A   | 2.78A | 3.22.4 | 3 00 A   | 3.00.5 | JTC JETC                       | 7360     | 2000   |
|                       |       |        |             |        |                         |       |       |  | T     |        |          |        |                                | 2001     | )      |
| 4.0                   | 3.45A | 4.45A  | 3.67A       | 2.33CD | 3.56A                   | 3.008 | 1.67A | 4.45A 3.67A 2.33CD 3.56A 3.00B 1.67A 1.44A 1.44A 2.89A 2.44A 2.11A 2.56C 1.89C 2.23C | 1.44A | 2.89.4 | 244      | 2.11A  | 2.56C                          | 1.89C    | 222C   |

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

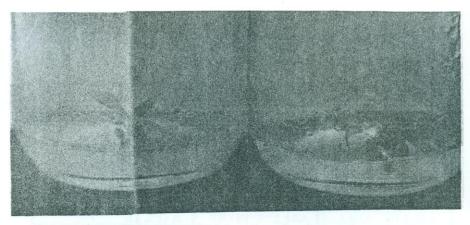


Photo (1): Effect of explant type on explant development. A = Shoots regenerated from nodal cutting. B = Shoots regenerated from shoot tip.

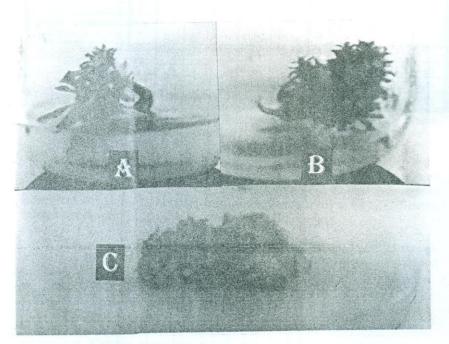


Photo (2): Effect of different BAP concentrations on proliferation of communis pear.  $A = 2.0 \ mg/L. \qquad B = 4.0 \ mg/L. \qquad C = 6.0 \ mg/L.$ 

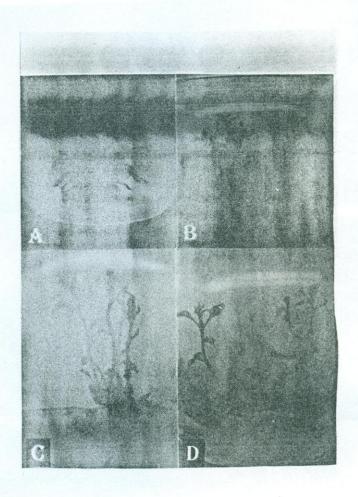


Photo (4): Steps of shoots elongation of communis pear.

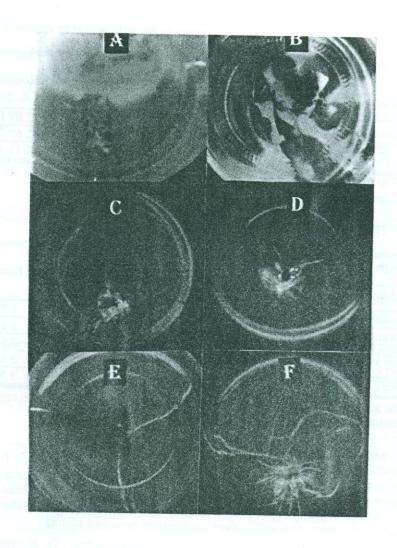


Photo (5): Steps of root development of communis pear.

Table (9): Effect of medium state on necrosis, growth, greening and rooting parameters of communis pear.

| Growth parameters |          |         |          |         |
|-------------------|----------|---------|----------|---------|
| Medium state      | Necrosis | Growth  | Greening | Rooting |
| Solid             | 1.67 B   | 2.67 A  | 3.33 A   | 2.89 B  |
| Semi-solid        | 2.78 A   | 1.67 B  | 2.00 B   | 1.67 C  |
| Liquid            | 1.67 B   | 2.33 AB | 3.78 A   | 4.11 A  |

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

Table (10): Effect of darkening treatments on growth and rooting of communis pear.

| Growth parameters        | 6       |          |        |          |         |
|--------------------------|---------|----------|--------|----------|---------|
| Darkening treatments     | Callus  | Necrosis | Growth | Greening | Rooting |
| Control                  | 1.11 D  | 2.00 A   | 3.00 A | 3.00 A   | 1.89 B  |
| Activated charcol        | 2.11 C  | 2.22 A   | 3.33 A | 3.11 A   | 1.67 B  |
| Surface coverage         | 3.00 B  | 2.00 A   | 4.44 A | 3.56 A   | 3.56 A  |
| Outer coverage           | 3.45 AB | 1.39 A   | 3.78 A | 3.78 A   | 3.44 A  |
| Surface + outer coverage | 3.89 A  | 1.33 A   | 3.44 A | 3.67 A   | 3.56 A  |

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

Generally the above results give basists suggest that both surface and outer coverage as well as their combination induced the best rooting. These results partially agreed with the findings of Kuhne, et al., (1988) on pome and stone fruits and Welander, (1991) on some apple rootstocks. They declared that darkening treatments encouraged rooting. Also, Farid, (1997) reported that both surface outer coverage as well as their combination increased rooting.

Photo (4) demonstrates different steps of shoot elongation after proliferation stage and Photo (5) shows rooting steps of communis pear.

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## إكثار اصل الكمثرى الكميونس في الأنابيب

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اجرى هذا البحث بمعمل زراعة الأنسجة بقسم البساتين بكلية الزراعة بمشتهر خلال الفترة من ١٩٩٥ إلى ١٩٩٧ و ذلك لتحديد البيئة المناسبة للحصول على و المتجانسة بأقل تكلفة و كذا تقليل اكبر عدد ممكن من النبتتات السليمة استيراد البذرة من الخارج.

أخذت القمم النامية و العقل ذات البرعم الواحد من الأفرع الحديثة لأصل الكمثرى الكميونس شهريا طوال السنة و تمت زراعتها على بيئات مختلفة (موراشيج و سكوج - لابوفير - اندرسون ) بحالات مختلفة (صلبة - شبه صلبة - سائلة) و قوى مختلفة (قوة كاملة - نصف قوة - ربع قوة - ثمن قوة) كما تمت دراسة تــ أثير أنــواع مختلفة من السيتوكينينات بتركيزات مختلفة و كذا تأثير قوى البيئة المختلفة تركيزات الجبريلين المختلفة على استطالة و تكوين مبادئ الجذور كما تمــت دراســة تأثير حالات البيئة المختلفة و أنواع مختلفة من الأكسنيات بمستويات مختلفة و كذا

تأثير معاملات الإظلام على تكوين الجذور .

وجد أن القمة النامية المنزرعة على بيئة موراشيج و سكوج الصلبة بقوة كاملة أو بنصف قوة أعطت أفضل النتائج من حيث تقليل موت االمنفصل النباتي و زيادة تطور االمنفصل النباتي و الاخضرار، كما اتضح أن اخذ المنفصل النباتي في الشهور من أبريل إلى يونيو أعطى نتائج ممتازة بينما تم الحصول على افضل زيادة عددية باستخدام البيئة شبه الصلبة مضافًا إليها ٤ مليجرام/لتر ٦-بنزيل أمينو بيورين و ساعد استخدام البيئة ذات القوة الكاملة أو نصف قوة مضاف اليها ٤ مليجرام/لتر جبريلين في الحصول على افضل استطالة للنبتة و كذا ثبت من النتائج إن استخدام البيئة السائلة مضافا إليها ١ مليجرام/لتر أندول حمض البيوتريك و تغطية الجزء السفلي من البرطمان (المنطقة السفلية من النبات المغمور في البيئة) سطحياً أو من الخارج أو بكليهما معا أعطت أفضل النتائج بالنسبة لتكوين الجذور.