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# MICROPROPAGATION OF PEAR ROOTSTOCK (*Pyrus betulaefolia* L.).

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## ABSTRACT

*In vitro* rapid propagation of pear rootstock (*Pyrus betulaefolia*) was established and achieved from shoot tips and nodal segment explants of mature tree grown in the greenhouse. Explants cultured on MS (Murashige and Skoog, 1962) basal medium in addition to Ca-pantothenate (10 mg/L) supplemented with two types of cytokinins (BA or Kin ) at the concentration of 0.0, 1.0, 2.0 and 3.0 mg/L singly or combined with IBA at 0.0, 0.5 and 1.0 mg/L were used. Shoot tip explant recorded the highest shoots number (7.6/shoot) as compared with single node explant (5.0/shoot) under the same BA concentrations (1.0 mg/L) and absence of IBA after 6 weeks of incubation at 25 ± 1ºC and 16 h photoperiod with a light intensity of 1500 lux using florescent lamps. Addition of cytokinin to the culture media was considered as limiting factor in shoot proliferation and was effective with the two explant types at low concentration of BA (1.0 mg/L) as compared with high concentrations (2.0 and 3.0 mg/L). Raised either BA or IBA concentrations alone or in combinations in the media reversely recorded lower shoot numbers in the two explant types compared with low concentration. Interestingly, single node that cultured on the medium contained BA (3.0 mg/L) and IBA (1.0 mg/L) obtained the lowest shoot number (2.4/shoot) as compared with all other studied treatments. The results revealed that BA was more effective than Kin in shoot formation at the same concentrations especially at 1.0 mg/L, since the number of shoots/explant (5.08) for BA against (3.23) for Kin. In the rooting stage, in general IBA was found more superior than NAA in root characters. Healthy shoots separated individually from the shoot clump and cultured on MS basal medium supplemented with IBA (0.5 mg/L) recorded the highest root formation (100 %), number of roots (5.7) as well as root length (2.5 cm) after 4 weeks of incubation. Well–developed pear plantlets transferred from rooting medium to acclimatization and the growing mixture of peat moss and perlite (1:1, v/v) obtained the highest plant survival (91.7 %), number of leaves (13.1 leaf) and plant height (17.7 cm) after 2 months in acclimatization.

**Keywords:***In vitro*, nodal explants, tissue culture, direct organogenesis, necrotic cultures,

## INTRODUCTION

Pear is one of the most important deciduous fruit trees all over the world it takes the second rank after apple in world production. Rootstocks play an important role in pear production since, the proper choice of rootstock is an important as the choice of variety and site (FAO, 2012)

Seedling rootstocks are not uniform in growth and productivity (Baviera *et al.,* 1989). Therefore, vegetative propagation methods like cutting and stooling are used to multiply pear rootstocks. In vitro propagation has shown promises for rapid and large scale clonal multiplication of disease free planting material throughout the year. In vitro propagation has been reported in several pear rootstocks like *P betulaefolia* L. (Hassanen and Gabr 2012).

The principal diseases of pear trees, which related to rootstocks, are fire blight *Pyrus betulaefolia* seedlings were used in the last few years in a commercial scale as rootstocks for Japanese cultivation (Stebbins, 1995). *Pyrus betulaefolia* is one of the best rootstocks, which is tolerant to wet and drought conditions. Resistant to decline, blight, root aphid and root rot (Paul and Silver 2002).

In Egypt, *P. betulaefolia* seeds were used to imported annually for production of rootstocks.

Biotechnological strategies, based on concepts of *in vitro* plant cell, and tissue and organ culture have been developed as an alternative and an ancillary measure in response to the problems related to the conservation of plant germplasm in the field (Vasanth and Vivier, 2011). These techniques also have the potential to overcome some of the limitations inherent to conventional methods of the conservation ex situ, and to facilitate the exchange of pest free germplasm with other research institutions (Ray and Bhattacharya, 2010).

*In vitro* propagation, due to high multiplication rate has been recognized as an efficient method for mass and clonal multiplication of elite species of the plant material (Shabbir *et al*. 2009).

Tissue culture techniques have been used for rapid plant propagation, plant breeding and for studying various aspects of plant growth and development in pear. Micropropagation is the rapid asexual *in vitro* multiplication of a desired plant. In pear, micropropagation was achieved for the first time in 1979 on pear rootstock OH x F 51 (Cheng, 1979) and scion variety Bartlett (Lane, 1979).

Since, these first reports, significant progress has been made in the different areas of *in vitro* pear culture. Recently, micropropagation in pear rootstock using axillary shoots proliferation from explants of nodes and shoot tips is the most desirable and safe method to minimize genetic variation (Rehman *et al.* 2014 a). The formation of healthy shoots with higher rates of multiplication is one of the prerequisite of an economically viable micropropagation protocol.

The present study was carried out to use the *in vitro* culture as a recent faster technique for establishing a large – scale clonal propagation and acclimatization protocol of *Pyrus betulaefolia* using shoot tips and single nodes as explants.

## MATERIALS AND METHODES

This study was carried out in the laboratory of Plant Tissue Culture, Dep. of Plant Biotechnology, Genetic Engineering and Biotechnology Institute, Sadat City Univ., Egypt during the period 2011 - 2013. The produced pear vitroplants were acclimatized under greenhouse conditions of Sadat City University. The shoot tips and single nodes explants of young branches of *P. betulaefolia* were used as experimental plant materials; it was collected in spring from greenhouse of private Nursery, Badr City, Egypt.

The MS (Murashige and Skooge, 1962) basal medium including sucrose (30 g/L)] in addition to Ca-pantothenate (10 mg/L) was used. The pH of the media was adjusted to 5.8 with 0.1 M KOH or 0.1 M HCl prior to gelling agent addition (agar 7.0 g/L). Media were dispensed either in a glass tubes (25 x 2.5 cm; Borosil ) capped with Bellco plastic caps containing 15 ml medium or into jars (325 ml) at the rate of 50 ml/jar. The jars were capped with polypropylene closures and autoclaved at 121°C and 1.2 Kg/cm² for 20 min.

**Preparation of experimental plant materials:**

Terminal shoots with 5-6 cm in length were excised using sharp knife. Leaves were carefully removed then, transferred to the laboratory and soaked under running tap water for two hours, followed by a soap solution treatment for 5 min then rinsed under running tap water for 30 min. each shoot was divided into two explants; shoot tip of 9-10 mm in length and single nodes. The excised explants were surface sterilized in the laminar air flow cabinet by immersed in sterilized mercuric chloride ($HgCl_2$) solution (0.1%) for 3 min, 2 drops/ 100 ml solution of Tween 20 (polyoxyethylenesorbitan monolaurate) as wetting agent were used. Followed by rinsed (4 times) with sterilized distilled water to remove all traces of the disinfectant.

**Effect of cytokinin type (BA or Kin) and concentration on number of shoot/explant:**

After surface sterilization, the explants were shortened to 5-6 mm in length and were planted on the surface of the solidified MS (Murashige and Skoog, 1962) basal medium supplemented with sucrose (30 g/L) and solidified with phyto agar (7 g/L) in addition to plant growth regulators. Two types of cytokinins were tested;

- BA (6-benzylaminopurine), at 0.0, 1.0, 2.0 and 3.0 mg/L singly or a combined with IBA (indole-3-butyric acid) at the concentration of 0.0, 0.5 and 1.0 mg/L.
- Or Kin (6-Furfurylaminopurine) at 0.0, 1.0, 2.0 and 3.0 mg/L singly or a combined with IBA (indole-3-butyric acid) at the concentration of 0.0, 0.5 and 1.0 mg/L.

Five jars (replicates) were used; each replicate consisted of one jar containing three explants. Cultures were incubated at 25 $^{o}$C ± 1 and photoperiod of 16-h light using florescent tubes with a light intensity of 1500 lux. After 6 weeks of incubation, the number of shoots per explant was recorded.

**Effect of auxin type (IBA or NAA) and concentration on root formation:**

In vitro regenerated shoots obtained during shoot proliferation stage were separated individually from the shoot clump and cultured on rooting medium. The MS basal medium supplemented with sucrose at 30 g/L supplemented with two types of auxins were tested; IBA or NAA at the concentration of 0.0, 0.5, 1.0 and 1.5 mg/L. The medium was solidified with phyto agar (7.0 g/L). Each treatment included 10 replicates (culture tubes) and each tube contained one shoot. The culture tubes were incubated under the same environmental conditions previously mentioned. After 4 weeks of incubation, root formation percentage, number of roots/shoot and the root length (cm) were recorded.

**Effect of soil and growing mixture types on acclimatization:**

Well-rooted healthy plantlets produced from rooting stage were washed with tap water to remove the remaining agar from the roots and then planted into plastic pots of diameter 6 cm. Those pots were filled with sand only, peat moss and sand (1:1, v/v), peat moss and sand (1:2, v/v), peat moss and perlite (1:1, v/v) and peat moss only.

The cultured pots were protected with plastic bags to maintain high relative humidity around the plants. The pots were maintained for a month under greenhouse environmental conditions at 25 ± 1ºC, relative humidity (80-90 %) and 16 h photoperiod with a light intensity of 4000 lux. A solution of one half strength MS salts was used weekly to fertigate the pots to enhance the development of plants. Each treatment included 12 replicates (plantlets). After 2 months, survival percentage, shoot height (cm) and leaves number/plant were recorded.

**Statistical analysis:**

Experiments were set up in a completely randomized block design. Data were statistically analyzed by analysis of variance (ANOVA) according to Gomez and Gomez (1984).The Least Significant Differences among levels of each treatment were compared using L.S.D. test at 5%, according to Steel and Torrie (1980).

## RESULTS AND DISCUSSION

Previous pre-elementary unpublished experiments were done on the same pear rootstock to avoid and control shoot tip necrosis obtained *in vitro* within pear tissue culture protocol, depending upon our long experience with date palm cultivars with the same physiological disorder phenomena (Hegazy and Aboshama, 2010 & Hegazy, 2014). It could be concluded that addition of Ca-pantothenate (10 mg/L) to MS basal medium in all *in vitro* pear growth stages obtained beneficial effect on avoiding shoot tip necrosis in pear rootstock c.v *betulaefolia*. Recently, Rehman *et al.* (2014a,b) reported in pear rootstock ( Kainth ) that necrotic culture on nodal explants was found to be influenced by type of media and growth regulator fortification during establishment stage. Rehman *et al.* (2014a) found in pear rootstock ( Kainth ) that least necrotic culture percentage in Kainth was observed by using MS medium supplemented with BA (1.5 mg/L) and IBA (0.01 mg/L). MS medium containing BA (1.5 mg/L) and IBA (0.25 mg/L) gave maximum explant establishment (52.80 %).

**Effect of cytokinin type (BA or Kin) and concentration on number of shoot/explant:**

The explants which excised during spring season obtained high survival percentage as compared with other season; it might be due to climate suitability in spring. Similar observation was detected by Bharate *et al.,* (2008) which found that, the intensity of oxidative browning was less during spring and increased by time tell reached the maximum during summer. As well as. Usage MS basal medium was a good choice for pear micropropagation. This is in agreement with Bell and Reed (2002) since

they reported that MS nutrient medium has been most commonly used for axillary shoot proliferation of pear.

Data presented in Table (1) and Fig. (1) showed the interaction between explant types and the different IBA and BA concentrations had a non-significant effect on the number of shoots per explant. However, the highest record of shoot numbers (7.60) was obtained with shoot tip explant cultured on medium containing BA (1.0 mg/L) only (Fig 1) followed by (6.50) with shoot tip explant and BA (1.0 mg/L) and IBA (0.5 mg/L). In this respect., Rehman *et al.* (2014a,b) reported in pear rootstock ( Kainth ) that necrotic culture on nodal explants was found to be influenced by type of media and growth regulator fortification during establishment stage. Our obtained results could be due to the modification we did to the basal MS medium by the addition of Ca-pantothenate (10 mg/L). Which may played an important role in avoiding the occurrence of the physiological disorder (shoot tip necrosis) in plant tissue (Hegazy and Aboshama, 2010 & Hegazy, 2014).

Results in Table (1) clearly showed the main effect of explant types on the number of shoots /xplant. Evidently, this growth character was significantly increased by used shoot tip as explant (3.61) as compared with that of single node (2.48). On contradictory, Hassanen and Gabr (2012) on *Pyrus betulaefolia*, they found that nodal explant showed a better response than shoot tip explants towards shoot regeneration. Data representative on the effect of IBA concentrations showed that IBA at 0.0 and 0.5 mg/L had significantly a similar higher effect on the number of shoots / explant, while at 1.0 mg/L a significant decrease was observed. The obtained data on the effect of BA levels revealed that no proliferated shoots were observed in the absence of BA, while was significantly increased by the presence of BA in the culture media, especially at 1.0 mg/L since the highest number (5.08) was recorded. Concerning the effect of explant types and IBA levels, data demonstrated in Table (1) indicated that at all IBA concentrations, the number of shoots/explant was significantly increased when shoot tip explant was cultured compared with single node explant. In addition, a gradual decrease was observed with raised IBA concentration up to 1.0 mg/L with both types of explants. Data of the interaction between explant type and BA levels indicated that no proliferated shoots were recorded in the absence of BA with both explant types. On the contrary, all levels of BA significantly increased the number of shoots especially with shoot tip explant. In this concern, the highest response was recorded (6.27) with shoot tip explant and BA (1.0 mg/L) However, other combinations of both growth regulators under study significantly increased this character. The highest number of shoots (6.30) was obtained with the medium devoid of IBA and contained BA (1.0 mg/L), while the lowest number (2.50) was obtained with the treatment of IBA (1.0 mg/L) and BA (3.0 mg/L). Results in agreement with those of Hutchinson (1981) who reported that MS media supplemented with BA was satisfactory for many species and cultivars of plant crops for their *in vitro* propagation. In general, BA is the most effective cytokinins for pear micropropagation (Thakure and Kanwar 2008 & Rehman *et al.* 2014a,b).

**Table (1): Effect of BA and IBA concentrations and explant type on number of shoots per explant of *Pyrus betulaefolia after 6 weeks in vitro.***

| Explant types(A) | IBA mg/L (B) | BA (mg/L) (C) | | | | Means of (A x B) | Means of (A) |
|---|---|---|---|---|---|---|---|
| | | 0.0 | 1.0 | 2.0 | 3.0 | | |
| | | No. of shoots/explant | | | | | |
| Shoot tip | 0.0 | 0.00 | 7.60 | 6.30 | 4.60 | 4.63 | 3.61 |
| | 0.5. | 0.00 | 6.50 | 4.50 | 3.10 | 3.53 | |
| | 1.0 | 0.00 | 4.70 | 3.40 | 2.60 | 2.68 | |
| Single node | 0.0 | 0.00 | 5.00 | 3.90 | 3.60 | 3.13 | 2.48 |
| | 0.5. | 0.00 | 3.90 | 3.00 | 2.70 | 2.40 | |
| | 1.0 | 0.00 | 2.80 | 2.50 | 2.40 | 1.93 | |
| Means of (C) | | 0.00 | **5.08** | **3.93** | **3.16** | Means of (B) | |
| Means of (B x C) | 0.0 | 0.00 | 6.30 | 5.10 | 4.10 | 3.88 | |
| | 0.5. | 0.00 | 5.20 | 3.75 | 2.90 | 3.96 | |
| | 1.0 | 0.00 | 3.75 | 2.95 | 2.50 | 2.30 | |
| (A x C) | Shoot tip | 0.00 | 6.27 | 4.73 | 3.43 | | |
| | Single node | 0.00 | 3.90 | 3.13 | 2.90 | | |

**LSD at 5% A = 0.18 B = 0.23 C = 0.26 A x B = 0.32 A x C = 0.37 B x C = 0.45 A x B x C = NS**

**Effect of Kin and IBA concentrations and explant types on number of shoots/explant**

Data on the main effect of explant type (Table, 2) revealed that shoot tip explants had a significant increase in shoot number compared with single node explants. Shoot tips from the current season's growth have been widely used as explants for *in vitro* propagation of pear (Thakur and Kanwar 2008).

Concerning the main effect of IBA, Table (2) revealed that with the gradual increasing of IBA concentrations up to 1.0 mg/L, number of shoots was gradually decreased. However, IBA-free medium showed the highest response. The effect of Kin showed that no shoots were obtained in the absence of Kin, while a significant increase in shoot number was observed with Kin addition to the culture media and the lowest level (1.0 mg/L) was more effective in this concern. The interaction effect between explant types and IBA concentrations indicated that there was no significant difference among these combinations on the number of shoots. Rehman *et al.* (2014a) studied the effect of various media {1/2 MS , MS and WPM and growth regulators (BA, IBA and NAA) on establishment, proliferation and rooting. Per cent necrotic culture was found to be influenced by type of media and growth regulator fortification during establishment stage. Data of the main effect of interaction between explant types and Kin concentrations obviously clear that there was no produced shoots in the absence of Kin, however a significant increase in this parameter was observed at all Kin levels compared with the control, particularly at 1.0 mg/L showed the highest recorded no of shoots with shoot tip and single node (3.73 and 2.73, respectively). Data in the same Table showed the effect of interaction between IBA and Kin concentrations; it is clear that in the absence of Kin, no shoots were proliferated at all IBA levels, however with decreasing levels of IBA from 1.0 to 0.0 mg/L and Kin from 3.0 to 1.0 mg/L, a gradual increase in shoot number was recorded.

Concerning the interaction between explant types and different concentrations of IBA and Kin, data in the same Table revealed that, higher responses were recorded (4.50) with the treatment contained Kin (1.0 mg/L) and an explant of shoot tip in the absence of IBA, Fig (1) followed by shoot tips explant (3.70) cultured on medium contained either Kin (1.0 mg/L) with IBA (0.5 mg/l) or Kin (2.0 mg/L) only recorded (3.70). However, these results did not reach to the significance level at 5%. In this regard, Sangwan and Harada (1977) reported that the morphogenetic responses of the different explants varied under the same hormonal treatment. In no case, there was direct regeneration of shoot buds from the mature tissue explants (stem, leaf, root and pedicel), while formation of multiple shoots was observed from apical meristem explants with cytokinin alone or along with NAA. The present result indicated that cytokinins can stimulate growth of lateral buds and thus suppress apical dominance (Te-Chato *et al.* 2008).

**Table (2):Effect of Kin and IBA concentrations and explant type on number of shoots per explant of *Pyrus betulaefolia after 6 weeks in vitro.***

| Explant types (A) | IBA( mg/L) (B) | Kin (mg/ L) ( c ) | | | | Means of (A x B) | Means of (A) |
|---|---|---|---|---|---|---|---|
| | | 0.0 | 1.0 | 2.0 | 3.0 | | |
| | | No. of shoots/explant | | | | | |
| Shoot tip | 0.0 | 0.00 | 4.50 | 3.70 | 3.00 | 2.80 | 2.33 |
| | 0.5. | 0.00 | 3.70 | 2.80 | 2.70 | 2.30 | |
| | 1.0 | 0.00 | 3.00 | 2.30 | 2.20 | 1.88 | |
| Single node | 0.0 | 0.00 | 3.40 | 3.10 | 2.70 | 2.30 | 1.98 |
| | 0.5. | 0.00 | 2.70 | 2.40 | 2.80 | 1.98 | |
| | 1.0 | 0.00 | 2.10 | 2.20 | 2.40 | 1.68 | |
| Means of (C) | | 0.00 | **3.23** | **2.75** | **2.63** | Means of (B) | |
| Means of (B x C) | 0.0 | 0.00 | 3.95 | 3.40 | 2.85 | 2.55 | |
| | 0.5. | 0.00 | 3.20 | 2.60 | 2.75 | 2.14 | |
| | 1.0 | 0.00 | 2.55 | 2.25 | 2.30 | 1.78 | |
| Means of (A x C) | Shoot tip | 0.00 | 3.73 | 2.93 | 2.63 | | |
| | Single node | 0.00 | 2.73 | 2.57 | 2.63 | | |

**LSD at 5% A = 0.14 B = 0.17 C = 0.20 A x B = NS A x C = 0.28 B x C = 0.34 AxBxC = NS**

From the results occurred in Tables (1 and 2), it is worth to mention that BA was more effective in shoot formation than Kin in all used treatments regardless the type of explant. However, the only exception was observed with the treatments of both cytokinins (BA and Kin) at 3.0 mg/L which showed similar results when each one of them combined with IBA (1.0 mg/L) with using single node explant.

**Fig. (1). Effect of cytokinins (BA or Kin) type at the same concentration (1.0 mg/L) on shoot proliferation obtained from Pear rootstock shoot tips explant cultured *in vitro* for 6 weeks.**

**Effect of type and concentration of auxin on root formation.**

Data on the main effect of auxin type showed in Table (3) and Fig. (2) indicated that well rooted plantlets cultured on MS basal medium supplemented with IBA (0.5 mg/L) after 4 weeks in vitro. While, NAA was more effective in increasing root formation percentage as compared with IBA. However, Rehman *et al.* (2014 b) reported on *in vitro* pear rootstock that no rooting was obtained irrespective of media using NAA. Concerning the main effect of concentrations, the same data indicated that, the presence of auxin resulted in increasing root formation percentage compared with auxin-free medium. The lowest record (23.3 %) was obtained in the absence of both auxins (control), whereas the high records (100%) were obtained with IBA or NAA at 0.5 mg/L and NAA at 1.0 mg/L respectively. Similar results were reported by Rehman *et al.* (2014 a) they found in pear rootstock (Kainth) that WPM medium supplemented with BA (3.0 mg/L) resulted in highest proliferated cultures (83.19%). Similarly the highest shoots/explant were obtained using WPM medium supplemented with BA (3.0 mg/L). Data showed that there was a significant increase in root numbers as a result of auxins addition compared with the control treatment. The highest number of roots (5.65) was obtained with the concentration 0.5 mg/L and a gradual decrease was observed with increasing the concentrations to 1.0 or 1.5 mg/L. However, Rehman *et al.* (2014 a) reported on pear that using MS medium in addition to NAA (1.0 mg/L) induced highest roots /explant (3.60 ). Although high records of root numbers were achieved with IBA or NAA at 0.5 mg/L but, all combinations among auxins types did not reach to the significance level at 5%. On the other hand, Rehman *et al.* (2014 b) reported on *in vitro* pear rootstock that the maximum no of roots 2.38 were recorded with 1/2 MS salt strength medium fortified with IBA (1.0 mgl/L).

Data in Table (3) also showed the effect of types and concentrations of auxins on root length. In this concern, data on the main effect of auxin

types reveal that IBA was more effective in increasing root length than NAA. Concerning the effect of auxin levels, data on the main effect indicated that the presence of auxin at all levels resulted in increasing this character as compared with the control. The highest root length (2.5 cm) was obtained at IBA (0.5 mg/L), while the lowest length (1.0 cm) was obtained at NAA (1.5 mg/L). The same data showed that there was no significant effect for combinations between types and levels of auxins on root length. However, high record of root length (2.5 cm) was obtained with 0.5 mg/L IBA. In this regard, Rehman *et al.* (2014 b) reported on *in vitro* pear rootstock (Patharnakh) that, roots of maximum length were scored using WPM medium supplemented with IBA (1.0 mg/L)

**Table (3): Effect of type and concentration of auxins on the formation of roots on shoots of *P. betulaefolia* after 4 weeks *in vitro.***

| Growth characters | Root formation % | | | Number of roots/shoot | | | s | | |
|---|---|---|---|---|---|---|---|---|---|
| Types(A) / Con. (B) | IBA | NAA | Mean (B) | IBA | NAA | Mean (B) | IBA | NAA | Mean (B) |
| 0.0 | 23.3 | 23.3 | 23.3 | 1.3 | 1.3 | 1.30 | 2.1 | 2.1 | 2.10 |
| 0.5 | 100.0 | 100.0 | 100.0 | 5.7 | 5.6 | 5.65 | 2.5 | 2.0 | 2.25 |
| 1.0 | 96.6 | 100.0 | 98.3 | 4.9 | 4.2 | 4.55 | 1.6 | 1.3 | 1.45 |
| 1.5 | 90.0 | 93.3 | 91.66 | 3.6 | 3.2 | 3.40 | 1.3 | 1.0 | 1.15 |
| Mean (A) | 77.48 | 79.15 | | 3.88 | 3.58 | | 1.88 | 1.60 | |
| L.S.D at 0.05% | | | | NS A = 0.53 B = NS AxB = | | | 1.96 2.78 NS | | |

**Fig. (2):Well rooted plantlets cultured on MS basal medium supplemented with IBA (0.5 mg/L) after 4 weeks *in vitro***

**Effect of soil mixture type on survival percentage, plant height (cm) and number of leaves of pear plantlets after 2 months in acclimatization stage in the greenhouse**.

Data in Table (4) and Fig. (3) demonstrated that there was significant difference among all the used growing mixtures for the collected growth parameters. The growing mixture of peat moss and perlite (1:1, v/v) scored the

highest survival percentage, plant height (cm) and number of leaves 91.7 %, 17.7 and 13.1 respectively. Similar results were obtained on *P. betulaefolia in vitro,* by Guo and Jin (1994) they found that when shoots were transferred into a mixture composed of perlite and carbonized chaff (1:1, v/v), survival of *ex-vitro* plantlets was 89.5 - 95% in the acclimatization stage. However, Bommineni *et al.* (2001) reported that pear had a low percentage of survival and acclimatization after transfer to the soil, perhaps since most of the roots were induced through callus instead of originating directly from the base of the main shoot.

On the other hand our results indicated that, sand soil treatment were recorded the lowest growth parameters values in survival percentage, plant height (cm) and number of leaves 33.3%, 9.6 and 6.5, respectively. Noteworthy, increase addition of sand to peat moss remarkable reduced survival percentage and all other growth parameters. Sand may made the growing mixture compact and
reduced the air space in the mixture.

**Table (4): Effect of soil mixture type on growth characters of pear plantlets after 2 months in acclimatization stage in the greenhouse**.

| Soil mixture type | Survival (%) | Plant height (cm) | No. of leaves |
|---|---|---|---|
| Sand | 33.3 | 9.6 | 6.5 |
| Peat moss + Sand (1:1, v/v) | 50.0 | 14.7 | 10.1 |
| Peat moss + Sand (1:2, v/v) | 41.7 | 13.7 | 8.2 |
| Peat moss + Perlite (1:1, v/v) | 91.7 | 17.7 | 13.1 |
| Peatmoss | 50.0 | 11.6 | 9.5 |
| L.S.D at 0.05% | 39.0 | 1.82 | 1.20 |

**Fig (3): Healthy P*. betulaefolia* vitroplant grown in growing mixture after 2 months in the greenhouse**

## REFERENCE

Baviera JA, J.L. Garcia and M. Ibarra (1989) Commercial in vitro micropropagation in pear cv. Conference. Acta Horticulturae 256:63-68.

Bell, R. L.and. M B, Reed. (2002). *In vitro* tissue culture of pear: Advances in techniques for micropropagation and germplasm preservation. *Acta. Hortic.* 596: 412–418.

Bharate, K. P.; D. U Guoqiag,.; Z.Yuxing,; J.Liu, and S. Qingchum, (2008). Studies on browning problem and phenols content on shoot of Yali, Aikansui and Abee Fete pears for *in vitro* culture. *Front. Agric. China*, 2(3):321-330.

Bommineni V. R.; H Mathews,.; B S Samuel,. M Kramer,. and D. R Wagner,. (2001). A New Method for Rapid *In Vitro* Propagation of Apple and Pear Hort Science 36 (6):1102–1106.

Cheng, T. V. (1979). Compact Fruit Trees. 12: 127-137.

Food and Agricultural Organization (FAO) Statistical Yearbook 2012. ISBN 978-92-5-106913-4. www.fao.org

Gomez, K. A. and A. A Gomez,. (1984). Statistical Procedures for the Agricultural Researches. John Wiley and Son, Inc. New York.

Guo, D. C. and M. X Jin,. (1994). Effects of different environment and medium on the transplanting survival rate of plantlets *in vitro*. *Acta Agriculturae Zhejiangensis*, 6 (3): 171 – 175.

Hassanen, S. and M.Gabr, (2012). *In vitro* propagation of pear *Pyrus betulaefolia* rootstock *American – Eurasian J. Agric. & Environ. Sci*., 12 (4): 484-489.

Hegazy, A. E. (2014). Promising protocol for in vitro direct organogenesis of date palm cv. Ekhlass. Fifth International Date Palm Conference , Abu Dhabi - UAE; 16 – 18 March 2014, p. 113

Hegazy, A. E. and H.M. Aboshama, (2010).An efficient novel pathway discovered in date palm micropropagation.Acta Hort.(ISHS) 882:167-176.

Hutchinson, J. F. (1981). Tissue culture propagation of fruit trees. In: Proc. Symp. On Tissue Culture of Economically Important Plants, A. N. Rao (Ed.) Singapore, pp: 113-120.

Lane, W. D. (1979). Regeneration of pear plants from shoot meristem tips. *Plant Sci. Lett*. 16: 337-342.

Murashige, T. and F. Skoog, (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*., 15: 473-497.

Paul, M. and D. Silver, (2002). Growing temperate tree fruit and nut crops for planting in the home garden and landscape. Univ. Of California, USA.

Ray, A. and S. Bhattacharya, (2010). Storage and conversion of Eclipta alba synseeds and RAPD analysis of converted plantlets. *Biologia Plantarum*, 54:547-550.

Rehman, H. U.; M. I. S Gill,.; W. S Dhillon,. and, , S. Bedi (2014 b). Micropropagation of Patharnakh(Pyrus pyrifolia (Burm f.) nakai) pear using explants obtained from forced cuttings.Int. J. Agric.Sc & Vet.Med.Vol. 2 (2): 54-65.

Rehman, H. U.; M. I. S Gill,.; G. S Sidhu,. and H. S Dhaliwal,. (2014 a). Micropropagation of Kainth (Pyrus pashia) – An important rootstock of Pear in Northern Subtropical Region of India. J. of Exp. Biology and Agric. Sci., Vol. 2(2): 188-196.

Sangwan, R. S. and H. Harada,(1977).Cellular totipotency in chrysanthemum tissues cultured *in vitro*. *Acta Hort*. (ISHS), 78: 237-242.

Shabbir, M. K.; H.; Nadeem, M. Anwar, and , M. W. Mumtaz (2009). Physico-chemical analysis and determination of various chemical constituents of essential oil in *Rosa centifolia* . *Pakistan Journal of Botany*, 41(2): 615-620.

Stebbins, R. L. (1995). Choosing Pear Rootstocks for the Pacific Northwest. pacific northwest extension publication Washington, Oregon and Idahostate Universities. Cooperative extension service, 1: 341-344.

Steel, R. G. and J. H. Torrie, (1980). Principles and Procedures of Statistics, a Biomerical Approach.
Mc Grow- Hill Book Company, New York, 469-517.

Te- Chato, S.; A Hilae,. and K,. In-Peuy (2008). Effect of cytokinin type and concentration on growth development of cell suspension culture of Oil palm. *J. Agric. Technol*., 4: 157-163.

Thakure, A. and J. S. Kanwar, (2008). Micropropagation of wild *Pyrus pyrifolia* (Burm F.) Nakai. 1 Explant establishment and shoot multiplication. *Not. Bot. Hort. Agrobot. Cluj* 36 (1), 103-108.

Vasanth, K. and M. A. Vivier, (2011). Improved cryopreservation procedure for long term storage of synchronised culture of grapevine. *Biologia Plantarum* 55(2): 365 – 369.

## الإكثار الدقيق لآصل الكمثرى بيتشلفوليا

**عادل السيد حجازى**

**جامعه مدينة السادات – معهد الهندسة الوراثية والتكنولوجيا الحيوية – قسم البيوتكنولوجيا النباتية**

كطريقه إكثار سريعة يمكن تطبيقها على النطاق التجارى تم تحديد جميع المراحل المختلفة والمطلوبة لإكثار الكمثرى معمليا. تمت المقارنة بين نوعين من الاجزاء النباتية هي القمة النامية والعقدة الساقية والتي فصلت من أصول أشجار ناضجة نامية فى الصوبة. زرعت هذه الاجزاء النباتية على بيئة موراشيج وسكوج والمضاف اليها بنثوسينات الكالسيوم (١٠ ملجم /لتر) وسكروز ( ٣٠ جرام /لتر ) والسيتوكينينات ( بنزيل ادينين أو كاينتين) بتركيزات صفر, ١, ٢ و ٣ ملجم / لتر منفردة أو مع أندول حمض البيوتيريك بتركيزات صفر, ٠.٥ و ١ ملجم /لتر. وقد أظهرت النتائج تفوق القمة النامية معنويا في عدد الأفرع الناتجة منها بالمقارنة بالعقدة الساقية. ولم ينتج أفرع على اى من الاجزاء النباتية المنزرعة ( العقدة الساقية أو القمة النامية ) في غياب البنزيل ادنين من بيئة الزراعة. كان أكبر عدد من الأفرع ( ٧.٦ ) واضحا مع البيئة المحتوية على١ ملجم / لتر بنزيل أدينين مع غياب الأوكسين وذلك عند إستخدام القمه الناميه, بينما ظهرأقل عدد من الأفرع ( ٢.٤) عند زراعة العقد الساقية على بيئة محتوية على ١ ملجم / لتر أندول حمض البيوتيريك مضاف اليها ٣ ملجم / لتر بنزيل أدينين. تفوق البنزيل أدينين على الكاينتين عند نفس التركيزات المختبرة وخاصة عند ١ ملجم / لتر مع غياب الأكسين حيث كان عدد الأفرع المتكونة عند هذا التركيز مع البنزيل أدينين ٧.٦ فى مقابل ٤.٥ مع الكاينتين. لتجذير الأفرع الناتجة تمت مقارنة نوعين من الأكسين هما أندول حمض البيوتريك ونفثالين حمض الخليك عند تركيزات صفر, ٠.٥, ١ و ١.٥ ملليجرام / لتر. تفوقت البيئة المحتوية على ٠.٥ أندول حمض البيوتريك من حيث عدد وطول الجذور المتكونة. وفى مرحلة الأقلمة أوضحت النتائج أن خليط البيتموس والبيرليت بنسبة ١:١ حجما قد تفوق من حيث عدد النباتات الحية (٩١.٧%) وكذلك من حيث عدد الاوراق على النبات (١٣.١ ) وإرتفاع النبات ( ١٧.٧ سم ) بعد شهرين من بداية الاقلمة.