In Vitro Propagation of some Pear Rootstocks

Abstract

The rootstock is very important to the growth and development of the tree as a necessary part of a grafted fruit tree. In this study, seeds of several pear species were collected for tissue culture, its germination, subculture and micro-propagation were investigated. The chilling requirement of different wild pear species also were compared to select the rootstock strains which need low chilling requirement and more suitable under Egypt condition. Three different media were tested for multiplication M1: BA at 2mg/l + Kin at 1mg/l + NAA at 1mg/l, M2: 2ip at 1mg/l + Kin at 0.5 mg/l + IBA at 0.5 mg/l, M3: BAP at 4 mg/l + TDZ at 1mg/l + IBA at 1mg/l.in ten pear rootstocks. It was different response among different rootstock genotypes in number of shoots and shoot length. The media M1 and M3 were recorded the higher value of shoot number, while M2 were recorded the highest value of shoots length. For rooting stage different concentration of IBA (1, 2 and 3 mg/l) and IAA (0.5, 1 and 2 mg/l) were tested and the result showed that, low concentration of IBA or IAA were more suitable for rooting percentage and root number, and the effect of IBA on rooting were better than IAA. *Pyrus. betulifolia* and *P. calleryana* had the highest rooting percentage and the IBA 1 mg/l was the best medium. *In vitro* propagation of some wild pear rootstocks can be achieved, different growth regulators of auxin and cytokinin had effect on pear proliferation stage. In the rooting stage, the low concentration of IBA were better than high concentration and IAA. *P. betulifolia, P. calleryana* and *P. serrulata* were high in survival percentage and were more suitable for *in vitro* propagation of pear rootstocks in Egypt, moreover they had low chilling requirement.

Key words: Pear rootstocks, Pyrus sp., Tissue culture, Micro-propagation, Seed germination, Auxins, Cytokines

Introduction

Pear is the third most important temperate fruit in the world production, next to grape and apple [1]. *Pyrus sp.* with proper ecophysiological [2] and medicinal [3, 4] properties is the second most naturally widespread species in the world. Pear planting in Egypt is limited for biotic and abiotic stress and needs to import seeds of rootstocks every year, so we tried to propagate pear rootstocks locally by tissue culture technique.

Wild pear fruit tree genotypes in natural arid ecosystems were evolved to resist a complex stressful condition [5] and potentially capable of preparing valuable rootstocks for fruit orchard establishment under water deficit and salts management models.The wide genetic variation in *Pyrus* makes micropropagation challenging for many genotypes [6]. Thakur and Kanwar [7] reported that BA (1.5 mg per litre) + IBA (0.5 mg per litre) was the best growth regulator combination for shoot multiplication in

wild pear as it produced sufficient number of shoots.The multiplication and growth of shoots were better on Pear Medium with higher concentrations of calcium chloride, potassium phosphate and magnesium sulfate than MS medium with 4.4 µM N6 benzyladenine (BA) [6].

Many countries such as Egypt can`t produce pear rootstocks and must import pear seeds every year, therefore the aim of the study to select suitable pear rootstocks for tissue culture and propagate them by tissue culture nationally.

Materials and Methods

This study was achieved through two successive years of 2020 to 2021 in the Tissue Culture Technique Laboratory, Central Laboratories Network - National Research Center - Dokki - Egypt.

Plant material

The seeds of wild pear rootstocks were provided under MoU between National Research Centre (NRC) and Research Institute of Pomology, Chinese Academy of Agricultural Sciences (IPCAAS). The wild pear rootstock trees were collected from different areas in China and planted in the orchard of IPCAAS under natural pollination, where the fruits were collected for seeds extraction. As in fig (1) and fig (2) we had five species *P. phaeocarpa, P. betulifolia, P. serrulata, P. calleryana* and *P. ussuriensis.* We noticed that the shape and size of fruit and seed of different trees were different, even of the same species such as in *P. betulifolia*.

Pear seeds germination

Complete and good seeds were selected which were not floating on the water surface.

Seeds sterilizing and stratification

For seeds sterilizing using 70-75% ethanol for 30 sec. Then $HgCl_2$ 0.1 % for 10 min. Then washing in sterilized water 3-5 times for 3-5 min with shaking. The seeds soaking in sterilized water (in sterilized jars) for 24 hours. For culture media of stratification and germination using ¼ MS without sugar and sterilized the media. Planting the seeds in dishes and jars under the hood sterilized condition, setting two treatments of 3 weeks and 4 weeks for stratification of each test species and planting 20 seeds each treatment. During the stratification stage the seeds keeping in refrigerator at 4 -7 ℃ for 3-4weeks and Checking every three days for germinating.

Germination

- After cold stratification the seeds incubated in dark at 25 ℃ for germinated.
- The germination percentage were recorded after 3 weeks and 4 weeks.
- Germination %= Number of germinated seeds / total seeds *100.

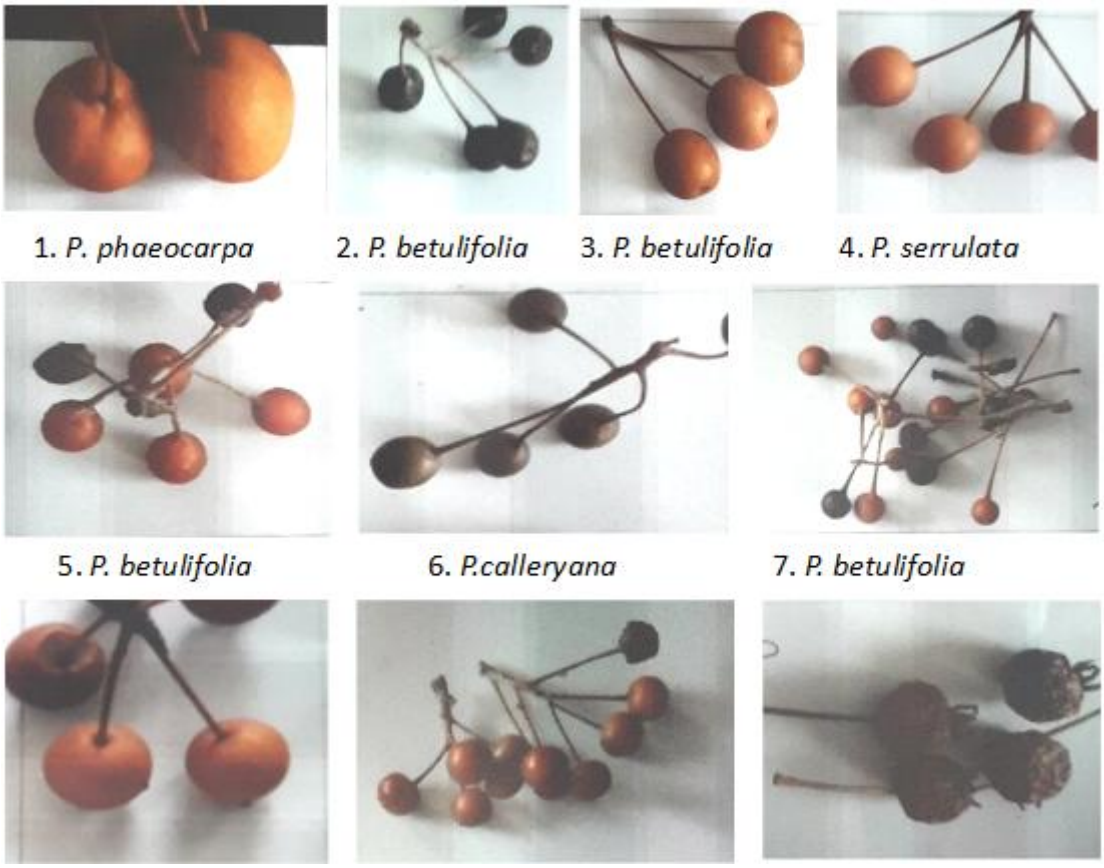

Fig. 1: Fruits of different wild pear rootstocks with different size and shape

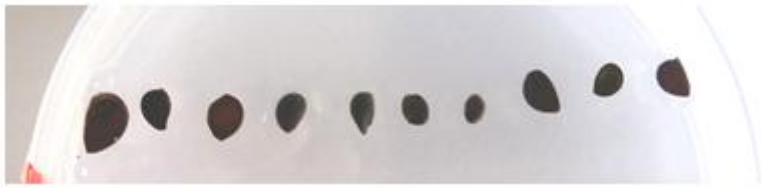

Fig. 2: Seeds of different rootstocks (from left to right 1 to 10) with different shape and size

Multiplication stage

After the seeds germinated in jars, the shoot tips of each seedling were cut and planted on MS medium separately to multiple shoots for two months as initiation stage, then the shoot tips from the same seedling were divided and cultured on MS with three combinations of different auxin and cytokinin concentrations (M1, M2 and M3) for proliferation under sterilized condition. 10 tips were planted for each treatment and replicated 4 times.

M1: BA at 2mg/l+ Kin at 1mg/l+ NAA at 1mg/l

M2: 2ip at 1mg/l+ Kin at 0.5 mg/l + IBA at 0.5 mg/l

M3: BAP at 4 mg/l+ TDZ at 1mg/l+ IBA at 1mg/l

BA (benzyle adenine), BAP (6- Benzyleaminopurine), Kin (Kinetin), TDZ (Thidizuron) and 2ip (isopentenyl adenine) are Cytokinin plant growth regulators.NAA (Naphthalene Acetic Acid) and IBA (Indol -3- butyric Acid) are plant hormones from auxin family.

MS medium [8] (Murashige & Skoog 1962) salts at full strength were supplemented with vitamins, Ino-sitol at 100 mg/l, glutamine at 200 mg/l, adenine at 100mg/l, citric acid at 150 mg/l, ascorbic acid at 150 mg/l during germination stage and shoot multiplication stage. All types of solid media used in this study were supplemented 30 g/l sucrose and 8 g/l purified agar. The pH was adjusted to 5.7 ± 0.02 by NaOH. The media were autoclaved at 121℃ for twenty five minutes, then the media were cooled and harden for 24 hours.

Shoot cultures were incubated in culture room at 26± 2 ℃ and under the day-light condition16 hours and dark condition 8 hours. Number of shoots/explants and average shoots length (cm) were recorded after eight weeks.

Rooting Stage

Effect of auxin type & concentration on rooting percentage and roots number of different pear seedling microshoots were investigated during rooting stage. Microshoots of pear seedling about 3-5 cm in length produced after the 3th subculture were transferred to MS rooting medium at half strength supplemented with:

R1: MS Free auxin
R2: MS + IBA at 1mg/l
R3: MS + IBA at 2 mg/l.
R4: MS + IBA at 3 mg/l
R5: MS + NAA at 0.5 mg/l
R6: MS + NAA at 1 mg/l
R7: MS + NAA at 2 mg/l.

Rooting percentage and number of roots /microshoots were recorded after two months. This experiment contained 2 auxin types × 3 concentration + MS Free auxin = 7 treatments. Each treatment represented by 9 microshoots. Experiment was coordinated in a completely randomized design.

Acclimatization stage

After rooting stage the plantlets of pear rootstocks about 8-10 cm in length were rinsed carefully with water distilled and sterile to remove adhering medium and transplanted into 20 cm plastic pots containing a mixture of peatmoss: perlite with ratio (1: 1) by volume.

Plantlets were grown in greenhouse condition and covered with clear polyethylene bag for two months, then the polyethylene bags were progressively removed. The plantlets were sprayed with MS medium salts solutions at half strength weekly. Survival percentages were recorded after three months from transplanting. Experiment was harmonious in a completely randomized design. Every treatment includes 3 replicates and each replicate contained one pot, each pot contained one plantlet.

Statistical analysis

The current study followed a complete randomized design, with three replicates for each treatment. The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran [9]using MSTAT software (1998), and significant differences among the various treatments were compared by Least Significant Difference (LSD) according to Duncan (1955) at significance level of 0.05.

Results and discussion

Pear rootstock seeds germination

The data in table (1) showed that the germination percentages of different rootstocks were different, *P. betulifolia* (R7, R8 and R9) has higher germination percentage and germinated earlier than the others. The R1 (*P. phaeocarpa)*, R6 (*P. calleryana)* and R10 (*P. ussuriensis)* had lower germination percentage and need more chilling hours than (*P. betulifolia).* R1 germinated after 6 weeks.

Table 1. Germination percentage of pear rootstocks after three and four weeks

Pear rootstocks	Germination percentage after three weeks stratification (%)	Germination percentage after four weeks stratification (%)	Mean of the germination percentage (%)
R1 *P. phaeocarpa*	0	0	0
R2 *P. betulifolia*	20	40	30
R3 *P. betulifolia*	0	70	35
R4 *P. serrulata*	40	70	55
R5 *P. betulifolia*	40	70	55
R6 *P. calleryana*	0	40	20
R7 *P. betulifolia*	100	60	80
R8 *P. betulifolia*	60	60	60
R9 *P. betulifolia*	60	80	70
R10 *P.ussuriensis*	20	30	25

Fig. 3: Seed germination after stratification

Multiplication stage

Effect of different auxin, cytokinin types and concentration on number of shoot/culture and average shoots length (cm) of different pear rootstock seedlings were investigated in the first ,second and third subcultures during Multiplication stage. Data in Table (2) showed that, the rootstock (R6) had the highest number of shoot/culture, and M3 treatment was the best as in Fig (4). The different rootstocks had different response with the three media, where rootstocks R1, R2, R3 and R4 had high value significant with medium M1 whereas the rest of rootstocks were with medium M3. On the other hand R1, R3, R4 and R9 had high significant value of shoot length with medium M1. Whereas the rootstocks R2, R5, R7, R8 and R10 had with medium M2

Table 2. Effect of different MS media on numbers of shoots and shoots length of different pear rootstocks in the three subcultures during multiplication stage

Rootstocks	Media	Number of shoots/culture				Average shoots length (cm)			
		1st subcu.	2nd subcu.	3rd subcu.	Mean	1st subcu.	2nd subcu.	3rd subcu.	Mean
R 1	M 1	2.0	3.5	3.2	2.9 efg	1.5	2.0	2.5	2.0 jkl
	M 2	2.0	1.7	1.7	1.8 hi	1.2	2.9	2.3	2.1 h-l
	M 3	1.5	1.0	0.7	1.0 i	1.6	3.2	2.2	2.3 h-l

R 2	M 1	3.2	4.5	6.7	4.8 bc	2.7	2.1	2.7	2.5 g-k
	M 2	2.2	4.0	2.0	2.7 fgh	2.7	3.9	5.2	3.9 bcd
	M 3	3.7	2.0	5.2	3.6 def	3.1	3.3	1.2	2.5 g-j
R 3	M 1	4.5	7.0	4.5	5.3 b	4.0	4.0	3.7	3.9 b-e
	M 2	1.2	2.2	2.5	2.0 ghi	3.8	3.7	1.6	3.0 d-h
	M 3	1.7	1.7	1.0	1.5 i	1.5	3.2	1.5	2.0 i-l
R 4	M 1	5.0	5.0	4.7	4.9 bc	1.7	3.1	7.2	4.0 bc
	M 2	5.7	5.7	2.5	4.6 bcd	4.0	3.5	3.2	3.5 b-f
	M 3	1.5	1.5	1.5	1.5 i	2.0	1.5	2.3	1.9 jkl
R 5	M 1	1.5	2.2	2.2	2.0 ghi	2.8	3.5	3.8	3.4 c-g
	M 2	1.5	1.0	1.2	1.2 i	4.7	3.9	3.1	3.9 b-e
	M 3	1.7	5.2	4.7	3.9 cde	2.7	3.7	2.5	3.0 e-i
R 6	M 1	1.5	6.2	5.2	4.3 bcd	1.7	3.5	3.8	3.0 e-i
	M 2	1.7	6.5	6.0	4.7 bc	4.5	4.7	4.3	4.5 b
	M 3	9.2	9.7	9.2	9.4 a	4.5	4.3	7.0	5.2 a
R 7	M 1	2.5	3.5	2.5	2.8 fgh	1.7	3.8	4.7	3.4 c-g
	M 2	1.5	2.5	1.7	1.9 ghi	3.1	3.5	5.6	4.1 bc
	M 3	3.2	2.2	4.0	3.1 ef	3.4	3.0	4.5	3.6 b-f
R 8	M 1	3.0	3.5	4.5	3.6 def	3.2	3.0	4.2	3.5c-f
	M 2	3.5	4.5	3.7	3.9 cde	4.5	4.7	4.2	4.5 b
	M 3	4.2	4.2	4.5	4.3 bcd	2.7	3.0	3.4	3.0 d-h
R 9	M 1	5.2	4.0	5.2	4.8 bc	4.0	3.7	5.2	4.3 bc
	M 2	2.0	2.0	1.5	1.8 hi	3.7	3.0	3.5	3.4 c-g
	M 3	7.0	8.0	10.7	8.5 a	3.2	1.8	1.6	2.2 h-l
R 10	M 1	1.5	1.0	2.2	1.5 i	1.5	1.3	1.7	1.5 l
	M 2	1.2	1.5	1.5	1.4 i	2.1	2.0	4.0	2.7 f-j
	M 3	1.5	1.2	2.5	1.7 hi	1.9	1.2	1.7	1.6 kl

Fig. 4: Effect different media M1, M2 and M3 on shoots number and shoots length in multiplication stage of R6

From the previous data we can concluded that different rootstocks showed different response in multiplication stage on different media. The rootstocks *Pyrus betulifolia* (R9), *Pyrus calleryana* (R6*)* and *Pyrus serrulata* (R4) were multiplicated better and with higher number of shoots and shoot length than the other rootstock species. The media M1 and M3 recorded the higher value of shoots number, while M2 recorded the highest value of shoots length.

Our result agreed with the data of Sedlaka and Paprstein [10] in which they reported that the proliferation didn't only depend on the concentration of cytokinins in the culture medium, but also on

the response of an individual genotype. The observed variation among genotypes of pear cultivars could result from the different genetic control mechanisms affecting tissue auxin and cytokinin metabolism under artificial in vitro cultivation conditions. Analí Lizárraga *et al*. [11] reported that BA and TDZ were proved to be the most effective cytokinins for multiplication of all tested apple and pear cultivars varying the optimal concentration according to the genotype.

Table 3. Effect of different concentration of auxin types (IBA&IAA) on rooting percentage of pear rootstocks

Pear Rootstocks	Rooting percentage							
	T1	T2	T3	T4	T5	T6	T7	Mean
R1	2.3 c-g	3.0 b-e	2.3 c-g	1.3 f-j	2.0 d-h	2.0 d-h	0.6 hij	1.90 abc
R2	2.6 b-f	4.6 a	3.0 b-e	0.6 hij	3.3 a-d	1.3 f-j	0.3 ij	2.20 a
R3	2.0 d-h	3.6 abc	1.3 f-j	0.33 IJ	3.0 b-e	0.7 hij	0.3 ij	1.60 bc
R4	3.0 b-e	2.0 d-h	1.0 g-j	0.33 ij	2.0 d-h	2.0 d-h	0.3 ij	1.50 cd
R5	2.0 d-h	3.3 a-d	1.3 f-j	0.7 hij	2.6 b-f	1.3 f-j	0.0 j	1.60 bc
R6	3.3 a-d	4.6 a	2.3 c-g	1.3 f-j	2.3 c-g	2.3 c-g	0.0 J	2.30 a
R7	2.0 d-h	3.3 a-d	1.0 g-j	2.3 c-g	1.3 f-j	1.3 f-j	2.6 b-f	2.10 ab
R8	4.0 ab	2.0 d-h	0.7 hij	3.0 b-e	1.3 f-j	1.0 g-j	0.7 hij	2.00 abc
R9	0.7 hij	1.0 g-j	1.3 f-j	1.6 e-i	1.0 g-j	0.3 ij	1.3 f-j	0.95 de
R10	0.7 hij	0.7 hij	0.0 j	0.33 ij	1.0 g-j	1.6 e-i	1.3 f-j	0.80 e
Mean	2.20 b	3.00 a	1.83 bc	0.77 d	2.33 b	1.40 c	0.57 d	

Rooting and survival stages

The data of rooting percentage of different pear rootstocks as shown in table (3) cleared that R2 (*P. betulifolia*) and R6 *(P. calleryana)* were the highest rooting percentage and the T2 treatment at IBA 1 mg/l was the best medium. Also R6 *(P. calleryana)* and (*P. betulifolia*) except R9 were high in roots number/ microshoots with free hormone and IBA 1 mg/l media as in table (4). R10 (*P.ussuriensis)* was the lowest ability for rooting and roots number with all treatments. In general the low concentration of hormone IBA or IAA is more suitable for rooting percentage and roots number and the effect of IBA on rooting is better than IAA. Although roots were developed on basal medium without any growth regulator. The root formation was varied among different pear rootstocks as in fig. (5).

The survival percentage was the highest value with R6 without different significant with R5, R7 and R9 (*P. betulifolia*) and the lowest survival percentage was R1 (*P. phaeocarpa*) and R10 (*P.ussuriensis*) as in table (5). The plantlets of the ten rootstocks shown in fig. (6).

Table 4. Effect of different concentration of auxin types (IBA&IAA) on roots number /microshoots of pear rootstocks

Pear Rootstocks	Roots number / Microshoots							
	T1	T2	T3	T4	T5	T6	T7	Mean
R1	2.0 d-i	3.3 a-e	4.0 abc	2.3 c-h	1.3 f-j	1.6 e-j	0.7 hij	2.19 b
R2	4.0 abc	4.6 ab	2.0 d-i	0.7 hij	2.6 c-g	1.3 f-j	0.7 hij	2.29 b

R3	3.3 a-e	3.3 a-e	1.3 f-j	0.7 hij	3.0 b-f	1.3 f-j	0.3 ij	1.91 b
R4	2.6 c-g	3.0 b-f	2.0 d-i	1.0 g-j	2.6 c-g	1.3 f-j	0.7 hij	1.91 b
R5	3.0 b-f	4.0 abc	2.3 c-h	1.0 g-j	1.6 e-j	2.0 d-i	0.0 j	2.00 b
R6	5.0 a	4.0 abc	3.0 b-f	2.0 d-i	3.0 b-f	4.0 abc	0.0 j	3.00 a
R7	2.3 c-h	4.0 abc	2.0 d-i	1.0 g-j	1.6 e-j	1.0 g-j	1.3 f-j	1.91 b
R8	3.6 a-d	3.3 a-e	2.3 c-h	0.7 hij	1.3 f-j	1.3 f-j	2.6 c-g	2.19 b
R9	1.6 e-j	0.7 hij	1.0 g-j	1.0 g-j	2.3 c-h	1.3 f-j	0.7 hij	1.24 c
R10	1.0 g-j	1.0 g-j	1.0 g-j	0.0 j	1.3 f-j	0.7 hij	1.0 g-j	0.86 c
Mean	2.8 a	3.1 a	2.1 b	1.0 c	2.1 b	1.6 b	0.8 c	

Table 5. Survival percentage during acclimatization stage of different pear rootstocks

Pear rootstocks	Survival percentage
R1	11.11 d
R2	66.60 b
R3	33.30 cd
R4	77.70 ab
R5	88.80 a
R6	93.30 a
R7	77.70 ab
R8	44.40 c
R9	77.70 ab
R10	11.11 d

These results agreed in many investigations, maximum roots were formed when medium was supplemented with IBA [12]. Most studies involving in vitro rooting of apple and pear use auxins, such as IBA and IAA. The use of IBA has been shown to play an important role in the rooting of these fruit trees as well as in different plants; however, the optimum concentration of IBA for maximum rooting differs between species and cultivars [11] (Analí Lizárraga *et al*. 2017).

Fig. 5: Rooting formation of partial trial pear rootstocks, from left to right R4,R5,R8,R9,R10.

Fig. 6: The plantlets of different pear rootstocks from left R1 to right R10

Conclusions

Our results confirmed that *in vitro* propagation of some wild pear rootstocks can be achieved. The different growth regulators of auxin and cytokinin had effect on pear proliferation stage. In the rooting stage, the low concentration of IBA were better than high concentration and IAA. *Pyrus betulifolia, Pyrus calleryana* and *Pyrus serrulata* were more suitable for *in vitro* propagation of pear rootstocks in Egypt and they had low chilling requirements.

List of Abbreviations

BA: Benzyl adenine

BAP: 6- Benzylaminopurine

KIN: Kinetin

TDZ: Thidiazuron

2ip: Isopentenyl adenine

NAA: Naphthaleneacetic acid

IBA: Indole -3- butyric acid

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