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Nyo Zin Hlaing
Plant Tissue Culture Laboratory Department of Biotechnology, Ministry of Education, Kyaukse, Myanmar

Fang Ming
Germplasm Resources and Breeding of Pear, Institute of Pomology (CAAS), Ministry of Agriculture, Xingcheng, 125100, Liaoning, China

Jiang Shuling
Germplasm Resources and Breeding of Pear, Institute of Pomology (CAAS), Ministry of Agriculture, Xingcheng, 125100, Liaoning, China

Correspondence:
Jiang Shuling
Germplasm Resources and Breeding of Pear, Institute of Pomology (CAAS), Ministry of Agriculture, Xingcheng, 125100, Liaoning, China
Email: jshling[at]163.com

Study of *In vitro* Root Induction and Hardening Responses of Four *Pyrus spp.*

*Nyo Zin Hlaing, Fang Ming, Jiang Shuling**

Abstract

Explants of three *P. betulifolia* (Bet 1, Bet 2 and Bet3) and *P. ussuriensis* (Uss) were tested for root induction in vitro and hardening in vivo. Bet 1 was the best rapid rooting response among four pears. The maximum rooting percent and the highest number of roots were 86.67% and 7.33 respectively that were observed in Bet 1 at ½MS basal medium supplemented with 0.3 mg/l IBA and quick-dip 15s of 2 mg IBA. Other Bet 2 and 3 were not different with each other. But Uss was produced the longest root length. It was 3.23cm at ½MS basal medium containing 0.2 mg/l IBA and 15s quick-dip of 2mg IBA. Almost 60 % of Bet 1 explants were hardened.

Keywords: Pyrus, *P. betulifolia*, *P. ussuriensis*, Root induction, Hardening, quick-dip.

INTRODUCTION

Advance of biotechnology provided additional techniques for quick manufacturing of high quality, disease elimination and homogenous planting materials. Biotechnological tools like *in vitro* background and micropropagation tender a beneficial opportunity in studies of fruit trees propagation, control of virus and administration of genetic resources. Micropropagation can manufacture high-qualities explants within short durations that don't need large areas. Their products are disease-free strong rooting and uniform growth qualities [2]. Fruit trees are multiplied onto rootstocks. The rootstock is the most essential reason in resolving the powerful and consecutive size of the tree. The numerous sizes can be actually affected. The alternative of rootstock is essential as it will establish the acceptable of the tree for the location and the development form. In fruit species, the different *in vitro* techniques (called biotechnologies) have observed several practical utilizations both for the clonal propagation and genetic improvement. Climatic conditions, plant diseases and pests combine several risks to the genetic resources conserved in field accumulations. Management of plant genetic resources via tissue culture could defend against those risks. Mass propagation of plants; especially axillary bud multiplications are identical of genotypically and phenotypically as the elite plant from which they were manufactured (Even, 1990). The quality of *in vitro* shoots come out roots affected several factors, containing differences between genotypes, culture processes, mineral nutrious, subculture durations, the stage of tissue developed and morphological maturity. Because of these factors, *in vitro* shoots showing the different of rooting reactions developed into plants that several demonstrated as easy-to-root and hard-to-root.

Pears are members of the subfamily Pomoideae and the family of Rosaceae. The genus *Pyrus* of pear is composed of 20 species. The natural habits of pear distribute a large area from Europe to Asia of china and japan. Most of pears are deciduous, medium-size tree and not ever green. Many pear fruits are globular but can vary size and shape depending on species [8]. Among *Pyrus* species, *P.betulifolia* called Duli and *P.ussuriensis*, called Ussurian or Harbin pear are Asian pears that are major commercial in China. Pyrus rootstocks are not easy to get root vegetative. The objectives of this research were to get the standard in vitro rooting method for pears and hardening in field trials of tissue culture explants.

MATERIALS AND METHODS

A. Explant Sources

The *in vitro* shoots of *Pyrus sps.* were applied as the sources of this study. They were obtained from Laboratory of Germplasm Resources and Pear Breeding, Institute of pomology, Chinese Academy of Agricultural Science, Xingcheng, Liaoning.

B. Multiplication for Pyrus spp.

The four *Pyrus* species: three *Pyrus Betulifolia* (Bet 1, Bet 2, Bet 3) and *Pyrus ussuriensis* (Uss) of *in vitro* shoots were multiplied on full MS medium adding with 0.5 and 1.0 mg/l BA, 0.2 mg/l Zt, 0.1 mg/l IBA, 0.4mg/l IAA and 0.1 mg/l GA3. Moreover, 3% sucrose and 0.6 % agar added on one liter media respectively. The media of pH was adjusted to 5.75 with 0.1 N KOH and then they were sterilized with autoclave (121˚C for 20 minutes). All culture bottles were set in a culture room after planting *in vitro* shoots at 20 ± 3˚C with a light power of 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ brightened by white phosphorescent. They were changed fresh media every 4 weeks.

C. Rooting Media of Four Pyrus spp.

Firstly, about 2-3cm of the four pears shoots obtaining from multiplication was used for *in vitro* root induction with two-steps procedure. The first one was that they were prepared to grow on ½ MS basal medium adding with 0.2 and 0.3 mg/L of IBA (Indole-3-butyri acid). The media were disinfected as the above multiplication stage. The shoots were cultured on this basal media under the disinfected laminar airflow. They were maintained in culture room under rotating of darkness 7 days and light 7 days.

After 2 weeks, the second step was changed that explants were 10 and 15 seconds quick dipped in 2mg/L IBA (50% Ethanol). And then, shoots were grown on free hormone of ½MS medium exception of the fourth salt concentrations later 2 weeks. But control plants were placed under only light condition and never changed media until 1 month. All bottles were maintained in a culture room at 20 ± 3˚C with a light power of 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ brightened by white phosphorescent. The four treatments in this research were:

T 1 = ½MS + 0.2 mg/l IBA + 10s dip IBA
T 2 = ½MS + 0.3 mg/l IBA + 15s dip IBA
T 3 = ½MS + 0.2 mg/l IBA + 15s dip IBA
T 4 = ½MS + 0.3 mg/l IBA + 15s dip IBA

D. Hardening of Pear Explants

Explants were removed from the medium of culture bottles and gently washed with water that had not adhesive medium. Then, they were moved to grow in plastic pots containing mixture of sterile soil and perlite. They were covered with plastic to avoid dry and to get full humidity. The coverings are gradually removed after 2 weeks and the plants are moved to higher light intensity under normal room. Finally, they were grown in field trial after 2 months.

E. Collection and Analysis of data

Roots from each bottle and roots from each shoot source were counted one month after transfer to rooting media. A randomized block experimental design was applied for LSD all-pairwise comparisons test. Mean values for each treatment were calculated using Statistix 8.0 that was identified differences among treatments at a 95% level of confidence ($P \leq 0.05$) and results of multiple range tests was used to separate homogeneous groups among treatments. Graphs were analyzed with Microsoft Excel 2010. The data was counted after one month. The below rooting counts were determined: rooted shoots, the number of roots, root lengths and the rooting percent.

RESULTS AND DISCUSSIONS

The results of this research were observed as the following suitable tables and graphs. In this study, the multiplication results were not showed because the main target was the root induction activities of pears.

i. *In vitro* Rooting Activities of Four Pears in Different Treatments

The four pears were studied of the rooted shoots among different rooting activities at different treatments in table I. Among these, treatment 2 was observed the best and the lowest rooted shoots in Bet 1 (4.00) and Bet 3(1.67). But 'Uss' (4.00) was also good in this rooted shoots activity at treatment 3. The other treatments were significant different each other in table I.

Moreover, the different root length results were counted among four pyrus species at 4 treatments. Bet 2 was significantly different with other species showed in Table 3. Minimum root length was 0.84 cm at treatment 2 of Bet 2.

Table 1: Means of the rooted shoots of the four pear species at different treatments after 1 month

Treatments	Bet 1	Bet 2	Bet 3	Uss
T 1	2.67b	2.67a	2.00a	2.67a
T 2	4.11a	2.39a	1.67a	3.56a
T 3	3.33ab	2.67a	2.33a	4.00a
T 4	4.33a	2.33a	1.67a	3.00a

(p<005), the different letters are significant different.

In this study, the maximum root length was 3.23cm in Uss of treatment 3. The above results were competitively shown in figure 1.

Table 2: Means of the number of roots depending on 4 treatments after 1 month on four types of pears.

Treatments	Bet 1	Bet 2	Bet 3	Uss
T 1	3.33b	4.67a	2.67a	6.33a
T 2	3.5b	3.78a	2.33a	6.61a
T 3	4.33b	4.33a	2.33a	4.33a
T 4	7.33a	4.33a	2.00a	4.67a

(p<005), the different letters are significant different.

Table 3: Showing means of the root length of four pears at different four treatments after 1 month

Treatments	Bet 1	Bet 2	Bet 3	Uss
T 1	2.53b	1.90d	2.12a	1.83b
T 2	1.83cd	0.84c*	2.67a	2.00ab
T 3	1.60d	1.87b	1.70a	3.23a
T 4	2.02c	1.07a	2.47a	1.54b

(p<005), the different letters are significant different.

Finally, the rooting percent of the four pears were shown in table IV and figure 2. The maximum and minimum of rooting percent were 86.67 at treatment 4 and 82.77 at treatment 2. Both results were observed in Bet 1. But Uss of the rooting percent was also better than the other two species. Treatment 1 is the lowest rooting percent in every species.

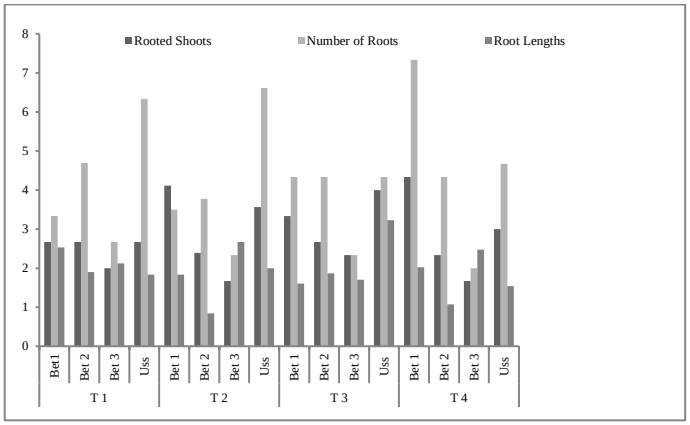

Figure 1: Competitive results of rooted shoots, number of roots and root lengths of the four pears among 4 treatments.

According to this data, IBA 0.3 mg/l was good for three P. betulifolia and early given for pear rooting. Among these species, Bet l was shown the early rooting responses within 2 weeks. Bet 2 was not different with other treatments except T 4. Bet 3 was the worst rooting response in the four pears.

Table 4: Means of the four pears of rooting percent on different treatments after 1 month.

Treatments	Bet 1	Bet 2	Bet 3	Uss
T 1	53.33b	53.3a	40.00a	53.33a
T 2	82.22a	47.7a	33.33a	71.11a
T 3	66.67ab	53.3a	46.67a	80.00a
T 4	86.67a	46.6a	33.33a	60.00a

(p<005), the different letters are significant different.

The four treatments gave clear roots without calli in four species. NAA had not given any responses but it was not shown in this research.

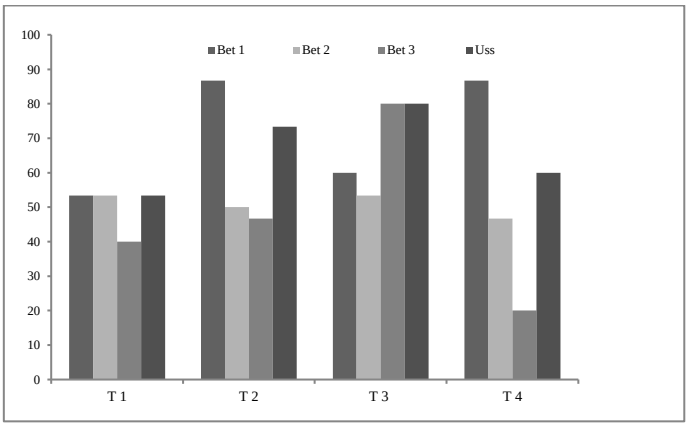

Figure 2: Different rooting percent of the 4 pears on different treatments after 1 month.

Shibli *et al*. (1997) reported that NAA, IBA and IAA were produced in vitro rooting of *P.syrica* and maximum 72 % rooting was performed at 17 µM IAA. But these two species *P.betulifolia* and *P.ussureinsis* were developed roots only IBA in this research. Moreover, Al. Maarri et.al (1994) was researched that IAA was not achieved roots in pears species. High concentrations of auxin in rooting media could be increased callus formation and reduced root elongation.

IBA containing media were placed in darkness 5-7 days, but 7 days treatment was the best and quick giving roots. The previous researches were showed that darkness treatments were long 5-20 days. The darkness time is suitable for 7 days because when the plants were long in darkness, the hardening step could not succeed. Darkness could be promoted cell devision and maintained auxin degradation because it reduced the activity of peroxidase. As other treatments, Reed (1995) was concluded that IBA and NAA dip treatment were produced for pear rooting because of unknown rooting potential. The results that *P.eleaegrifolia* was dipped in 10-20 mM IBA for in vitro rooting were not different with culturing in 5 µM IBA supplemented media in dark. The four pears in this research could be induced root at quick-dip of 2mg IBA (50% ethanol).

In some plants, *in vitro* rooting was only occurred under the light. Also the other findings in some other species were controlled the light as a rooting delayed and about one week in the dark would be chanced rooting. Dark condition was be slow up the effect of photochromic and auxin content such as IBA under a short dark period could be promoted the cell division and later increased the rooting percent.

ii. Expplants Hardening

ion period, new leaves were added and roots become stronger and plants needed more water requirement to fill the loss of water by dessication in land plant. And then, they were moved to the greenhouse over two months later.

60 % of Bet 1 explants were hardend in pots. Uss was not resistance in hardening so they could get hardening just 33.33%. The other Bet 2 and 3 were stabled in hardening almost 28-35%. Finally, Bet 1 was not only the best rooting response but also hardening activity this research.

CONCLUSIONS

To conclude this paper, *P.betulifolia* species were the best rooting responses at IBA 0.3 mg/l + quick-dip 15s in this research. But *P.ussureinsis* was suitable for IBA 0.2 mg/l. Dark condition held to produce in vitro root of the four pears. The combination of low IBA, quick-dip of IBA, darkness and hormone free media changing must be induced rooting response of pear but should be researched the other methods of rapid root formation of pear, hardening and production of commercial tissue culture pear plants.

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